

SCHOOL OF PH.D. STUDIES  
SEMMELWEIS UNIVERSITY

---

# ALMANAC

## 6

2009-2010



Simmelweis Publishers

[www.semmelweiskiado.hu](http://www.semmelweiskiado.hu)

B u d a p e s t , 2 0 1 2

Compiled by     Anita Marosfalvi  
                      Emőke Márton  
                      Tímea Rab

Edited by        Dr. Károly Rácz

© School of Ph.D. Studies, Semmelweis University, Budapest, 2012

**ISSN 2063-2207**

All rights reserved. No part of this work covered by the copyright hereon may be reproduced or copied in any form or by any means—graphic, electronic or mechanical, including photocopying, recording, taping on information storage and retrieval systems—without written permission of the author and the publisher.



Responsible editor: László Tancos

Make-up editor: János Békésy

Cover design: László Tancos

SKD: 383

Printed and bound by: Avaloni Nyomda Kft.

---

# TABLE OF CONTENTS

---

<b>SCHOOL OF PH.D. STUDIES, SEMMELWEIS UNIVERSITY .....</b>	<b>5</b>
General Overview .....	5
Organizational Structure .....	6
Program Overview .....	7
Admission and Tuition .....	8
Doctoral Council .....	9
Permanent Committees of the Doctoral Council .....	10
Educational Board .....	10
Quality Control and Evaluation Board .....	10
Committee of Disciplinary Procedure .....	12
Doctoral Secretary Office .....	13
Doctoral Students' Union .....	14
Intranet .....	15
‘VERITAS ET VIRTUS’ AWARD IN MEMORY OF DR. ZSOLT FARKAS JR. ....	16
PH.D. COURSES .....	17
PH.D. SCIENTIFIC DAYS .....	24
EXCELLENT PH.D. SUPERVISOR AWARD .....	25
AWARDING OF THE DOCTORAL DEGREE WITH DISTINCTION .....	25
 <b>1. BASIC MEDICINE DOCTORAL SCHOOL .....</b>	<b>26</b>
Program 1/1. Physiology and clinics of the heart and coronary diseases .....	26
Program 1/2. Mechanisms of normal and pathological functions of the circulatory system .....	38
Program 1/3. Biological effects of ionizing and non-ionizing radiation .....	45
Program 1/4. Fluid and electrolyte balance in healthy and pathological regulation of blood pressure and circulation .....	48
Program 1/5. Clinical and experimental cardiology and atherosclerosis .....	57
 <b>2. CLINICAL MEDICINE DOCTORAL SCHOOL .....</b>	<b>63</b>
Program 2/1. Oxidative stress and immunological reaction in liver diseases .....	63
Program 2/2. Fetal and neonatal medicine .....	67
Program 2/3. Prevention of chronic diseases in childhood .....	68
Program 2/4. Gastroenterology .....	79
Program 2/5. Dental research .....	91
Program 2/6. Clinical haematology .....	104
Program 2/8. Physiology and pathology of the musculoskeletal system .....	109
Program 2/9. Pulmonology .....	120
Program 2/10. Ophthalmology .....	127
Program 2/12. Clinical and experimental research in angiology .....	132
Program 2/13. Hormonal regulatory systems .....	135
Program 2/14. Clinical and experimental research on urological diseases .....	144
Program 2/15. Molecular genetics, pathomechanisms, and clinical aspects of metabolic disorders .....	150
Program 2/16. Dermatology and venerology .....	160

<b>3. PHARMACEUTICAL SCIENCES DOCTORAL SCHOOL .....</b>	<b>167</b>
Program 3/1. Modern trends in pharmaceutical sciences .....	168
Program 3/2. Experimental and clinical pharmacology .....	193
<b>4. MENTAL HEALTH SCIENCES DOCTORAL SCHOOL .....</b>	<b>202</b>
Program 4/1. Psychiatry .....	203
Program 4/2. Behavioral sciences .....	215
Program 4/3. Group of research topics: mental health sciences .....	235
<b>5. SPORT SCIENCES DOCTORAL SCHOOL .....</b>	<b>237</b>
Program 5/1. Training and adaptation .....	237
Program 5/2. Physical training, regulation and metabolic transport .....	241
Program 5/3. Sport and social sciences .....	251
<b>6. JÁNOS SZENTÁGOTHAÏ NEUROSCIENCES DOCTORAL SCHOOL .....</b>	<b>269</b>
Program 6/1. Neuromorphology and cell biology .....	269
Program 6/2. Neuroendocrinology .....	285
Program 6/3. Functional neuroscience .....	290
Program 6/4. Clinical neuroscience .....	300
Program 6/5. Clinical neurological investigations .....	308
Program 6/6. Biological psychiatry .....	314
<b>7. MOLECULAR MEDICINE DOCTORAL SCHOOL .....</b>	<b>318</b>
Program 7/1. Cellular and molecular physiology .....	318
Program 7/2. Pathobiochemistry .....	323
Program 7/3. Embryology, theoretical, experimental and clinical developmental biology .....	336
Program 7/4. Basis of human molecular genetics and gene diagnostics .....	340
Program 7/5. Immunology .....	350
<b>8. PATHOLOGICAL SCIENCES DOCTORAL SCHOOL .....</b>	<b>356</b>
Program 8/1. Oncology .....	356
Program 8/2. Alterations of cells fibres and extracellular matrix, and diagnostic pathomorphological studies in the course of heart and vascular diseases and in certain tumours. Experimental and diagnostic pathomorphological studies .....	374
Program 8/3. Study of the immunobiological effects of microorganisms and of their components at molecular and cellular level and in microorganisms .....	385
Program 8/4. Public health .....	390
Program 8/5. Clinical and experimental transplantation .....	403
Program 8/6. Health sciences .....	407

---

## GENERAL OVERVIEW

---

Semmelweis University's School of Ph.D. Studies' foundations were laid in the early 1990s, following *Act LXX of 1993 on Higher Education*, which for the first time gave individual universities the right to issue doctoral degrees.

There are hundreds of Ph.D. students enrolled in one of the more than 40 training programmes offered by the University's eight doctoral schools. These students work under the supportive guidance of the School's dedicated tutors, and can choose from among the 60–120 courses that are offered by the School each semester.

The School's professors and tutors come either from one of Semmelweis University's six faculties, or from the institutes and laboratories of various other Hungarian universities. This level of interdepartmental and interuniversity collaboration has led to the creation of new research centres that function as true melting pots.

The supportive guidance and knowledge of the School's experienced professors and talented tutors has a great impact on the research work of young candidates, whose motivation is augmented from being able to meaningfully interact with experts in their particular field. Indeed, by involving the greatest authorities from within, as well as from without the university, the School of Ph.D. Studies has, from the beginning, been able to preserve the quality and prestige of the degree.



## ORGANIZATIONAL STRUCTURE

---

The School of Ph.D. Studies is an autonomous educational body of Semmelweis University; its activities are subject to the decisions made by the University's Doctoral Council, which meets every second month of the academic year. The Doctoral Council determines the content of the Ph.D. programmes, the admission procedures and the admission fee. The work of the Doctoral Council is supported by the Doctoral Secretariat, which is also responsible for providing detailed information about these to the applicants.

The School of Ph.D. Studies is organised around doctoral schools, which serve as umbrellas for related branches of science and their respective programmes. Each doctoral school has its own council, which serves as its central administrative body. Nonetheless, the individual programmes continue to enjoy a considerable amount of independence.

Currently the School of Ph.D. Studies at Semmelweis University has eight doctoral schools:

1. Basic Medicine
2. Clinical Medicine
3. Pharmaceutical Sciences
4. Mental Health Sciences
5. Sport Sciences
6. Neurosciences (János Szentágothai)
7. Molecular Medicine
8. Pathological Sciences.

The School of Ph.D. Studies integrates research groups and programmes from all the faculties of Semmelweis University that are entitled to issue Ph.D. degrees. The University's largest faculty, the Faculty of Medicine, is represented in almost every doctoral school. The faculties of Dentistry and Health Sciences each have their own study programmes, while the Faculty of Pharmacy and the Faculty of Physical Education and Sport Sciences are represented by their own doctoral schools.

The basic unit of the School's complex educational system is "one student—one tutor". Together, they enjoy a high level of freedom and autonomy in conducting their research, within the limits set by the School's rules and regulations.

## PROGRAM OVERVIEW

---

The Ph.D. program at Semmelweis University consists of two parts: the Educational Phase (Phase I) and the Qualification Phase (Phase II).

### ***Phase I: Educational Phase***

The aim of Phase I is to train students to become scientists through coursework and research activity. It is in this phase that students select the specific scientific topic that will become the core of their final dissertation. Research is conducted in collaboration with faculty members, while a qualified tutor supervises each student.

### ***Phase II: Qualification Phase***

Phase II provides students with the opportunity to evaluate the results of experiments and publish them in acknowledged scientific journals. Naturally this is, or can be, an ongoing activity in Phase I as well. At the end of this phase, students are required to pass a comprehensive examination and to write and defend a dissertation. Since dissertations are required to be made available to the public prior to its defence, an online database containing hundreds of full-text doctoral theses, and their respective synopses has been set up by the School, in order to facilitate this process. Although Phase II logically follows Phase I, students may choose to skip the first phase and go straight into Phase II, provided that the necessary prerequisites and requirements have been met.

### ***Ph.D. Courses***

There are a number of courses announced on the School's website each semester. The list of required courses, which have to be taken during the Ph.D. training period, is finalised each year a few weeks after registration. Beginning in 2002, the doctoral schools have published a study plan for the entire training period, while a database of available courses is made accessible through the School of Ph.D. Studies' website, giving students the freedom to easily construct their own individual study plan.



## ADMISSION AND TUITION

---

The School of Ph.D. Studies offers three forms of education:

- Full-time, entering Phase I as students
- Part-time, entering Phase I as students
- Individual studies, entering Phase II as candidates.

Both full-time and part-time students are required to meet the same admission requirements. Individuals who join the programme in Phase II are not considered students, and will not be given a record book or student identity card; rather, they are considered candidates for the doctoral degree.

### **Admission Requirements and Process**

Doctoral applicants must

- be university graduates or students registered for their final semester of university studies
- possess at least a certified C type (oral and written) state foreign-language exam or an equivalent certificate if applying admission to the Hungarian-language program. Applicants for the English-Language program must have a good command of English.

When applying to the programme, applicants are required to state the specific training programme and research topic they wish to pursue within one of the University's doctoral schools.

The admission procedure is based on evaluating the candidate's

- general knowledge and personal ability
- topic-related knowledge and academic competence
- previous scientific activity and contribution.

The admission board of each doctoral school creates a ranked list of candidates which is submitted to the University's Doctoral Council. The Doctoral Council makes the final decision regarding admissions.

### **Tuition Fees**

Certain costs of education, scientific training and official procedures are covered by students and candidates. Most of the fees are equal to or close to what is ordinarily paid by undergraduate students.



## DOCTORAL COUNCIL

---

*President of the Doctoral Council: Dr. Károly Rácz*

*Members of the Doctoral Council:*

<b>Dr. József Tímár</b>	Vice-President
<b>Dr. Ágoston Szél</b>	Vice-Rector of Semmelweis University
<b>Dr. Miklós Tóth</b>	Vice-Rector for Scientific and International Affairs
<b>Dr. Gábor Makara</b>	President of the Educational Board
<b>Dr. János Rigó</b>	President of the Quality Control and Evaluation Board
<b>Dr. László Rosivall</b>	Basic Medicine Doctoral School
<b>Dr. Zsolt Tulassay</b>	Clinical Medicine Doctoral School
<b>Dr. Éva Szőke</b>	Pharmaceutical Doctoral School
<b>Dr. István Bitter</b>	Mental Health Sciences Doctoral School
<b>Dr. Dániel Bereczki</b>	János Szentágotthai Neurosciences Doctoral School
<b>Dr. György Nagy</b>	President of the Disciplinary Procedures Committee
<b>Dr. József Mandl</b>	Molecular Medicine Doctoral School
<b>Dr. Emil Monos</b>	Basic Medicine Doctoral School
<b>Dr. József Tihanyi</b>	Sport Sciences Doctoral School
<b>Dr. Pál Magyar†</b>	Representative of the Faculty of Medicine
<b>Dr. Gábor Varga</b>	Representative of the Faculty of Dentistry
<b>Dr. Kálmán Magyar</b>	Representative of the Faculty of Pharmacy
<b>Dr. Péter Tamás Sótónyi</b>	Representative of Szent István University
<b>Dr. Zsolt Radák</b>	Representative of the Faculty of Physical Education and Sport Sciences
<b>Dr. István Szabolcs</b>	Representative of the Faculty of Health Sciences



## PERMANENT COMMITTEES OF THE DOCTORAL COUNCIL

### Educational Board (EB)

The Educational Board (EB) meets at least once in a half year. It expresses opinion on course proposals and requests for financial support for courses. If needed, the EB can alter courses. It can make proposals in the following matters: (1) the structure of teaching, (2) the coordination of courses, (3) the nature of the method of course registration and registration deadlines, (4) the establishment of credit points which can be given to each course and (5) the recognition of credit points.

Students receiving tuition are advised to choose those courses which are promoted by the Doctoral Council of Semmelweis University. Theoretical knowledge and skills necessary for research are obtained from the compulsory course modules. Throughout the year the Ph.D. schools organize optional courses. Some of them organize courses which are obligatory for all students who are registered in that particular school.

***President of the Educational Board: Dr. Gábor Makara***

***Members of the Educational Board:***

<b>Dr. László Rosivall</b>	Basic Medicine Doctoral School
<b>Dr. Béla Molnár</b>	Clinical Medicine Doctoral School
<b>Dr. Éva Szökő</b>	Pharmaceutical Sciences Doctoral School
<b>Dr. László Tringer</b>	Mental Health Sciences Doctoral School
<b>Dr. Gábor Pavlik</b>	Sport Sciences Doctoral School
<b>Dr. Emília Madarász</b>	János Szentágothai Neurosciences Doctoral School
<b>Dr. Ágota Vér</b>	Molecular Medicine Doctoral School
<b>Dr. Károly Nagy</b>	Pathological Sciences Doctoral School
<b>Tamás Sticz</b>	Representative of the Doctoral Students' Union

Members of this body debate and decide whether a candidate has a thorough grounding in academic work by examining his/her previous performance in education and research and the elected topic for research. The board decides whether the candidate will or will not be able to produce a thesis three or four years after his/her entrance into the system.

### Quality Control and Evaluation Board (QCEB)

The efficiency of the doctoral training is shown in the frequency of completed doctoral theses and academic publications. The standards of these works are judged by scientometric indicators. This process evaluates both the academic competence of individual Ph.D. candidates and also the academic standards of the Doctoral School.

One of the most important acts of quality control is that everybody involved in the process complies with the instructions given in the qualification phase by the Doctoral Council (DC). The importance of this issue led to the establishment of The Quality Control and Evaluation Board (In Hungarian: VMB). This first evaluating forum controls

whether the submitted work fulfils the conceptual, structural and formal requirements of a doctoral thesis. Consequently, a formal opinion is released stating whether the Ph.D. candidate acquired the scientometric indicators prescribed in the Book of Regulations of the Doctoral School or not.

***President of the Quality Control and Evaluation Board: Dr. János Rigó***

***Members of the Quality Control and Evaluation Board:***

<b>Dr. Tamás Ivanics</b>	Basic Medicine Doctoral School
<b>Dr. László Herszényi</b>	Clinical Medicine Doctoral School
<b>Dr. István Antal</b>	Pharmaceutical Sciences Doctoral School
<b>Dr. Katalin Hegedűs</b>	Mental Health Sciences Doctoral School
<b>Dr. Gyöngyi Szabó (Földesiné)</b>	Sport Sciences Doctoral School
<b>Dr. András Csillag</b>	János Szentágothai Neurosciences Doctoral School
<b>Dr. Miklós Csala</b>	Molecular Medicine Doctoral School
<b>Dr. Janina Kulka</b>	Pathological Sciences Doctoral School
<b>Katalin Dezső</b>	Representative of the Doctoral Students' Union

Considering the proposals given by the QCRB (VMB in Hungarian) the Doctoral Council decides whether the doctoral thesis can be sent to the opponents. At a first glance this process might seem unnecessary because the control of requirements looks like a simple administrative issue. However, the process of evaluation described above—which applies the general rules (sometimes with a great deal of empathy)—is highly desirable for a number of reasons: there is a great variety of research topics, the issues discussed range from molecular biology to behavioral sciences which must be able to win academic recognition in Hungary and abroad.

Apart from works which meet all the standards there are doctoral theses which are inadequate in their content and structure and are therefore unacceptable. Candidates who do not fulfill the main requirements are advised to withdraw their dissertation prior to a detailed, expert review. We are proud that only thoroughly controlled doctoral theses are given in the hands of official opponents and reviewers. It is worth mentioning some of these requirements which doctoral candidates must take into consideration.

- (a) Only those candidates deserve scientific degrees who are able to publish their results intelligibly and based on a coherent analysis. Summarizing the content of two or three excellent publications is not equal to a doctoral dissertation.
- (b) Some articles which were published in high-rank international journals with a sophisticated title and a high impact factor are not sufficient to create the basis for Ph.D. qualification. Only those articles are potential items for recognition which are the result of real academic work. Therefore, articles summarizing the academic literature of a particular topic or commenting on the academic work of others as “letters to the editor” are not acceptable. By contrast, articles published in a journal under the latter heading could include original scientific results. But this must become clear from the presentation of scientific methods and/or from the editor’s professional opinion.

- (c) The QCRB during its evaluation takes into consideration the grammatical correctness and style of the English or Hungarian language, the length as well as the external appearance of the thesis and the quality of illustrations.

These examples illustrate that the board has a high level of responsibility in defining the academic quality of Ph.D. qualifications. At the same time the board is meticulously tactful in giving criticisms. Therefore, written judgments also include constructive suggestions for correcting the deficiencies discovered. This professional opinion is sent to both the candidate and the head of the training program. This process creates grounds for the necessary corrections and gives the possibility of legal remedies.

When this evaluation process is applied in practice, for instance, nine doctoral theses out of ten are found acceptable. The other one is rejected usually because basic requirements are not fulfilled, i. e. the shortage of sufficient acceptable publications. Furthermore, it occurs that two or three theses are rejected in form (i. e. the outward appearance and/or some of the essential requirements are missing from the package which was submitted to the QCRB). In these cases the professional review of the doctoral theses will be delayed until the corrections are completed and necessary supplements are submitted.

One of the major duties of the QCRB is to report its experience to the Doctoral Council and to suggest proposals concerning the alterations in the Rules of the Doctoral School if necessary. Apart from the duties described above the QCRB has authority over any stage of the doctoral procedure in scientific matters. Furthermore, it passes judgments on applications, looks into the matters of complaints and makes decisions concerning naturalizations (e. g. the recognition of qualifications).

Since 2000 the Quality Control and Evaluation Board has had this role of quality evaluation within the Doctoral School.

## **Disciplinary Procedures Committee**

The activity of this committee is needed only in exceptional cases, of which plagiarism and disharmony between student and tutor have given some work to the committee in the past years.

***President of the Disciplinary Procedures Committee: Dr. György Nagy***

***Members of the Disciplinary Procedures Committee:***

<b>Lilla Fang</b>	Clinical Medicine Doctoral School
<b>Dr. István Antal</b>	Pharmaceutical Sciences Doctoral School
<b>Emese Ficsor</b>	Pharmaceutical Sciences Doctoral School
<b>Ákos Gerencsér</b>	Mental Health Sciences Doctoral School



## DOCTORAL SECRETARY OFFICE

---

**Emőke Márton**

Head

**Anna Marádi (Pintérné)**

Financial officer

**Anita Marosfalvi**

Adviser

**Tímea Rab**

Adviser

The administrative duties of the Doctoral School are managed by the Doctoral Secretary Office which creates a link between the Doctoral Council, the Ph.D. training programs and the Ph.D. students. It maintains permanent contact with the educational bodies outside of the university (e.g. Ministry of Education, Hungarian Accreditation Board, etc.). The Secretary is available for Ph.D. students on an office-hours basis, however, they are ready to help anytime in urgent cases. In one little room practically every major step of the degree obtaining process is handled, starting from the first inquiries and paper work of the entrance examination, all the way up to the preparation of the diplomas. (Address: H-1085 Budapest, Üllői út 26, ground floor Nr. 9.)



## DOCTORAL STUDENTS' UNION (DSU)

---

The DSU (In Hungarian: DHÖK) is a body elected by the doctoral students in order to legally represent their interests both inside and outside the university; e.g. in the Doctoral Council and the National Association of Doctoral Students. Members of the union participate in the *ad hoc* commissions dealing with matters in their interest and are responsible for organizing the Scientific Ph.D. forums. The head office of the DSU is: The Students' Office of Semmelweis University (H-1089 Budapest, Nagyvárad tér 4, ground floor Nr. 18).

Members of the Union of the Doctoral Students are also accessible through the Doctoral Secretarial Office.

### *Members of the Doctoral Students' Union:*

<b>Lilla Fang</b>	Basic Medicine Doctoral School
<b>Árpád Patai</b>	Clinical Medicine Doctoral School
<b>Emese Ficsor</b>	Pharmaceutical Sciences Doctoral School
<b>Ákos Gerencsér</b>	Mental Health Sciences Doctoral School
<b>Kinga Kiszela</b>	Sport Sciences Doctoral School
<b>Viktória Reményi</b>	János Szentágotthai Neurosciences Doctoral School
<b>Sándor Békási</b>	Molecular Medicine Doctoral School
<b>Tamás Sticz</b>	Pathological Sciences Doctoral School



---

## INTRANET

---

**Website:** <http://www.phd.sote.hu>

The website of the Doctoral School at Semmelweis University is an essential means for organizing doctoral education. Hundreds of Ph.D. students take the courses as part of more than thirty training programs of eight doctoral schools under the supervision of hundreds of tutors in different locations. Every half year the school organizes 60–120 courses; the lectures and seminars are given in more than 50 locations within the university.

There are great advantages stemming from the intensity and variety of our education system. Therefore, it is a high priority to publish information which is clear-cut and accessible by everybody. The gradual augmentation of the website leads to the accumulation of information about every training program and sub-program and of every teacher and doctoral defense since 2000. Importantly, the website provides information about on-going courses and application possibilities. All application forms and documents, which are necessary for the administration of doctoral matters and the resolutions of the Doctoral Council, are also accessible on the website. Other detailed information and curricula are also available electronically. The website provides addresses, telephone numbers and e-mail addresses.

The regulations, the forms, the course and program data, the decisions of the Doctoral Council and the invitations to all defense ceremonies are accessible via the Internet. News on important conferences, university events, calls for proposals are also not missing from the repertoire.

The website opens a possibility for course leaders to put the information about their courses directly to the website. The Doctoral Secretarial staff manages and publishes all the relevant information: e. g. general news, advertisements and Ph.D. defenses without the assistance of a web supervisor.

The website has considerable web traffic according to statistical figures. The database system of presenting doctoral theses on the internet was set up. It is required that doctoral theses be available to the public before the defense so they may be accessed electronically in full through the internet simultaneously with the announcement of the Ph.D. defense.

The “Dissertation Abstracts”, a trademark of ProQuest, allows for the availability of each recently defended thesis all over the world. Older works are not available yet, however we try to put out as many of our precious creations as possible.

## ‘VERITAS ET VIRTUS’ AWARD IN MEMORY OF DR. ZSOLT FARKAS JR.

---

After the tragic death of Dr. Zsolt Farkas Jr., a Ph.D. student of the Doctoral School, his parents, Dr. and Mrs. Zsoltné Farkas established a foundation in memory of their late son. The aim of the foundation is to support financially research by Ph.D. candidates.

Some of the aspects of the charter are: *“The aims of the Foundation are to subsidize the work of Ph.D. students under thirty-five years who are concerned primarily with physiological research in the Doctoral School of Semmelweis University. Additionally, it contributes to the realization of the aims of the Doctoral School, i. e. to improve the quality of doctoral education, to facilitate the acquisition of widely recognized scientific degrees, to provide financial support for the expansion of accredited doctoral research programs, to establish pre-doctoral scholarships and to improve scientific communication. (...) Those Ph.D. students are able to benefit from the payments of the foundation whose submitted work wins a public competition advertised by the trustees of the Foundation. The type of work submitted can be in the process of publication but the candidate must be its first author. Other details are defined by the committee of trustees who are responsible for both advertising and reviewing the submitted work. (...) The awards must be transferred ceremonially to the winners every year on the Dies Academicus (first Saturday of November).”*

The Office of the Foundation is: H-1085 Budapest VIII., Üllői út 26. The trustee committee is the Advisory Board of the Foundation. The president of the Advisory Board of the Foundation is always the current head of the Doctoral Council at the Semmelweis University, at present, university professor, Dr. Ágoston Szél.

### *Recipients of ‘Veritas et Virtus’ Awards*

2009	<b>Balázs Hangya</b>	János Szentágothai Neurosciences Doctoral School
	<b>Anita Garas</b>	Clinical Medicine Doctoral School
	<b>Péter Pócza</b>	Molecular Medicine Doctoral School
2010	<b>Kornélia Baghy</b>	Pathological Sciences Doctoral School
	<b>Sándor Spisák</b>	Clinical Medicine Doctoral School
	<b>Ágnes Szappanos</b>	Clinical Medicine Doctoral School
	<b>Zsófia Lázár</b>	Clinical Medicine Doctoral School



## PH.D. COURSES

Every semester there are a number of courses (60–120) announced on the website. Previously, the Educational Board of the Doctoral School filtered down the number of courses to between sixty and seventy. The Doctoral Council of Semmelweis University took into consideration the views the Educational Board and restricted the number of courses which can be run by each departmental doctoral school in one semester to between five and seven.

Since then the following procedure has been established concerning Ph.D. courses, i. e. the Doctoral Committee of each Departmental Doctoral School proposes between five and seven courses at the beginning of each semester which are entered the database of current courses. However, the Educational Board can recognize and award credit points for participation in Ph.D. courses of the appropriate standard at other universities. In this case the Educational Board needs the recommendation of the tutor and the head of the departmental doctoral school of the student in question.

The database of available courses is accessible at the website of the Doctoral School. Consequently, students are able to access the database and construct their own individual study plan.

<b>Compulsory courses</b>	<b>Course leaders</b>	<b>Semester</b>
Interactions, side effects and simultaneous effects in phytotherapy	Ágnes Kéry	2009/2010/2
Links between information, communication and sport	Ágnes Kokovay	2010/2011/1
Instrumental analysis of drugs	András Gergely	2009/2010/2
Role and detection of cell junction structures and molecules	András Kiss	2009/2010/1, 2010/2011/1
Cell adhesion molecules. The FISH technique in pathological diagnosis	András Kiss	2009/2010/2
Novel knowledge on the pathophysiology of diabetes mellitus and its treatment modalities	Anikó Somogyi	2009/2010/1
Theoretical and practical studies for successful Ph. D. degree	Anna Blázovics	2009/2010/1, 2010/2011/1
Effects of globalization on diseases development	Anna Tompa	2009/2010/1
Role of neuropeptides in the function of the nervous system		
Diagnostic and therapeutic techniques in clinical practice	Attila Nemes	2009/2010/1
Frontier issues of surgery—Applied sciences in surgery—Thoughtful surgery	Attila Szijártó	2009/2010/1–2, 2010/2011/1

Prognosis of tumor progression	Balázs Győrffy, József Tímár	2009/2010/1, 2010/2011/1
Aspiration cytology in practice	Balázs Járay	2009/2010/1, 2010/2011/1
Different aspects of liver transplantation with special respect to hepatitis C virus	Balázs Nemes	2010/2011/1
Structure and function of biological membranes	Balázs Sarkadi	2010/2011/1
Pediatric research: from ideas to publications	Barna Vásárhelyi	2009/2010/1
Special research methodology (statistics, content analysis)	Bea Ehmann, András Ittész	2009/2010/1
Sportpsychology for the performance	Csaba Nagykáldi	2009/2010/1, 2010/2011/1
Theory of action-efficacy	Csaba Nagykáldi	2009/2010/2
Nutritional science	Csaba Nyakas	2009/2010/2
Endocrinology and sport	Csaba Nyakas	2010/2011/1
Genomic background of multifactorial diseases	Csaba Szalai	2009/2010/2
Research methods in clinical neurology	Dániel Bereczki	2009/2010/1
Cognitive psychotherapy and social competence	Dóra Perczel- Forintos	2010/2011/1
Introduction to biometry	Elek Dinya	2009/2010/1, 2010/2011/1
Clinical cardiovascular physiology	Emil Monos, Márk Kollai	2009/2010/2
Radical changes in personality (phenomenology of conversion)	Endre Nagy	2009/2010/1
Phytochemistry	Éva Lemberkovics	2010/2011/1
Pharmaceutical biotechnology	Éva Szőke	2009/2010/2
Interfacial behaviour of macromolecular colloids	Ferenc Csempez	2009/2010/1
Nanosystems in biomedical sciences	Ferenc Csempez	2009/2010/1
Bioinformatics and the study of antibiotic resistance and its epidemiology	Ferenc Rozgonyi	2009/2010/2
History of philosophy	Ferenc Takács	2009/2010/1–2, 2010/2011/1
Sport aesthetics	Ferenc Takács	2009/2010/2
Surgical treatment of heart failure— theory and practice	Gábor Balázs Szabó	2009/2010/2

Stem cells in cardiac research and therapy	Gábor Földes	2009/2010/1
Planning and evaluation of experiments	Gábor Makara	2009/2010/2
Physiology of fitness	Gábor Pavlik	2009/2010/2, 2010/2011/1
Medical physiology	Gábor Pavlik	2009/2010/1–2
Application for grants, project financing and management in EU and Hungary in the field of health sciences	Gábor Pörzse	2009/2010/1, 2010/2011/1
Introduction to innovation management in the fields of health and life sciences	Gábor Pörzse	2009/2010/1
Receptors and signal transductions in the immune system	Gabriella Sármay	2009/2010/1, 2010/2011/1
Sport in contemporary society	Gyöngyi Szabó (Földesiné)	2009/2010/1–2, 2010/2011/1
Neurochemistry, pharmacology and models of behaviour	György Bagdy	2009/2010/2
Molecular virology and molecular epidemiology	György Berencsi	2009/2010/1
Electrophysiological research methods of the neuromuscular system	György Karmos	2009/2010/2
Electrophysiological assays and protheses	György Karmos	2010/2011/1
Asthma bronchiale	György Losonczy	2009/2010/1
Pathomechanism of obstructive pulmonary disorders. Changes in mechanics of respiration	György Losonczy	2009/2010/1
Autoimmunity	György Nagy	2009/2010/1, 2010/2011/1
Chromatographic analysis of xenobiotics	Huba Kalász	2009/2010/1
Application of HPLC in pharmacology	Huba Kalász	2010/2011/1
Clinical and physiological basis of respiration and respiratory disorders	Ildikó Horváth, Márk Kollai	2009/2010/1, 2010/2011/1
Ophthalmology	Ildikó Süveges	2009/2010/2, 2010/2011/1
Impaired cell regulation in cancer	Ilona Kovalszky	2009/2010/2
Bioanalytic methods in pharmacokinetics	Imre Klebovich	2009/2010/1, 2010/2011/1
Developmental biology	Imre Oláh	2009/2010/1–2, 2010/2011/1
XVIII. International Semmelweis Symposium —2008—New trends, innovations and technology in urology	Imre Romics	2009/2010/1

Disturbances of movement coordination and mentality	Imre Szirmai	2010/2011/1
Pharmaceutical applications of NIR spectroscopy	István Antal	2010/2011/1
Clinical psychopharmacology	István Bitter	2010/2011/1
Bioinformatics and pathway analysis	István Miklós	2010/2011/1
Bases of nutrigenetics and nutrigenomics	István Szabolcs	2009/2010/2
Dietotherapy	István Szabolcs	2009/2010/2
Clinical endocrinology	István Szabolcs, Miklós Góth	2009/2010/1
Pharmacology of endocrine disorders	István Takács	2009/2010/1
Isotope use methodology and radiation protection	István Voszka	2009/2010/2
Basics of society medicine	Iván Forgács	2009/2010/1, 2010/2011/1
Hepatology	János Fehér†	2009/2010/2
Sport pedagogy	János Gombocz	2009/2010/1–2, 2010/2011/1
Research methodology in sport science	József Bognár	2009/2010/1–2
Research methods	József Bognár	2009/2010/1–2, 2010/2011/1
Neuromechanics of movements	József Laczkó	2009/2010/2
Molecular epidemiology of adenoviruses in immunosuppressed conditions and tumors	József Ongrádi	2009/2010/1
Disorders of adaptation to society	József Rácz	2009/2010/2, 2010/2011/1
Biomechanics of the musculoskeleton system	József Tihanyi	2009/2010/2
General pathology for biologists	József Tímár	2010/2011/1
Basic aspects of hematologic research	Judit Demeter	2010/2011/1
History of health and science	Judit Forrai	2009/2010/1
Studies on the process of becoming a nurse	Judit Mészáros	2010/2011/1
Molecular virology: human retroviruses (HIV, HTLV) and their roles in immuno-pathological disorders	Károly Nagy	2009/2010/2
History of philosophy I.	Katalin Vermes	2009/2010/1
History of science	Katalin Vermes	2010/2011/1
Interpretation of the physical body in philosophy and psychology	Katalin Vermes	

Pharmacodynamics	Klára Gyires, Kornélia Tekes	2010/2011/1
Introduction to medical toxicology	Kornélia Tekes	2009/2010/1–2
From neurobiologic basis of stress to stress-related neurological disorders	Krisztina Kovács, Zsuzsanna Tóth	2010/2011/1
Functional organization of the cerebral cortex	László Acsády, Tamás Freund	2009/2010/1
Elements of molecular biology	László Buday, Mária Sasvári, András Váradi	2009/2010/1
Modern research and measuring methods in experimental and clinical medicine (selected chapters)	László Dézsi	2009/2010/1, 2010/2011/1
Clinical and experimental electrophysiology	László Gellér	2010/2011/1
Health economics and pharmaceutical economics	László Gulácsi	2009/2010/2
Evidence based medicine and health technology assessment with particular emphasis on prevention and treatment of communicable diseases	László Gulácsi	2009/2010/2
Selected chapters from molecular cell biology	László Homolya	2009/2010/1–2
Structure and function of membrane receptors	László Hunyady	2010/2011/1
Clinical oncology	László Kopper	2009/2010/1–2, 2010/2011/1
Chemotaxis: its biological and clinical significance	László Kőhidai	2009/2010/2
Budapest Nephrology School (Nephrology, Hypertension, Dialysis, Transplantation)	László Rosivall	2009/2010/2
Nanomedicine for targeted pharmacotherapy	László Rosivall, János Szebeni	2009/2010/1
Pharmacometrics and applied clinical biostatistics	László Tóthfalusi	2009/2010/2, 2010/2011/1
Psychotherapy in medical practice	László Tringer	2009/2010/1, 2010/2011/1
Methods of literature research	Lívia Vasas	2009/2010/1–2, 2010/2011/1
Bibliometry serves successful publication	Lívia Vasas	2010/2011/1
Changing paradigm in neurology and psychiatry	Mária Judit Molnár	2010/2011/1

Principles and practice of personalized medicine	Mária Judit Molnár	2010/2011/1
Neurogenetics in clinical practice	Mária Judit Molnár	2009/2010/1
Basis of behavioural medicine	Mária Kopp	2010/2011/1
Methodology of behavioural sciences	Mária Kopp	2009/2010/1–2
Quality of vegetables and fruits—its role in healthy nutrition	Mária Pankotai Gilingerné	2009/2010/2
Theory of sport games	Mariann Reigl	2009/2010/2
Management of events (organizational aspects of major sport events)	Mihály Nyerges	2009/2010/1
Clinical immunology and allergology	Miklós Benczúr, Péter Gergely	2009/2010/1–2, 2010/2011/1
Biotransformation	Miklós Csala	2009/2010/1
Introduction to biology of reactive oxygen derivatives	Miklós Geiszt	2009/2010/2
Molecular biomechanics	Miklós Kellermayer	2009/2010/1, 2010/2011/1
Biomedical light microscopy and computational image analysis	Miklós Kellermayer	2009/2010/2
Plant fermentation in producing active substances of medicinal plants	Miklós László	2009/2010/2
Research methods	Miklós Zsidegh	2009/2010/1, 2010/2011/1
Biometrics	Miklós Zsidegh	2009/2010/2
Bariatric surgery. Infertility—gynecologic, endocrine and andrologic aspects. International endocrine guidelines	Miklós Góth	2010/2011/1
From synaptic neurotransmission to network oscillations	Norbert Hájos, Zoltán Nusser	2009/2010/2, 2010/2011/1
Theoretical and methodological fundamentals of PE Curricula	Pál Hamar	2009/2010/2
The role of profession in the practice of health care	Péter Balázs	2009/2010/1–2, 2010/2011/1
Neurobiology of epilepsy	Péter Halász	2010/2011/1
Neurobiology of sleep	Péter Halász	2010/2011/1
Genetics and genomics in endocrinology	Péter Igaz, Attila Patócs	2010/2011/1
Disorders of calcium homeostasis 2010	Péter Lakatos, István Takács	2009/2010/1, 2010/2011/1
Genetic and immunologic background of gastroenterological disorders	Péter László Lakatos	2010/2011/1

Design of active substances	Péter Mátyus	2009/2010/1
Reconstructive genitoplasty	Péter Nyirády	2010/2011/1
Pathopsychologic and psychiatric aspects of artistic imagery	Péter Rajna	2010/2011/1
Questions of health care and medical justness in Hungarian judiciary practice	Péter Sótonyi	2009/2010/1, 2010/2011/1
Examination of the role of inositol lipids and phosphates in signal transmission of mammary cells	Péter Várnai	2009/2010/1
Experiments with laboratory animals	Piroska Anderlik	2009/2010/1, 2010/2011/1
Pharmaceutical aspects of quality assurance	Romána Zelkó	2009/2010/2
Paradigm of nursing and education at the 21 <sup>st</sup> century	Sándor Hollós	2010/2011/1
Clinical evaluation, mechanism of action and rational use of anticancer drug	Sándor Kerpel-Fronius, András Telekes	2010/2011/1
Genetics of sex	Sára Tóth	2009/2010/1, 2010/2011/1
Biopharmacy	Sylvia Marton	2009/2010/2
Clinical and pathophysiological aspects of polycystic ovary syndrome	Szabolcs Várbíró	2010/2011/1
Research and innovation management training	E-learning	2009/2010/2
Treatment of patients with severe sepsis in intensive care units	Tibor Gondos	2009/2010/1, 2010/2011/1
Hemodynamic monitoring	Tibor Gondos	2009/2010/2
Neurobiologic principles of stroke	Zoltán Nagy	2009/2010/1–2
Biometrics I.	Zsolt Csende	2009/2010/1–2
Motor control and learning	Zsolt Csende	2009/2010/1–2
Modern view of male infertility	Zsolt Kopa	2010/2011/1
Neuroendocrinology	Zsolt Liposits	2009/2010/2
Pedagogical aspects of training theory and methods	Zsolt Radák	2009/2010/2
Gastroenterology	Zsolt Tulassay	2010/2011/1
Financial planning of innovative biotechnological projects	Zsombor Lacza	2009/2010/1
Entrepreneurship basics for biotechnology innovation projects	Zsombor Lacza	2010/2011/1
Care of diabetes mellitus in clinical practice	Zsuzsa Kerényi, Gyula Tamás	2009/2010/1

Clinicopathological approach in research	Zsuzsa Schaff	2009/2010/2
Dermopathology	Zsuzsa Schaff	2009/2010/2

## PH.D. SCIENTIFIC DAYS

The Ph.D. training program provides opportunities for every candidate to acquire practical knowledge of the methodology of presenting results gained in scientific research. Ph.D. students therefore are required to present their work regularly both among fellow workers and in a wider professional environment. The need for an overall Ph.D. conference of the Doctoral School was promoted even though the departmental doctoral schools organize scientific forums for their own Ph.D. students. The primary objective was that participants would be able to familiarize themselves with the scientific work of each program. On these occasions Ph.D. students and candidates had the opportunity to present their work in several sections with a jury. Candidates with works of a high standard gained awards in each section.

Every academic year highly regarded professionals, normally holders of the distinction “Excellent Ph.D. Supervisors” have been invited to give plenary lecture with great success.

### *Plenary Lecturers*

- 2009     **József Tihanyi** (Sport Sciences Doctoral School)  
*Causes and magnitude of increased stretch in response to muscle rendering*
- György Bagdy** (Mental Health Sciences Doctoral School & János Szentágothai Neurosciences Doctoral School)  
*Serotonin in the central nervous system: excursion from neurobiology and genetics toward pharmacology, psychiatry and neurology*
- Péter Gergely** (Clinical Medicine Doctoral School)  
*Importance of immunologic tests in the prediction of activity and prognosis of systemic lupus erythematosus*
- 2010     **Péter Sótónyi** (Pathological Sciences Doctoral School)  
*Novel investigation prospects in crime verification*
- Gábor Makara** (János Szentágothai Neurosciences Doctoral School)  
*Personal views on Ph. D. training*

The plenary speakers are carefully selected from among those who have been awarded with the distinction of the “Excellent Ph.D. Supervisors”.



## EXCELLENT PH.D. SUPERVISOR AWARD

---

Nominations for the Excellent Ph.D. Supervisor Award are made by the heads of individual Ph.D. Schools, and the University Doctoral Council decides on the final list of awardees. The number of awardees is limited.

2009	<b>János MÉSZÁROS</b>	Sport Sciences Doctoral School
	<b>Béla NOSZÁL</b>	Pharmaceutical Sciences Doctoral School
	<b>Gábor MAKARA</b>	János Szentágothai Neurosciences Doctoral School
	<b>Miklós RÉTHELYI</b>	János Szentágothai Neurosciences Doctoral School
	<b>Péter SÓTONYI</b>	Pathological Sciences Doctoral School
2010	<b>István MUCSI</b>	Basic Medicine Doctoral School
	<b>Gábor PAVLIK</b>	Sport Sciences Doctoral School
	<b>Zsuzsa SCHAFF</b>	Pathological Sciences Doctoral School
	<b>Ágoston SZÉL</b>	Molecular Medicine Doctoral School

## AWARDING OF THE DOCTORAL DEGREE WITH DISTINCTION

---

The President of the Republic consented to the awarding of the doctoral degree to **Miklós Antal** (2009) with the distinction *‘Promotio sub auspiciis praesidentis Rei Publicae’*. It was Dr. László Sólyom, the President of the Republic in person who handed over the Diploma and the traditional Golden Ring to the inaugurated doctor. This ceremonial program was part of the Dies Academicus of Semmelweis University.

---

# SCHOOL OF PH.D. STUDIES

---

## 1. BASIC MEDICINE

---

***Chairman:*****László ROSIVALL M.D., Ph.D., D.Sc.**

Institute of Pathophysiology

4 Nagyváradi sq, Budapest H-1089

Tel: +36 1 210 2956

Tel./fax: +36 1 210 4409

E-mail: [rosivall@net.sote.hu](mailto:rosivall@net.sote.hu)

### **General overview**

The Doctoral School of Basic Medicine at Semmelweis University consists of five multidisciplinary research and training Ph.D. Programs. These Programs are closely related to the physiological sciences, and are chaired by internationally recognized professors as coordinators. At first, each Program was accredited individually in 1994, then all the Ph.D. Programs were integrated into a Doctorate (Ph.D.) School in 2002.

The major aims of the Ph.D. Programs are focused on investigating the mechanisms of diseases with high morbidity and mortality statistics in Hungary (e.g. cardiovascular and renal diseases, hypertension, obesity), and to study those environmental effects (UV and X-Radiation) which may influence the whole society. Investigating the molecular-cellular background of physiological and pathophysiological processes, and integration of knowledge at organ and organism levels lead us to new scientific results and discoveries which may promote the development of up-to-day methods for health prevention, diagnostics and therapy. In addition to several basic research projects offered to Ph.D. students, applied clinical studies are also incorporated into the Programs of the School.

### ***PROGRAM 1/1.***

---

## **PHYSIOLOGY AND CLINICS OF THE HEART AND CORONARY DISEASES**

***Coordinator:*****Béla MERKELY M.D., Ph.D., D.Sc.**

Heart Center

68 Városmajor st, Budapest H-1122

Tel./fax: +36 1 458 6840

E-mail: [titkarsag@kardio.sote.hu](mailto:titkarsag@kardio.sote.hu)

### **Program overview**

The complex program is directed to well-qualified students who are interested in cardiovascular research. The spotlight is on regulatory aspects and treatment of different cardiovascular diseases. According to the scientific interest of most of coordinators, the main problems are connected to pathophysiology (clinical physiology) of myocardial function,

coronary regulation and arrhythmogenesis. (However, as the list of the topics shows, other circulatory topics are included, too.)

The program prepares students for careers in either clinical science (especially invasive and non-invasive cardiology, anesthesiology, and cardiovascular surgery) or basic sciences. Preference is given to those who are ready to study overlapping territories of these sciences. Although the individual postgraduate trainings have an overall general similarity in their logistic aspects, the main characteristic of the entire educational process is the flexibility. Consequently, that research work can be tailored to the tutor's mutual interest within the territory covered by the general aims.

### ***Titles of research projects***

### ***Supervisors***

New aspects in the non-pharmacological therapy of tachyarrhythmias	Béla Merkely
Heart failure: pathomechanisms and new methods in the pharmacological and non-pharmacological treatment	Béla Merkely
Electrophysiology of ventricular arrhythmias	Béla Merkely
Role of endogenous agents in arrhythmogenesis	Béla Merkely
Challenge in the field of interventional cardiology	Béla Merkely
The role of contrast echocardiography in diagnostics and assessment of efficacy in the treatment of acute coronary syndrome	Péter Andrásy
Interventional radiology in the treatment and follow-up of patients with vascular diseases	Viktor Bérczi
External and internal noxa-induced secondary circulatory damage: clinical symptoms and therapy	András Csókay
Role of cardiac and endothelial progenitor cells in the remodeling and regeneration of the myocardium: <i>in vivo</i> and <i>in vitro</i> studies	Gábor Földes
Causes of recurrent stenosis after carotid surgery or other vascular surgical approaches. Researches in vascular surgery	László Entz
Clinical and experimental electrophysiology	László Gellér
Vasoactive peptides in heart diseases and their experimental models	Ferenc Horkay
Complications of interventional treatment of the vascular diseases	Kálmán Hüttl
Cardiovascular and cardioprotective effects of endogenous peptides in myocardial ischemia: experimental and clinical studies	Violetta Kékesi
Local myocardial interactions of cardiogenic agents: experimental studies	Violetta Kékesi
Mechanism of metabolic autoregulation in the coronary circulation	Violetta Kékesi
Mechanisms of the actions of cardiovascular regulatory agents: <i>in vitro</i> investigations	Violetta Kékesi
Diastolic dysfunction and heart failure	Mária Lengyel †
Cardiovascular effects of brain death. Donor management and selection	Gábor Szabó
Heart insufficiency in the era of modern cardiac surgery	Gábor Szabó
Tissue injury during and after cardiac surgery. Novel strategies to prevent reperfusion injury and acute and chronic rejection	Gábor Szabó
Pharmacogenomic investigations in the cardiovascular system	Zsolt Szélid
Non-cardiac risk factors in cardiac surgery	Andrea Székely

Role of inflammatory processes in the development of cardiovascular diseases	Gábor Széplaki
Nuclear cardiology in the diagnosis of ischaemic heart disease	István Szilvási
Molecular mechanisms of cardiac hypertrophy especially in the early and decompensated phase of the disease	Miklós Tóth
Identification of origin and mechanisms of wide QRS complex tachycardias using new algorithm combined with neuronal network schemes	András Vereckei
Investigation of epidemiological and mortality indices, pathophysiology, therapeutic processes and prognostic factors of complex cardiopulmonary resuscitation	Endre Zima

### ***Ph.D. students***

Astrid Apor	pt
Zsolt Bagyura	ft
Enikő Barnucz	ft
György Bárczi	pt
Andrea Dósa	pt
Tamás Erdei	ft
Zoltán Gonda	pt
Mihály Károlyi	ft
Máté Kerekes	ft

Bálint Kozman	pt
Valentina Kutýifa	ft
Zsuzsanna Lendvai	ft
Árpád Lux	ft
Csaba Mihályi	ft

Mónika Moravszki	pt
István Osztheimer	pt
György Szabó	pt
Dávid László Tárnoki	pt

Ádám Domonkos Tárnoki	pt
Roland Tóth	ft
Katalin Túri	ft
Eszter Mária Végh	ft

### ***Ph.D. candidates***

Balázs Berta	ft
Tamás Breuer	ft
Csaba Diószeghy	i
Pál Maurovich Horváth	pt
Zoltán Jambrik	i
Zsolt Béla Komka	pt
Andrea Nagy	ft

### ***Supervisors***

Péter Andrásy
Béla Merkely
Gábor Szabó
Péter Andrásy
Zsolt Szelíd
Mária Lengyel †
László Entz
Béla Merkely
Violetta Kékesi,
László Dézsi
András Vereczkei
Béla Merkely
Gábor Földes
Zsolt Szelíd
Violetta Kékesi,
László Dézsi
István Szilvási
László Gellér
Béla Merkely
Viktor Bérczi,
Ildikó Horváth
Viktor Bérczi
Andrea Székely
Violetta Kékesi
László Gellér

### ***Supervisors***

Béla Merkely
Miklós Tóth
Béla Merkely
Béla Merkely
Béla Merkely
Ferenc Horkay
Violetta Kékesi

Balázs Nemes	i	Kálmán Hüttl
Balázs Sax	ft	Violetta Kékesi
Gabriella Veress	ft	Béla Merkely

***Ph. D. graduates***

Dávid Becker	i
Mónika Dénes	ft
Csaba András Dézsi	pt
Kristóf Hirschberg	ft
Tímea Kováts	i
Andrea Ágnes Molnár	pt
Attila Róka	ft
Szabolcs Szilágyi	ft
Gábor Veres	pt

***Supervisors***

Béla Merkely
Mária Lengyel †
Béla Merkely
László Entz
Miklós Tóth
Viktor Bérczi
Béla Merkely
Béla Merkely
Gábor Szabó

ft, full-time; pt, part-time; i, individual

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****DÁVID BECKER (2009)****Treatment of ST-elevation myocardial infarction with percutaneous coronary intervention: analysis of quality parameters**

*Supervisor: Béla Merkely*

In our country, the majority of patients die due to acute myocardial infarction. The best treatment option today for acute ST-elevation myocardial infarction is percutaneous coronary intervention. Many factors influence the success of this type of treatment: the shortest possible transfer time, interventional team on site, number of patients with infarction treated by the given center, number of PCIs performed by the interventional cardiologist per year. One of the most important factors is how well care is organized. We analyzed in detail the data of 1890 patients with ST-elevation myocardial infarction treated with percutaneous coronary intervention at the Heart Center, Semmelweis University between 2003 and 2005 with 100% one-year follow-up. Most of the patients (n=1219; 64.5%) arrived during off-hours, almost every fifth patient (17.7%) was in severe condition; the rate of old patients was high (11.5% over the age 80). The prognosis was relatively good even in the very old patient population; approximately 60% of patients over the age of 80 are still alive after one year. The rate of patients who called the ambulance first was low (34.7%), however prognosis of these patients was better (one-year mortality: 12.7% vs. 16.1% p=0.042). The prognosis of patients transferred directly was also better (one-year mortality: 12.6% vs. 16.7% p=0.028). Early mortality of patients in severe condition (resuscitated or in cardiogenic shock) was high, however prognosis of patients surviving the acute stage is relatively good. In women mean age, as well as co-morbidity is higher and were in more severe condition on admission, however there was no difference in mortality corrected for age. Patients who had rescue PCI were younger, more were

hemodynamically unstable, but mortality was not higher. Patients who had stent thrombosis, despite more aggressive antithrombotic treatment, had more frequent further cardiovascular events. Patients in good general condition, treated with successful PCI without complications can be discharged early and safely. Long term mortality is also low in this patient group (3.2% vs. 21.1%). In those patients, where the clinical signs suggested myocardial infarction, but the coronarography was negative (n=14), acute heart MR examination could help in establishing the correct diagnosis. With this new method we were able to support (n=2) or exclude (n=10) infarction in this questionable patient group. We can state from the results that it is possible to achieve relatively good prognosis even in the most severe patient groups with the help of organized, network-based interventional infarction treatment. As part of complex infarction, treatment cardiac MR has a significant role.

- Becker D, Maurovich-Horvat P, Szabo G, Barczy G, Fulop G, Molnar L, Zima E, Apor A, Berta B, Nagy A, Belicza E, Merkely, B (2009) Life after coronary stent thrombosis. *Med Sci Monit* 15(5): CR236–241.
- Merkely B, Toth-Zsamboki E, Becker D, Beres BJ, Szabo G, Vargova K, Fulop G, Kerecsen G, Preda I, Spaulding C, Kiss RG (2009) Very late drug eluting stent thrombosis after non-steroidal antiinflammatory drug treatment despite dual antiplatelet therapy. *Can J Cardiol* 25(4): 229–232.
- Becker D (2007) Acut myocardialis infarctus. *Orv Hetil* 148(12): 569–557.

## MÓNKA DÉNES (2010)

### Assessment of diastolic dysfunction and heart failure with integrated Doppler echocardiography

*Supervisor: Mária Lengyel †*

Heart failure is a new epidemic of our era, which presents mainly as diastolic heart failure in the elderly. Asymptomatic diastolic dysfunction is also common at this age. Classification of diastolic dysfunction has been based on the use of integrated Doppler echocardiography.

The aim of the thesis was to address some controversial issues in the literature related to diastolic function and heart failure by both methodological and clinical approach.

Between 2004 and 2008 standard and tissue Doppler echocardiography was performed and analyzed in a total of 1350 subjects.

The main results of the methodological studies were the followings: 1. tissue Doppler velocities obtained by different echocardiography machines are not compatible; 2. pulmonary venous flow analysis is necessary to demonstrate moderate diastolic dysfunction in nearly half of the cases.

In clinically oriented studies we found an excellent correlation between diastolic tissue Doppler velocities of the right and left ventricle, and age-related impairment of diastolic function. We demonstrated, that diastolic dysfunction with elevated filling pressure and diastolic heart failure is associated with longitudinal systolic dysfunction.

Heart failure was confirmed in only half of elderly patients with suspected heart failure, out of whom 40–40% had systolic and diastolic heart failure, and 20% had heart failure with “preserved ejection fraction”, which was closely related to atrial fibrillation.

One third of elderly, asymptomatic hypertensive patients with preserved ejection fraction had moderate-to-severe diastolic dysfunction. Although patients with diastolic heart failure had better outcome than patients with systolic heart failure, the long-term event-free survival is only 46%.

In conclusion a detailed analysis of diastolic function in health and disease provided new findings to better understand of diastolic dysfunction and heart failure.

- Dénes M, Kiss I, Lengyel M (2009) *Assessment of diastolic dysfunction in elderly hypertensive patients using integrated Doppler echocardiography*. *Blood Press* 18(3): 135–141.
- Dénes M, Farkas K, Erdei T, Lengyel M (2010) *Comparison of tissue Doppler velocities obtained by different types of echocardiography machines. Are they compatible?* *Echocardiography* 27(3): 230–235.

## CSABA ANDRÁS DÉZSI (2009)

### Short-term and long-term effect of non-pharmacological rate control on symptomatic atrial arrhythmia reflected by the cardiopeptide serum level changes

*Supervisor: Béla Merkely*

Atrial arrhythmia with high ventricular rate may cause an elevation of the endogenous peptides (EP) produced in the heart, and in the long term it may result in tachycardiomyopathy. The radiofrequency catheter ablation (Rate Control: RC) of the atrioventricular node, followed by pacemaker implantation combined with sepal ventricular stimulation proved to be an effective therapy of drug refractory treatment of symptomatic supraventricular tachyarrhythmia. The elevation of serum levels of certain cardiopeptides during cardiovascular diseases is a commonly observable phenomenon, while regarding the pathogenesis of arrhythmia there are still several open questions. Our aim was the examination of changes in serum endothelin-1 (ET-1), big endothelin (BET), atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels, and clinical parameters during symptomatic drug refractory tachyarrhythmia and after its non-pharmacological RC. We studied the possible relationship between the pacemaker implantation procedure and the changes in endogenous peptide serum levels. In our clinical research it was proven that rate control performed with AV node ablation and PM implantation for patients suffering in atrial tachyarrhythmia with high ventricular rate resulted in a significant improvement of the contraction function and the functional stage, particularly at patient groups with DCM and heart failure. It was also proven that in parallel, the concentration of some of the endogenous peptides—and in the case of patients suffering from atrial fibrillation—peptides showing higher initial serum level concentration are also under change. Early and sustained decrease of ET-1 levels was discovered in all patients and in the subgroups alike. In contrary, the serum level of BET showed only temporary decrease, and did not decrease significantly in any of the examined subgroups after rate control. On the other hand, rate control of atrial fibrillation resulted in early and sustained remission of the ANP levels. Contrary to ANP levels, BNP production decreased significantly only in the groups of DCM and heart failure.



- Dézsi CsA, Szűcs A, Szűcs G, Róka A, Kiss O, Becker D, Merkely B (2006) Short-term effect of rate control on plasma endothelin levels of patients with tachyarrhythmias. *Exp Biol Med* 231(6): 852–856.
- Dézsi Cs A, Szűcs G, Kiss O, Szűcs A (2008) Pitvarfibrilláció nonfarmakológias frekvencia kontrolljának hosszú távú hatása cardiopeptidek szérum szintjének változása tükrében. *Card Hung* 38(2): 123–131.
- Szűcs A, Róka A, Soós P, Szilágyi Sz, Vágó H, Keltai K, Dézsi CA, Gellér L, Merkely B (2004) Effect of incessant ventricular tachyarrhythmias on serum endothelin and big endothelin levels. *J Cardiovasc Pharmacol* 44(Suppl 1): S402–406.

## KRISTÓF HIRSCHBERG (2010)

### New genetic risk factors and therapeutic possibilities in case of recurrent stenosis after caroid interventions

Supervisor: László Entz

Long-term success of surgical and endovascular treatment of atherosclerotic stenosis is limited by restenosis, a complication that has not yet been completely solved. The incidence of restenosis varies according to the method (stenting, endarterectomy), to the treated vascular region, and to several other factors, but the pathomechanics and risk factors are similar. Risk factors for restenosis can be divided into systemic (e. g. diabetes, gender) and local (e. g. type and length of the atherosclerotic plaques) ones. In addition, evidence is growing that restenosis development can be genetically determined as well. In the present work, we investigated these issues; clinical as well as experimental studies have been performed. In a retrospective study, incidence of restenosis following carotid artery stenting (CAS) and carotid endarectomy (CEA) has been compared by duplex ultrasound. In the prospective study, the role of the estrogen receptor alpha gene polymorphisms in recurrent carotid stenosis has been investigated by PCR-RFLP. Endothelial injury and ischemic/reperfusion injury during vascular interventions trigger the pathological processes leading to restenosis, which consist of the following four overlapping stages: (1) platelet activation and aggregation, (2) inflammation, (3) vascular smooth muscle cell (VSMC) migration and proliferation and (4) extracellular matrix deposition. Neointimal hyperplasia caused by VSMC migration is primarily responsible for lumen loss. According to multicentric trials, the use of drug-eluting stents covered by anti-inflammatory or antiproliferative drugs significantly reduced coronary in-stent restenosis. In the present work, we investigated the nitric-oxide(NO)-cyclic guanosine monophosphate(cGMP) pathway in neointima formation under phosphodiesterase-5(PDE-5)-inhibitor treatment, in an experimental carotid endarectomy model. Results from the clinical studies suggest, that incidence of severe (above 70%) restenosis is higher after CEA than after CAS. On the other hand, postoperative neurologic complications, especially transient ischemic attack, has been recorded more often after CAS than following CEA. According to a genetic association study, significantly higher restenosis rates have been found in patients carrying AA genotype of the XbaI polymorphisms of estrogen receptor alpha, as compared to patients carrying AG or GG genotypes (23.4% vs. 10.5%,  $p=0.02$ ). Regarding the other polymorphism tested, we found significantly higher restenosis rates in patients carrying TT or TC genotypes as compared to patients with CC genotype (19.3% vs. 3.1%,  $p=0.02$ ).



These associations were shown in the whole patient population, but especially in the subgroup of female patients undergoing carotid endarterectomy. We demonstrated by multiple logistic regression analysis, that T-A haplotype carriers (homozygous or heterozygous) had an increased risk of restenosis after carotid interventions, which was independent of age, gender and presence of recurrent stenosis. (adjusted Odds ratio: 7.85, confidence interval: 1.01–60.98) We found intensive alpha-smooth muscle actin and transforming growth factor  $\beta 1$  immunoreactivity in the control neointima, which indirectly confirms the migration and proliferation of VSMC's. Phosphodiesterase-5 inhibitor treatment significantly reduced the stenosis grade ( $24.64 \pm 7.46\%$  vs.  $54.12 \pm 10.30\%$  in the control endarterectomy group;  $p < 0.05$ ) and the neointima/media area ratio ( $0.50 \pm 0.15$  vs.  $1.03 \pm 0.13$  in the control endarterectomy group,  $p < 0.05$ ) as well as the expression of both neointimal markers. PDE-5 inhibitor treatment was correlated with local (neointimal) and systemic elevation of cGMP concentration, that was demonstrated by immunohistology and enzyme immunoassay, respectively. Estrogen hormone has antiproliferative effects, and increases cGMP signaling by genomic and nongenomic activation of endothelial nitric-oxide synthase. Our results suggest that neointimal hyperplasia can be effectively attenuated by phosphodiesterase-5 inhibitory treatment. The biggest long-term benefit of a genetic association study like ours may be that it encourages regular check-ups and individual therapy for patients with higher genetic predisposition to recurrent stenosis.

- Hirschberg K, Gombos T, Dósa E, Somorjai A, Szilágyi Á, Szabó G, Füst G, Entz L (2009) Association between estrogen receptor alpha gene polymorphisms and early restenosis after eversion carotid endarterectomy and carotid stenting. *Atherosclerosis* 206(1): 186–192.
- Hirschberg K, Radovits T, Loganathan S, Entz L, Beller CJ, Gross ML, Sandner P, Karck M, Szabó G (2009) Selective phosphodiesterase-5 inhibition reduces neointimal hyperplasia in rat carotid arteries after surgical endarterectomy. *J Thorac Cardiovasc Surg* 137(6): 1508–1514.
- Hirschberg K, Dósa E, Hüttl K, Selmei L, Nemes B, Szabó A, Merkely B, Acsády G, Entz L (2009) Early restenosis after eversion carotid endarterectomy versus carotid stenting: a single-centre retrospective study. *J Cardiovasc Surg (Torino)* 50(5): 655–663.

## TÍMEA KOVÁTS (2009)

### Molecular mechanism of natriuretic peptide secretion and adaptation to disease in animal model and clinical practice

Supervisor: Miklós Tóth

Cardiac natriuretic peptides (atrial natriuretic peptide: ANP, B-type natriuretic peptide: BNP) are important regulators of the cardiovascular homeostasis in health and disease, and the measurement of their plasma concentration facilitates diagnosis and prognosis in the clinical practice of several disorders. This thesis reports (1) *in vitro* and *ex vivo* experiments for the better understanding of the physiologic mechanism of ANP secretion from cardiomyocytes and the cardiac localization and molecular forms of the pro-ANP convertase corin; (2) *in vivo* studies of altered natriuretic peptide and endothelin secretion in response to acute hemodynamic load in animal model of alloxan-induced type I diabetes mellitus; and finally, (3) evaluation of natriuretic peptide secretion in bradycardia in the clinical

practice. Cell culture studies have shown that syntaxin 4 and Munc18c, which are essential regulators of secretion in other specific secretory cells, interact with each other and are co-localized to the plasma membrane in pro-ANP containing cardiomyocytes. Functional studies have shown that cardiac secretion of ANP is triggered by increased  $\text{Ca}^{2+}$  and is specifically regulated by Munc18c. Further experiments demonstrated that corin, the enzyme responsible for pro-ANP activation to active peptide ANP, is present on the cell-surface of pro-ANP-containing cardiomyocytes. Native corin is a glycosylated protease that exists on the plasma membrane in both zymogen and catalytically active forms. Animal studies have shown that NT-proANP plasma levels are increased in response to hemodynamic load both in diabetes and in metabolically healthy controls. Higher NT-proANP levels in diabetic animals supported the need for different cutoff values for diagnostic purposes with concomitant diabetes mellitus. Finally, a clinical example demonstrated that bradycardia can manifest in highly elevated natriuretic peptide plasma level even in the absence of heart failure signs. Cessation of the junctional escape rhythm responsible for the bradycardia resulted in a rapid fall in the NT-proBNP serum level. This study accentuated that any pathophysiology that leads to increased myocardial wall stretch might cause clinically elevated natriuretic peptide level.

- Kováts T, Tomcsányi J (2009) Bradycardia and B-type natriuretic peptide. *Int J Cardiol* 135(2): 238–239.
- Gladysheva IP, Robinson BR, Houg AK, Kováts T, King SM (2008) Corin is co-expressed with pro-ANP and localized on the cardiomyocyte surface in both zymogen and catalytically active forms. *J Mol Cell Cardiol* 44(1): 131–142.
- Kováts T, Wettstein A, Nagy E, Tomcsányi J (2008) Bradycardia can induce increased serum natriuretic peptide-level. *Int J Cardiol* 123(2): e43–44.

## ANDREA ÁGNES MOLNÁR (2009)

### Non-invasive, *in vivo* biomechanical assessment of human veins

Supervisor: Viktor Bérczi

Study of the physiological and pathophysiological properties of the human deep veins can help in the better understanding of the pathomechanism of venous disease. Our aim was the *in vivo*, non-invasive assessment of the biomechanical properties of human veins in different populations: elderly patients without history of chronic venous insufficiency, young patients with history of unilateral deep venous thrombosis (also examining the veins unaffected by thrombosis), thrombophilic patients without history of clinically apparent deep venous thrombosis; using young healthy subjects as a control group. With ultrasound we measured the anteroposterior and mediolateral diameters of the common femoral vein, the internal jugular vein and the axillary vein during a pressure-controlled Valsalva test in reclining and standing position. Venous distensibility was calculated from the change in diameter and pressure. In all patient groups, in the physiologically low pressure range, the common femoral vein and the internal jugular vein were distensible, while in the high pressure range the venous wall became stiff. The distensibility of the axillary vein was negligible in all pressure ranges, in all patient groups. We found that the distensibility of the common femoral vein and the internal jugular vein was significantly decreased in the elderly patient group compared to the control group. We measured

decreased distensibility in the young patient group with history of deep venous thrombosis, not only in the common femoral vein affected by thrombosis but also on the contralateral side and in the internal jugular vein too. Decreased distensibility was registered even in the thrombophilic patient group unaffected by deep venous thrombosis. Based on our results we conclude that the local and sometimes subclinical venous disorders can lead to the generalized, functional-biomechanical remodelling of the venous wall.

- Bérczi V, Molnár AÁ, Apor A, Kovács V, Ruzics C, Várallyay C, Hüttl K, Monos E, Nádasy GL (2005) *Non-invasive assessment of human large vein diameter, capacity, distensibility and ellipticity in situ: dependence on anatomical location, age, body position and pressure.* *Eur J Appl Physiol* 95(4): 283–289.
- Molnár AÁ, Apor A, Kristóf V, Nádasy GL, Préda I, Hüttl K, Acsády G, Monos E, Bérczi V (2006) *Generalized changes in venous distensibility in postthrombotic patients.* *Thromb Res* 117(6): 639–645.
- Molnár AÁ, Apor A, Kristóf V, Nádasy GL, Szeberin Z, Monos E, Acsády G, Préda I, Bérczi V (2008) *Generalized alterations in the biomechanical properties of large veins in non-thrombotic thrombophilic young patients.* *Int Angiol* 27(3): 247–252.

## ATTILA RÓKA (2009)

### Investigation of cardiac resynchronization therapy and pathomechanism of atrial fibrillation

*Supervisor: Béla Merkely*

The diseases of the heart are the most common causes of mortality, their importance is increasing with the growing ratio of elderly population. We investigated the pathomechanism and the optimization of non-pharmacological treatment of its common forms—heart failure and atrial fibrillation—in experimental and clinical studies. We were the first to utilize MRI study during biventricular pacemaker upgrade to select the optimal left ventricular stimulation site in a patient with post-infarction ischemic cardiomyopathy, severe heart failure, permanent atrial fibrillation and intraventricular conduction disorder. Phrenic nerve stimulation is a common complication of left ventricular epicardial stimulation. We were able to avoid phrenic nerve stimulation in 83% of patients using optimal stimulation wave form based on different excitability properties of myocardium and motor nerve. The pathomechanism of atrial fibrillation has not been elucidated yet despite the extensive research in the field, study data are often controversial. One of the causes can be the numerous methods used for induction, whose effect on the characteristics of the induced arrhythmia was not investigated. In an experimental model we proved that with electrical atrial fibrillation induction the applied method affects the characteristics of the arrhythmia during the induction, but the effect diminishes after ceasing induction, if the atrial fibrillation persist after discontinuing induction. The data regarding randomness of ventricular rhythm in atrial fibrillation is controversial. We proved that several statistical parameters show individual characteristics in patients with atrial fibrillation. Adequate ventricular rate control is sometimes difficult in patients with heart failure and atrial fibrillation due to side effects of antiarrhythmics, which are more common in this groups. We were the first to perform parasympathetic cardiac nerve stimulation with chronically implanted coronary sinus lead. There were no complications. Our results may

contribute to the effective utilization of non-pharmacological treatment of heart failure and atrial fibrillation.

- Roka A, Simor T, Vago H, Minorics C, Acsady G, Merkely B (2004) Magnetic resonance imaging-based biventricular pacemaker upgrade. *Pacing Clin Electrophysiol* 27(7): 1011–1013.
- Vago H, Roka A, Acsady G, Merkely B (2004) Parasympathetic cardiac nerve stimulation with implanted coronary sinus lead. *J Cardiovasc Electrophysiol* 15(5): 588–590.
- Roka A, Toth E, Szilagyí S, Merkely B (2008) Electrical atrial fibrillation induction affects the characteristics of the induced arrhythmia. *J Electrocardiol* 41(2): 131–137.

## SZABOLCS SZILÁGYI (2010)

### New methods in the non-pharmacological treatment of heart failure and ventricular arrhythmias

*Supervisor: Béla Merkely*

Heart failure and ventricular arrhythmias play a leading role in cardiac mortality. We investigated new therapeutic methods in the non-pharmacological treatment of these diseases. Biventricular pacing is an effective therapy in severe, drug-refractory heart failure combined with ventricular conduction disturbances. In some patients instability of the coronary sinus (CS) lead in the optimal position is an important problem. We described a new method to anchor the lead with stent implantation into the side branch of the CS. Control impedance measurements did not show injury of the lead insulation, mechanical complications were not detected, the position of the lead remained stable, and it was possible to extract the electrode if it was necessary. Distal position of the CS lead may result in phrenic nerve stimulation, which is often intolerable for the patient. We developed a new, minimal invasive method without opening the pacemaker pocket for the reposition of the CS lead. Withdrawn of the CS electrode via the femoral vein using an ablation catheter is an effective and safe method for the treatment of distal CS lead position causing phrenic nerve stimulation. If transvenous CS lead implantation is unsuccessful and surgical epicardial implantation is not recommended or it is refused by the patient, transseptal endocardial left ventricular lead implantation may be an alternative. We applied successfully in two cases the method described in the literature and modified it using electro-anatomical mapping system to find the place of the transseptal puncture and the region of the latest activation in the left ventricle. Frequent or incessant ventricular tachycardia (VT) is a life-threatening arrhythmia especially in patients with structural heart disease. We performed a number of radiofrequency catheter ablations because of VT after myocardial infarction. Our results are similar to the data in the literature. In Hungary our working group described first successful epicardial catheter ablation of incessant VT late after myocardial infarction, and successful ablation of a patient suffering from frequent VT episodes late after surgical correction of tetralogy of Fallot. Our results may contribute to the effective utilization of non-pharmacological treatment of heart failure and ventricular tachycardia

- Szilagyí Sz, Merkely B, Zima E, Fülöp G, Kutyifa V, Szűcs G, Molnár L, Gellér L (2008) Minimal invasive coronary sinus lead reposition technique for the treatment of phrenic nerve stimulation. *Europace* 10(10): 1157–1160.

- Szilágyi Sz, Merkely B, Róka A, Zima E, Fülöp G, Kutya V, Szűcs G, Becker D, Apor A, Szabolcs Z, Gellér L (2007) Stabilization of the coronary sinus electrode position with coronary stent implantation to prevent and treat dislocation. *J Cardiovasc Electrophysiol* 18(3): 303–307.
- Szilágyi Sz, Merkely B, Zima E, Róka A, Gellér L (2008) A sinus coronarius elektróda pozíciójának stabilizálása stent implantációval: új módszer a diszlokáció kezelésére és megelőzésére. *Card Hung* 38(Suppl A): 30–34.

## GÁBOR VERES (2010)

### New drug therapies reduce bleeding in cardiac surgery

Supervisor: Gábor Szabó

Cardiac surgery procedures with CPB can produce a certain amount of blood loss and in serious cases one of the most important tasks is to minimise post-operative bleeding. There are only a few anti-fibrinolytic drugs available which have this effect (aprotinin, tranexamic acid,  $\epsilon$ -aminocaproic acid). Bovine-derived aprotinin has been a widely accepted drug used for this purpose, however there have been several side effects including allergic reactions and infectious diseases. The withdrawal of aprotinin from the market created a major problem as there was no replacement available which gave the same benefits. A new group of serine-protease inhibitors based on aprotinin appeared to be a possible replacement. In the initial study, we demonstrated that the effectiveness of recombinant aprotinin on blood loss and coagulation parameters were equivalent to those of bovine-derived aprotinin. Neither recombinant aprotinin nor bovine aprotinin impaired the endothelium-dependent vasodilative function of coronary arteries. As recombinant aprotinin probably reduces the risk of allergic reaction and the transmission of animal disease, and bovine aprotinin was withdrawn (2007), it should be utilized clinically though further investigations would be needed. The studies with novel low molecular weight serine-protease inhibitors CU-2010 and CU-2020 in a non-ischemic model of CPB, were shown to reduce blood loss in an equivalent manner to aprotinin and to have improved anti-coagulative properties (100,000-fold impact on fXa and fXIa in comparison with aprotinin). As these small synthetic molecules have numerous advantages over aprotinin, we believe they may offer a true alternative in the “post-aprotinin” era. In our final study, we demonstrated several beneficial effects of CU-2010 in an ischemic model of CPB: (1) CU-2010 dose-dependently reduces postoperative blood loss after CPB which is comparable to aprotinin (2) it has an improved antikoagulatory property (elongated aPTT, ACT) relative to aprotinin (3) at higher dosages, because of the anti-inflammatory effect it may improve left ventricular function recovery (4) CU-2010 improved coronary endothelial function and this improvement was comparable to aprotinin. We believe that this compound may provide an “all-in-one” solution, addressing major issues (such as bleeding, inflammation, cardioprotection) in cardiac surgery and therefore deserves further investigations for clinical application. However, learning a lesson from the current debates, several safety trials should be performed before moving into the clinical arena.

- Veres G, Radovits T, Schultz H, Lin LN, Hütter J, Weigang E, Szabolcs Z, Szabó G (2007) Effect of recombinant aprotinin on postoperative blood loss and coronary vascular function in a canine model of cardiopulmonary bypass. *Eur J Cardiothorac Surg* 32: 340–345.

- Veres G, Radovits T, Szabolcs Z, Szabó G (2008) *A rekombináns aprotinin hatása a posztoperatív vérvesztésre és a koronáriák vaszkuláris funkciójára kardiopulmonális bypass kutyamodelljén.* *Cardiol Hung* 38: 247–253.
- Szabó G, Veres G, Radovits T, Haider H, Krieger N, Bährle S, Miesel-Gröschel C, Niklisch S, Karck M, Van de Locht A (2010) *Effects of novel synthetic serine-protease inhibitors on postoperative blood loss, coagulation parameters and vascular function after cardiac surgery.* *J Thorac Cardiovasc Surg* 139:181–188.

## PROGRAM 1/2.

### MECHANISMS OF NORMAL AND PATHOLOGICAL FUNCTIONS OF THE CIRCULATORY SYSTEM

#### **Coordinators:**

**Emil MONOS M.D., Ph.D., D.Sc.**

**Zoltán BENYÓ M.D., Ph.D., D.Sc.**

Institute of Human Physiology and Clinical  
Experimental Research

Basic Medical Science Center

37–47 Tűzoltó st, Budapest H–1094

Tel.: +36 1 210 6038, Tel./fax: +36 1 334 3162

E-mail: [monos.emil@med.semmelweis-univ.hu](mailto:monos.emil@med.semmelweis-univ.hu)

#### **Program overview**

The program consists of 12 research sub-programs with several special projects completed with appropriate theoretical courses for postgraduate students. Different aspects of normal and disturbed regulatory processes of the cardiovascular system are in the focus. Each Ph.D. student is working on his/her own individual research project under the guidance of a qualified scientific advisor. Successful completion of the Program including publications in recognized international journals provides an opportunity to summarize the results in a Ph.D. thesis.

#### ***Titles of research projects***

Pathophysiology of the cerebral circulation

Cardiovascular adaptational mechanisms in the whole body,  
as well as the myocardium and the brain cortex

Role of bradykinin receptors in the circulatory adaptation under  
normal and pathological conditions; interactions with other  
mechanisms affecting blood pressure

Spatio-temporal correlation of coupled hemodynamics and  
neuronal activities in the brain

Role of postmenopausal hormonal deficiencies in altering the  
fractal structuring of hemodynamic fluctuations in the brain cortex

Impact of cerebrovascular sclerosis in altering the fractal structuring of  
cerebrocortical hemodynamic fluctuations

#### ***Supervisors***

Zoltán Benyó

László Dézsi

László Dézsi

András Eke

András Eke

András Eke



Effects of blood substitutes on tissue hemodynamics and oxygenation	András Eke
Functional integrity of the cardiopulmonary system	Ildikó Horváth
Regulation of calcium homeostasis in the myocardial tissue	Tamás Ivanics
Alterations of the intracellular calcium homeostasis in progressive heart failure	Tamás Ivanics
Comparative evaluation of clinical and epidemiological diagnostic methods in the assessment of cardiovascular autonomic and peripheral sensory neuropathy	Péter Kempler
The mechanism of action of cell-based regenerative therapies in myocardial infarct	Levente Kiss
Investigation of vascular functions affecting the autonomous cardiovascular tone and reflex activity	Márk Kollai
Cardiovascular autonomous neural system	Márk Kollai
The role of mitochondria in ischemic and degenerative diseases	Zsombor Lacza
Adaptation mechanisms of hemodynamic functions and network properties of the vascular system to physiological and to pathological loading	György Nádasy
Alterations of the biomechanical properties of extremity arteries and veins during angiogenetic processes	György Nádasy, Emil Monos
Alterations of biomechanical and network properties of intramural coronary resistance arteries with aging, hypertension and in other angiogenetic processes	György Nádasy, Emil Monos
Videomicroscopic analysis of ureteral movements. Pharmacological and pathological effects	György Nádasy, Imre Romics
Ischemia-induced molecular-biological changes of the blood-brain barrier	Péter Sándor
The role of the female sex hormones in the regulation of the cerebral blood flow	Péter Sándor
Role of oxidative stress in pathophysiology of cardiovascular system	Csaba Szabó
Study of promoting and inhibiting factors in cardiovascular aging	Béla Székács
Hormon-dependent cardiovascular adaptation mechanisms in normo- and hypertension in females	Szabolcs Várbíró

### ***Ph. D. students***

Péter Antal	pt
Attila Cselenyák	ft
András Csepregi	pt
Katalin Fekete	ft
András Hartmann	pt
András Iring	ft
Péter Kemecsei	ft
Tímea Martos	ft
Katalin Módis	ft
Nóra Németh	ft
Tamás Németh	ft (a)
Ágoston Pasztuhov	pt
Alexandra Pintér	ft

### ***Supervisors***

György Nádasy
Zsombor Lacza
András Temesvári
Zoltán Benyó
András Eke
Zoltán Benyó
Tamás Ivanics
Péter Kempler
Csaba Szabó
Zsolt Rónai
Zoltán Benyó
Péter Tóth
Márk Kollai



Levente Sára	pt	Szabolcs Várbíró
Ádám Domonkos Tárnoki	pt	Viktor Bérczi
Dávid László Tárnoki	pt	Viktor Bérczi
Bernadett Trencsényiné Balázs	ft	Ákos Zsembery
Gabriella Vác	ft	Zsombor Lacza
Miklós Weszl	ft	Zsombor Lacza

***Ph.D. candidates***

Zsolt Hermányi	pt
Gábor Lenzsér	ft
Miriam Leszl-Ishiguro	ft
Petra Őrsy	ft
Eszter Pankotai	ft
István Portörő	ft
Éva Ruisanchez	ft (a)
Emese Szelke	na

***Ph.D. graduates***

Rita Benkő	ft
Eszter Mária Horváth	ft
Ildikó Istenes	ft
Levente Kiss	ft
Zsuzsanna Putz	ft

***Supervisors***

Péter Kepler
Péter Sándor
Zoltán Benyó
Zoltán Benyó
Zsombor Lacza
András Eke
Zoltán Benyó
Péter Sándor

***Supervisors***

Csaba Szabó
Csaba Szabó
Péter Kempler
Csaba Szabó
Péter Kempler

a, absolutorium; ft, full-time; pt, part-time; i, individual; na, not affiliated

***Abstracts of Ph.D. theses successfully defended in 2009 and 2010*****rita benkő (2009)****The role of poly(ADP-ribose) polymerase-1 in the pathogenesis of chronic cardiovascular diseases**

*Supervisor: Csaba Szabó*

Increased production of reactive oxygen and nitrogen species has recently been implicated in the pathogenesis of cardiac and endothelial dysfunction associated with atherosclerosis, hypertension, and aging. Oxidant-induced cell injury triggers the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP). Oxidant-mediated activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) plays a role in the development of endothelial dysfunction and the pathogenesis of various cardiovascular diseases, including diabetes, reperfusion injury, circulatory shock, and aging. Earlier studies found increased amounts of poly(ADP) ribosylated proteins in diabetic heart, vessels in both human and animal samples, and also in kidneys of Leprdb/db(BKsJ) mice, suggesting increased PARP activity. The aim of the present studies were to investigate (1) the effect of a new PARP inhibitor, INO-1001, on cardiac and endothelial dysfunction associated with advanced aging using Millar's new Aria pressure-volume conductance system and isolated aortic

rings; (2) the effects of INO-1001 on the development of diabetic endothelial dysfunction of Leprdb/db(BKSJ) mice, an experimental model of type 2 diabetes; (3) whether the activation of PARP contributes to the development of endothelial dysfunction in the apolipoprotein E (ApoE) deficient mice; (4) as peroxynitrite is a potent cytotoxic oxidant produced from nitric oxide (NO) and superoxide anion during conditions of oxidative stress, and it leads to PARP activation, the purpose of our next study was to determine the effects of a peroxynitrite decomposition catalyst (WW85) on the endothelial dysfunction and neointima formation in a rat model of carotid artery injury; (5) whether activation of the nuclear enzyme PARP contributes to the development of angiotensin II-induced endothelial dysfunction. In these studies, certain links were investigated between ageing, diabetic complications, atherosclerosis, hypertension on the level of reactive species formation and PARP activation using animal models. We observed that pharmacological inhibition of PARP protected the cardiovascular system in these pathological states.

- Benko R, Pacher P, Vaslin A, Kollai M, Szabo C (2004) Restoration of the endothelial function in the aortic rings of apolipoprotein E deficient mice by pharmacological inhibition of the nuclear enzyme poly(ADP-ribose) polymerase. *Life Sci* 75: 1255–1261.
- Pacher P, Vaslin A, Benko R, Mabley JG, Liaudet L, Hasko G, Marton A, Batkai S, Kollai M, Szabo C (2004) A new, potent poly(ADP-ribose) polymerase inhibitor improves cardiac and vascular dysfunction associated with advanced aging. *J Pharmacol Exp Ther* 311: 485–491.
- Szabo C, Biser A, Benko R, Bottinger E, Susztak K (2006) Poly(ADP-ribose) polymerase inhibitors ameliorate nephropathy of type 2 diabetic Leprdb/db mice. *Diabetes* 55: 3004–3012.

## ESZTER MÁRIA HORVÁTH (2009)

### Poly (ADP-ribose) polymerase activation in circulating leukocytes

Supervisor: Csaba Szabó

PARP activation significantly contributes to the pathogenesis of various conditions, such as endotoxin shock and myocardial infarction. Pharmacological inhibitors of PARP move toward clinical testing for a variety of indications including cardioprotection and malignant tumors. Our aim was to identify possible novel modulators of PARP, that may influence the outcome of these studies and to test whether measuring PARP activity in circulating leukocytes may serve as a sentinel test reflecting the degree of PARP activation and the efficiency of PARP inhibition.

Our results showed that in LPS treated female mice/rats LPS-induced TNF- $\alpha$  production and endothelial dysfunction were markedly attenuated, and in contrast to male mice/rats, pharmacological inhibition of PARP failed to provide further protection. The gender difference in TNF- $\alpha$  production is partially diminished by ovariectomy. In circulating leukocytes, the PARP inhibitor PJ34 only inhibited LPS-induced PARP activation in males. Our observations demonstrate that there is an interrelated regulation of the endotoxin-induced inflammatory and vascular responses by gender and PARP.

We demonstrated that insulin therapy in a rat model of endotoxemia blocks PARP activation and prevents inflammatory mediator production. Insulin treatment prevented LPS-induced hyperglycemic response, blocked PARP activation in circulating leukocytes and blunted LPS-induced TNF- $\alpha$  response. Insulin treatment caused a slight reduction in PARP activity of mononuclear cells and HUVECs in elevated glucose conditions *in vitro*.

In the examined population of cardiovascular patients STEMI followed by PCI is accompanied by increased nitrosative stress, PARP activation, and consequent AIF translocation in circulating leukocytes. These data provide evidence for PARP activation for the first time in humans suffering from myocardial infarction.

In all studies PARP activity of mononuclear cells reflected the pathological condition and the efficiency of PARP inhibition therapy.

Our observations indicate that estrogen is a novel endogenous inhibitor of PARP. Gender differences and PARP-inhibitory effect of insulin therapy has to be considered in pharmacological development and upcoming clinical trials of PARP inhibitors. Measuring the PARP activity of circulating leukocytes may serve as potential sentinel in these studies.

- Horvath EM, Benko R, Gero D, Kiss L, Szabo C (2008) Treatment with insulin inhibits poly(ADP-ribose)polymerase activation in a rat model of endotoxemia. *Life Sci* 82: 205–209.
- Toth-Zsamboki E, Horvath E, Vargova K, Pankotai E, Murthy K, Zsengeller Z, Barany T, Pek T, Fekete K, Kiss RG, Preda I, Lacza Z, Gero D, Szabo C (2006) Activation of poly(ADP-ribose) polymerase by myocardial ischemia and coronary reperfusion in human circulating leukocytes. *Mol Med* 12: 221–228.
- Mabley JG, Horvath EM, Murthy KG, Zsengeller Z, Vaslin A, Benko R, Kollai M, Szabo C (2005) Gender differences in the endotoxin-induced inflammatory and vascular responses: potential role of poly (ADP-ribose) polymerase activation. *J Pharmacol Exp Ther* 315: 812–820.

## ILDIKÓ ISTENES (2009)

### Relationship between hypertension, autonomic neuropathy and the cardiovascular reflextests in type 2 diabetes

Supervisor: Péter Kempler

The early diagnosis of cardiovascular autonomic neuropathy is very important, since it is a serious complication of diabetes. Therefore, the correct evaluation of the five standard cardiovascular reflextests is of pivotal importance. Onehundred and twenty-five diabetic patients and onehundred and twelve healthy control subjects underwent the five standard cardiovascular reflextests. According to our data, heart rate changes to deep breathing are affected by the patient's age, therefore it has to be taken into consideration at the evaluation of the results in order to avoid overestimating the prevalence of parasympathetic autonomic dysfunction. Orthostatic hypotension, a sign of sympathetic dysfunction, can be affected either by the initial systolic blood pressure values or the presence of parasympathetic autonomic neuropathy (through sympathetic dominance). Therefore using the measurement of orthostatic hypotension as a single test is not recommended for the diagnosis of autonomic neuropathy. Both hypertension and autonomic neuropathy are associated with an increased risk of cardiovascular disease and death. Both are also common occurrences in subjects with diabetes, but the nature of any relationship between the two is far from clear. In our cross-sectional studies we have found that diminished heart rate variability was associated with hypertension in Type 2 diabetic patients with hypertension and the association between autonomic dysfunction (based on the five standard cardiovascular reflextests) and elevated blood pressure values is present even in nor-

moalbuminuric Type 2 diabetic patients without prior history of hypertension. In this latter group, the prevalence of unrecognized hypertension was twice as many in patients with autonomic neuropathy compared to those with normal autonomic function. Our results support the hypothesis that relative sympathetic overactivity (due to the loss of parasympathetic counter-regulation) plays an important role in the pathogenesis of hypertension in Type 2 diabetes. Our results indicate that by relying simply on clinic blood pressures, hypertension is frequently unrecognized and therefore untreated. Subjects with autonomic neuropathy should therefore be screened aggressively for hypertension including with 24-hour blood pressure monitoring, if necessary and *vice versa*, hypertensive patients should be screened for autonomic neuropathy as well.

- Istenes I, Keresztes K, Hermányi Zs, Gyarmati G, Vargha P, Kempler P (2002) *A szívfrekvencia-variabilitás beszűkülése és a hypertonia összefüggése 2-es típusú diabetes mellitusban. Diabetol Hung 10: 15–22.*
- Keresztes K, Tislér A, Istenes I, Sipos É, Vargha P, Kempler P (2004) *Az autonóm neuropathia és a 24 órás ambuláns vérnyomásprofil összefüggése normalbuminuriás, 2-es típusú diabeteszes betegekben. Hypertonia és Nephrologia 8: 193–199.*
- Istenes I, Keresztes K, Tündik A, Hermányi Zs, Putz Zs, Vargha P, Kertész T, Emery C, Gandhi R, Tesfaye S, Kempler P (2007) *Blood pressure response to standing in the diagnosis of autonomic neuropathy: are initial (supine) values of importance? Diabet Med 24 (3): 325–327.*

## LEVENTE KISS (2009)

### Oxidative stress and the role of downstream pathways in cardiovascular diseases

*Supervisor: Csaba Szabó*

Oxidative and nitrosative stress play an important role in the pathogenesis of several cardiovascular diseases, but the involved downstream pathways are still incompletely understood. Based on recent investigations, poly(ADP-ribose) polymerase and hydrogen sulfide may play an important part in these processes. The purpose of the present thesis was to further elucidate the role of poly(ADP-ribose) polymerase and hydrogen sulfide in models of cardiovascular diseases involving free radicals. In our first experiments we investigated the effects of the main oxysterol 7-ketocholesterol on the activity of endothelial poly(ADP-ribose) polymerase and on endothelium-dependent vasorelaxant function. In further experiments we investigated the role of poly(ADP-ribose) polymerase in the process of cardiac remodeling and heart failure in a mouse model of heart failure induced by transverse aortic constriction. Finally, we investigated whether hydrogen sulfide has a potential to ameliorate myocardial ischemia-reperfusion injury *in vivo* and to explore what could be the possible underlying mechanism of action. Our experimental results indicate that: (1) although 7-ketocholesterol can activate poly(ADP-ribose) polymerase in endothelial cells, it is not sufficient on its own to cause impairment in the endothelium-dependent vascular reactivity; (2) poly(ADP-ribos)ylation plays an important role in the pathogenesis of banding-induced heart failure; (3) hydrogen sulfide may be of value in cytoprotection during the evolution of myocardial infarction and that either administration of hydrogen sulfide or the modulation of endogenous production may be of clinical benefit

in ischemic disorders. These results stress the importance of oxidative and nitrosative stress in the investigated cardiovascular diseases and may lead to novel approaches in the therapies of atherosclerosis, ischemic heart disease and chronic heart failure.

- Xiao CY, Chen M, Zsengellér Zs, Li H, Kiss L, Kollai M, Szabó C (2005) *Poly (ADP-ribose) polymerase promotes cardiac remodeling, contractile failure and translocation of apoptosis-inducing factor in a murine experimental model of aortic banding and heart failure* *J Pharmacol Exp Ther* 312: 891–898.
- Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabó C, Kimura H, Chow CW, Lefer DJ (2007) *Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function.* *Proc Natl Acad Sci USA* 104: 15560–15565.
- Kiss L, Chen M, Gerő D, Módos K, Lacza Zs, Szabó C (2006) *Effects of 7-ketocholesterol on the activity of endothelial poly (ADP-ribose) polymerase (PARP) and on endothelium-dependent relaxant function.* *Int J Mol Med* 18: 1113–1117.

## ZSUZSANNA PUTZ (2009)

### Characteristics of autonomic and sensory nerve dysfunction in subjects with impaired glucose tolerance

*Supervisor: Péter Kempler*

Autonomic and peripheral neuropathy are considered to be major complications of unfavourable prognosis in diabetes, alcoholic and non-alcoholic chronic liver disease and chronic kidney disease, which appears in more than half of type 2 diabetic patients. Due to the long-lasting development of type 2 diabetes, diabetes specific complications may occur even in the prediabetic state. The aim of our study was to evaluate the clinical symptoms as well as cardiovascular autonomic and peripheral sensory nerve function in patients with impaired glucose tolerance. According to our results, autonomic and sensory neuropathy are frequent complications among patients with impaired glucose tolerance. The autonomic dysfunction which may affect both parasympathetic and sympathetic nerve system can be considerable even in case of impaired glucose tolerance. As novel finding we demonstrate the attenuation of time domain measure of heart rate variability (HRVti) in patients with impaired glucose tolerance. Several prospective studies in diabetic patients confirmed that cardiovascular autonomic neuropathy is associated with poor prognosis. Cardiovascular autonomic neuropathy may contribute to the increased cardiovascular risk of impaired glucose tolerance. According to our results, unmyelinated small fibre damage is the characteristic feature of neuropathy in subjects with impaired glucose tolerance. Among the quantitative tests used for the assessment of sensory function, the 5 Hz current perception threshold of the Neurometer and the hot detection threshold of the Medoc device seem to be appropriate for the early detection of small fibre damage. Our data suggest that these non-invasive methods may serve as an alternative for the invasive punch skin biopsy used for the detection of early neuropathy among subjects with impaired glucose tolerance.

- Putz Zs, Jermendy Gy (2003) *Szokatlan aetiológiájú lábsérülés diabeteses neuropathia talaján.* *Diabetol Hung* 11: 275–277.

- Istenes I, Keresztes K, Tündik A, Hermányi Zs, Putz Zs, Vargha P, Kertész T, Emery C, Gandhi R, Tesfaye S, Kempler P (2007) Blood pressure response to standing in the diagnosis of autonomic neuropathy: are initial (supine) values of importance. *Diabet Med* 24: 325–327.
- Putz Zs, Jermendy Gy (2007) Öngyógyítás okozta súlyos égési lábsérülés diabeteszes neuropathia talaján. *Diabetol Hung* 15: 40–42.

## PROGRAM 1/3.

## BIOLOGICAL EFFECTS OF IONIZING AND NON-IONIZING RADIATION

### **Coordinator:**

**Miklós KELLERMAYER M.D., Ph.D., D.Sc.**

Institute of Biophysics

34–47 Tűzoltó st, Budapest H–1094

Tel.: +36 1 267 6261

E-mail: [miklos.kellermayer@eok.sote.hu](mailto:miklos.kellermayer@eok.sote.hu)

### **Program overview**

Biological effects, induced by different physical and chemical environmental factors (e.g. ionizing and non-ionizing radiations, pollutants) endanger the whole biosphere including humans. The conscious environmental protection and the prevention of several human diseases due to these environmental factors can be effective only knowing and quantifying the sources (natural radiation background, nuclear disaster, solar radiation, ozone depletion, chemical pollution, etc.). In this Program field and laboratory measurements of monitoring of ionizing and ultraviolet radiations are offered with particular interest to the quantification of their biological effects on global, cellular and molecular level.

### **Titles of research projects**

Self-assembling amyloid filament systems

Self organizing and nanomechanical properties of the myosin motor protein

Nanobiotechnology

Nanomechanics of nucleoprotein systems

Molecular biophysics of the giant muscle protein titin

Measurement of the environmental UV (ultraviolet) radiation, evaluation of health risk of the population caused by environmental and artificial UV radiation

Study of protein conformational dynamics related to function: experimental and computational approaches

Studies of the effects of antioxidants and photosensitizers on cell cultures and liposomes

Mechanism of action of photosensitizers and their application in microbial inactivation

### **Supervisors**

Miklós Kellermayer

Miklós Kellermayer

Miklós Kellermayer

Miklós Kellermayer

Miklós Kellermayer

Györgyi Rontó

Judit Fidy

Pál Gróf

Gabriella Csík



Computational and experimental investigation of the structure, dynamics and function of ABC transporters Tamás Hegedűs

### ***Ph.D. students***

Tamás Bozó	ft
Judit Somkuti	ft
Sándor Dániel Veres	ft

### ***Supervisors***

Miklós Kellermayer
László Smeller
Levente Herényi

### ***Ph.D. candidates***

Balázs Kiss	ft
Zsolt Mártonfalvi	ft
Csilla Ünige Murvai	ft

### ***Supervisors***

Miklós Kellermayer
Miklós Kellermayer
Miklós Kellermayer

### ***Ph.D. graduates***

Georgina Fröhlich	ft
Csilla Pesznyák	na

### ***Supervisors***

Tibor Major
Gyöngyi Rontó

ft, full-time; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2010***

### **GEORGINA FRÖHLICH (2010)**

#### **Domestic evaluation of image-based interstitial conformal prostate and breast brachytherapy**

*Supervisor: Tibor Major*

My research project includes dosimetric analysis of conformal interstitial prostate and breast brachytherapy (BT) supported by tomographic imaging and dose optimization. I proved that US based treatment planning in high dose rate (HDR) and permanent seed prostate BT, and preimplant CT imaging for target volume definition and catheter placement planning in HDR breast BT result in appropriate dose distribution regarding dose coverage, homogeneity and conformality while the doses to organs at risk are below acceptable limits. In HDR prostate BT the dose to rectum can be described with dose in reference point, but the dose to urethra can be characterized more accurately with a volumetric parameter of the dose to the most exposed 1% urethra volume. The best dosimetric parameters can be achieved with the use of medium number of needles (15–17). At uniform needle distribution the dose distribution in the prostate is more uniform and the dose to rectum is lower. At real needle distribution the geometrical optimization (GO) can partly compensate for the non-uniform needle distribution. The graphical optimization (GRO) can further improve the quality of the plan. At dose-volume based inverse optimization the dose coverage is slightly inferior, the conformality is superior and the dose to urethra is smaller than at forward optimization. At prostate seed BT the



dose coverage of target is smaller, the high dose volumes are larger, the relative dose in rectum is larger and the dose distribution is less homogeneous than at HDR prostate BT. In prostate seed BT the non-uniform seed arrangement increases the target volume coverage and dose homogeneity, decreases the dose to urethra but increases the dose to rectum compared to uniform seed arrangement. In HDR breast BT to characterize the dose to organs at risk the use of  $V_{10\text{Gy}}$  and  $V_{5\text{Gy}}$  for the lung and  $V_{5\text{Gy}}$  for the heart is recommended. To get the most accurate catheter reconstruction as large degree between the catheters and CT gantry plane as possible has to be used. Furthermore, small CT slice thickness ( $\leq 3\text{mm}$ ) and GO for planning are recommended to apply. GRO and dose prescription based on around 75% of mean central dose can further improve the plan quality. Conformal dose planning with the use of dose points placed on the target surface can result in high conformality and less maximal skin dose, but only at the cost of decreased dose homogeneity.

- Fröhlich, G, Ágoston, P, Lövey, J, Somogyi, A, Fodor, J, Polgár, Cs, Major, T (2010) *Dosimetric evaluation of high-dose-rate interstitial brachytherapy boost treatments for localized prostate cancer. Strahlenther Onkol* 186: 388–395.
- Fröhlich, G, Ágoston, P, Lövey, J, Polgár, Cs, Major, T (2010) *The effect of needle number on the quality of high-dose-rate prostate brachytherapy implants, Pathol Oncol Res* 16: 593–599.
- Polgár, Cs, Jánváry, L, Major, T, Somogyi, A, Takácsi-Nagy, Z, Fröhlich, G, Fodor, J (2010) *The role of high-dose-rate brachytherapy boost in breast-conserving therapy: long-term results of the Hungarian National Institute of Oncology. Rep Pract Oncol Radiother* 15: 1–7.

## CSILLA PESZNYÁK (2010)

### Some questions of the quality control in the megavoltage therapy (physical and informatical aspects)

Supervisor: Gyöngyi Rontó

The aim of the investigation is to give answer to some questions of the QC in the megavoltage therapy for the sake of making the treatments more trouble-free. We investigated the terms of the usage of CT and PET/CT equipments in treatment planning that were made originally for diagnostic purposes. We compared the calculation algorithms of the Varian CadPlan™ and CMS XiO® treatment planning systems (TPS) for photon and electron radiations of different energy. We also investigated the terms of usage of the PTW EPID QC PHANTOM® in the quality control of the EPID's and the portal images, as well. On the base of the measurements, it can be stated that on photon energies the superposition algorithm can be used for patient treatments in the case of the CMS XiO® TPS while in the case of Varian CadPlan™ TPS the pencil beam modified Batho convolution-algorithm is the proper choice. It is not allowed to use the TPS without inhomogeneity correction. The CIRS Thorax IMRT phantom can be used for electron measurement only at higher than 10 MeV since only the Farmer chamber can be inserted into the holes of the phantom. On the base of the electron measurements, it can be stated that both planning systems give good results in soft tissue. In lung equivalent material the calculated values of the Varian CadPlan™ are in better agreement with the measured values, but the

calculated values behind the bones are not accurate enough. In the QA/QC process the PTW EPID QC PHANTOM® is usable not only for the amorphous silicon EPID's but the image quality can be analysed on the video based devices and on EPID's operating with liquid filled ionisation chamber array detector and even on port films. In the protocol for measurements, the usable file format should be given since the DICOM implementation is not complete in the case of these systems.

- Pesznyák Cs, Fekete G, Mózes Á, Kiss B, Király R, Polgár I, Zaránd P, Mayer Á (2009) *Quality control of portal imaging with PTW EPID QC Phantom®. Strahlenther Onkol 185: 56–60.*
- Pesznyák Cs, Zaránd P, Mayer Á (2007) *Digitalization and networking of analog simulators and portal images. Strahlenther Onkol 183: 117–120.*
- Pesznyák Cs, Weisz Cs, Király R, Kiss B, Zelić S, Polgár I, Zaránd P (2009) *Computer-tomográfias készülékek minőségellenőrzése besugárzástervezés szempontjai alapján (magyarországi helyzetelemzés). Magyar Onkológia 53: 247–251.*

## **PROGRAM 1/4.**

### **FLUID AND ELECTROLYTE BALANCE IN HEALTHY AND PATHOLOGICAL REGULATION OF BLOOD PRESSURE AND CIRCULATION**

#### ***Coordinator:***

**László ROSIVALL M.D., Ph.D., D.Sc.**

Institute of Pathophysiology

4 Nagyvárad sq, Budapest H-1089

Tel.: +36 1 210 2956, Tel./fax: +36 1 210 2956

E-mail: [rosivall@net.sote.hu](mailto:rosivall@net.sote.hu)

#### **Program overview**

Our Ph.D. Program in pathophysiology/nephrology received accreditation in 1993. The goal of this Program is to foster the continued development of traditionally and internationally recognized basic and clinical nephrology research in Hungary. Participating experts in this Program represent various fields of physiology, pathophysiology, internal medicine, pediatrics, transplantation and clinical nephrology, and share a complex, multidisciplinary view of nephrology research and education. In our research activities, special emphasis is placed on the control of fluid and electrolyte balance, blood pressure and regulation of kidney function. With the discipline of translational research, modern experimental techniques are used at various levels from molecule to bedside. Research topics for doctorate degree graduates in Nephrology and Hypertension (41 Ph.D.-s so far) are quite extensive. Our research team has gained international recognition and is a leading expert on the regulation of renal hemodynamics and microcirculation, the mechanisms and progression of various kidney diseases including chronic renal failure, diabetic nephropathy, fibrosis and kidney allograft rejection. We are studying intracellular signal mechanisms, cell-cell communication, TGF-beta and the renin-angiotensin system, and their interaction with the control of renal hemodynamics. By studying the morphology

and function of the afferent arteriole and juxtaglomerular apparatus, we have described a novel regulatory mechanism of glomerular filtration.

### ***Titles of research projects***

### ***Supervisors***

Risk factors in diabetes nephropathy. Analysis of the correlations among serum relaxin, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF- $\beta$ ) and angiotensin II levels.	László Rosivall
Prevention of cardiovascular death	
Renin-angiotensin system (RAS)	László Rosivall
Intracellular signalling mechanisms of transforming growth factor-beta and angiotensin II	László Rosivall
Factors associated with the outcome of kidney transplantation	László Rosivall
Novel concepts in the regulation of blood pressure and kidney function	László Rosivall
Genetic factors inhibit the progressive renal fibrosis.	László Rosivall
Exploration of molecular transcriptic mechanism inhibiting profibrotic effects of TGF-beta	
Effects of VEGF, Angiotensin II, relaxin and renin (prorenin) on endothelial fenestration and permeability	László Rosivall
Dialysis therapy, biocompatibility, quality of life	László Rosivall
Characterization of leucocyte subpopulation to follow/prevent the rejection of kidney in transplanted patients	László Rosivall
Dialysis therapy, biocompatibility, quality of life	István Mucsi
Intracellular signalling mechanisms of transforming growth factor-beta and angiotensin II	István Mucsi
Diagnosis and treatment of renal osteodystrophy	István Mucsi
Obstructive sleep apnea syndrome as a cardiovascular risk factor in chronic kidney disease patients	István Mucsi
Factors associated with the outcome of kidney transplantation	István Mucsi
Cell-cell and cell-matrix interactions in the progression of chronic renal fibrosis	István Mucsi
Cardiovascular risk, calcium, phosphorus and bone metabolism in chronic kidney disease patients	István Mucsi
Cardiovascular and renal pathophysiology of aging	Zoltán Ungvári
Pathophysiology of nano-medicines with particular focus on nephrology and circulation	János Szebeni
New prognostic and morphological approaches to diagnosing HIV	János Szebeni
Pathophysiology of the complement system, with particular focus on the mechanism of drug-induced acute activations, their consequences and inhibition	János Szebeni
Novel concepts in the regulation of blood pressure and kidney function	János Peti-Peterdi
Endogeneous diuretic substances in the development of cardiac hypertrophy: experimental and clinical studies	Miklós Tóth
Effect of hypertension on microcirculation	Ákos Koller
Therapeutic utilisation of RNA interference to prevent ischemia-reperfusion injury of the kidney	Péter Hamar

Investigating the dual role of TGF-beta in atherosclerosis in a double gene-modified mouse strain	Péter Hamar
Molecular mechanisms of renal allograft rejection	Péter Hamar
The role of zinc in regulation of intracellular $\text{Ca}^{2+}$ and cAMP concentrations. Effects of zinc in the regulation of transepithelial ion transport	Ákos Zsembery
Cardiovascular diseases and renal failure. Prevention of renal hyperparathyroidism and osteodystrophy in early stage renal failure	András Szabó
SPECT analysis of cerebrovascular dysfunction induced by free radicals following cerebral trauma	Kinga Karlinger
Hypertension in pregnancy and molecular mechanisms of toxicosis	Miklós Molnár
The role and mechanisms of epithelial-mesenchymal transition during fibrosis and tumor progression	Attila Sebe
Malnutrition and inflammation in patients with chronic kidney disease	Miklós Zsolt Molnár
Study of the receptor-ligand binding and their interactions by paramagnetic nanoparticles: binding force of epidermal growth factor (EGF) to the receptors and the interactions in the gastrointestinal tissues	Tivadar Zelles
Study of the paramagnetic blood components by magnetooptical spectroscopy	Tivadar Zelles
Molecular mechanisms of progression in renal fibrosis	Gábor Kökény

***Ph. D. students***

Lilla Fang	ft
Andrea Dunai	ft
Tamás Kaucsár	ft
Zalán Németh	pt
Zsuzsanna Rác	ft (a)
Csaba Révész	ft
Imre Csaba Szalay	ft
Petra Szoleczky	ft
Ákos Újszászi	ft
Gergő Vőfély	ft
Ali Vatankhah	it
Ágnes Orsolya Wéber	ft

***Supervisors***

Gábor Kökény
István Mucsi
Péter Hamar
Péter Hamar
Péter Hamar
Péter Hamar
Péter Hamar
Gábor Kökény
Miklós Zsolt Molnár
Attila Sebe
László Rosivall
István Mucsi

***Ph. D. candidates***

Csilla Almási	ft
Tivadar Tamás Dankó	ft
Adrienn Marton	pt
Zsófia Klára Németh	pt
Ghafari Seyed Mohammed	it
Ádám Remport	na
Csaba Mihály Rikker	pt
Károly Varga	na
Zoltán Veresh	ft

***Supervisors***

István Mucsi
Ákos Zsembery
István Mucsi
István Mucsi
László Rosivall
László Rosivall
László Rosivall
László Rosivall
Ákos Koller

***Ph. D. graduates***

Csaba Ambrus	pt
Boglárka Banizs	na
Szilveszter Dolgos	pt
Arnold Sipos	ft
Eszter Panna Vámos	ft
Tamás Gábor Zóber	pt

***Supervisors***

István Mucsi
László Rosivall
László Rosivall
János Peti-Peterdi,
László Rosivall
István Mucsi
László Rosivall

a, absolutorium; ft, full-time; pt, part-time, it, international; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010***

**CSABA AMBRUS (2010)**

**Bone mineral disorders in patients on maintenance hemodialysis and after kidney transplantation**

*Supervisor: István Mucsi*

One of the most common complications of chronic kidney insufficiency is bone mineral disease that can lead to bone loss, bone fracture and contribute to the high cardiovascular morbidity and mortality in this population. Although the pathological pathways of the disease are not completely understood, changes in parathyroid hormone synthesis and the resistance of the bone to the parathyroid hormone are certainly main determinants of the altered bone metabolism in uremia. In this study, I investigated the associations between parathyroid function, vitamin D insufficiency, bone mineral density and bone fracture in patients on maintenance hemodialysis. In addition, I examined the characteristics of mineral bone disease and its associations with clinical parameters in a large prevalent cohort of patients after kidney transplantation. I demonstrated that relative hypoparathyroidism in hemodialysis patients, likely corresponding to adynamic bone, was not associated with reduced bone mineral density. In relative hypoparathyroidism, parathyroid hormone was not associated with bone mineral density and biochemical markers of bone turnover. There was a negative correlation between biochemical markers of turnover and mineral density. I reported the relationship between vitamin D insufficiency and decreased mineral density of cortical bone, an association that is likely mediated by the parathyroid hormone. The results further suggest a possible effect of vitamin D on bone ultrastructure, as assessed by quantitative bone ultrasound. Supporting this theory, vitamin D insufficiency was an independent predictor of bone fracture and this was independent from parathyroid function. Further important predictors of bone fracture were relative hypoparathyroidism and decreased bone mineral density at the radius. The study of renal transplant recipients showed that disturbances of mineral metabolism are common in this population and parameters of bone mineral metabolism are strongly correlated with the graft function. The duration of dialysis prior to transplantation is an important independent predictor of both hyperphosphatemia and hyperparathyroidism. Treatment of bone and mineral disorders is not optimal when compared to the NKF-KDOQI guidelines for non-transplanted patients with kidney disease. The lack of appro-

appropriate guidelines is probably the most important factor resulting in suboptimal management of mineral bone disease in this population.

- Marton A, Ambrus Cs, Mucsi I (2003) *A hiperfoszfátémia jelentősége és kezelése krónikus veseelégtelen betegekben. Hypertonia és Nephrologia* 7: 86–92.
- Ambrus Cs, Almasi Cs, Berta K, Deak Gy, Marton A, Nemeth Zs, Horvath Cs, Lakatos P, Szathmari M, Mucsi I (2011) *Bone mineral density and parathyroid function in patients on maintenance hemodialysis. Int Urol Nephrol* 43: 191–201.
- Ambrus Cs, Molnar MZ, Czira E, Rosivall L, Kiss I, Rempert A, Szathmari M, Mucsi I (2009) *Calcium, phosphate and parathyroid metabolism in kidney transplanted patients. Int Urol Nephrol* 41: 1029–1038.

## BOGLÁRKA BANIZS (2010)

### The role of epithelial primary cilium in the development of hydrocephalus

Supervisor: László Rosivall

Hydrocephalus is a progressive pathological condition characterized by excessive accumulation of cerebrospinal fluid (CSF) in the brain ventricles. The treatment strategies for this condition are fairly limited; a better understanding of the underlying pathomechanism is expected to provide novel therapeutic avenues. Cilia are complex organelles involved in sensory perception and fluid/cell movement. They are constructed through a highly conserved process called intraflagellar transport (IFT). Mutations in IFT genes, such as *Tg737*, result in severe developmental defects and disease including cystic kidney disease, and hydrocephalus. While cilia on the ependymal cells are motile and loss of motility has been associated with hydrocephalus, the function of cilia on the choroid plexus remains enigmatic. Our hypothesis is that cilia function is required for normal regulation of pathways governing CSF production and homeostasis. Here, we explore the connection between cilia dysfunction and the development of hydrocephalus by using the *Tg737<sup>orpk</sup>* mutants. Our analysis indicates that cilia on ependyma of *Tg737<sup>orpk</sup>* mutant mice are severely malformed. These defects lead to disorganized beating and impaired CSF movement. However, the loss of the cilia beat and CSF flow is not the initiating factor, as the pathology is present prior to the development of motile cilia on these cells and CSF flow is not impaired at early stages of the disease. Rather, our results suggest that loss of cilia leads to altered function of the choroid plexus epithelium, as evidenced by elevated intracellular cAMP levels and increased chloride concentration in the CSF. To evaluate hydrocephalus association with defects in ion transport in *Tg737<sup>orpk</sup>* mice, we compared the steady-state pH<sub>i</sub> and Na<sup>+</sup>-dependent transport activities of isolated CP tissues from *Tg737<sup>orpk</sup>* mutant and wild type mice. The data indicate that *Tg737<sup>orpk</sup>* mutant CP epithelium have lower pH<sub>i</sub> and higher Na<sup>+</sup>-dependent HCO<sup>3-</sup> transport activity. In addition, wild-type choroid plexus epithelium could be converted to a mutant phenotype with regard to the activity of Na<sup>+</sup>-dependent HCO<sup>3-</sup> transport by addition of dibutyryl-cAMP and mutant choroid plexus epithelium toward the wild-type phenotype by inhibiting PKA activity with H-89. Together, these data suggest that cilia function is necessary for regulating ion transport and CSF production, as well as for CSF flow through the ventricles and that ciliary dysfunction in *Tg737<sup>orpk</sup>* mutants disrupts a signaling pathway leading to elevated intracellular cAMP levels and aberrant regulation of pH<sub>i</sub> and ion transport activity.



- Banizs B, Pike MM, Millican CL, Ferguson WB, Komlosi P, Sheetz J, Bell PD, Schwiebert EM, Yoder BK (2005) Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development* 132: 5329–5339.
- Banizs B, Komlosi P, Bevensee MO, Schwiebert EM, Bell PD, Yoder BK (2007) Altered pH(i) regulation and Na(+)/HCO3(-) transporter activity in choroid plexus of cilia-defective Tg737(orpk) mutant mouse. *Am J Physiol Cell Physiol* 292: C1409–1416.

## SZILVESZTER DOLGOS (2010)

### Bone mineral density and associated changes after kidney transplantation

Supervisor: László Rosivall

Solid organ transplantation (SOT) is the best treatment of choice for patients with advanced organ failure. While patient and graft survival are obviously still the primary goals, increasing efforts have been directed towards long-term complications such as bone disease and alteration of body composition. The aims of our studies were to measure bone mineral density (BMD) and identify risk factors of low BMD in patients with chronic renal failure at the time of renal transplantation (RTx) (n=133), to quantify the early changes in body composition and identify risk factors of these changes following RTx (n=102), and to describe the magnitude of early post-transplant bone loss with corresponding changes in biochemical bone markers (n=44). In addition, BMD was compared in candidates for lung-, liver-, kidney- or heart transplantation (n=291). BMD and body composition measurements were performed by dualenergy x-ray absorptiometry at the single transplant centre in Norway. In body composition, we found a marked increase in body fat mass with a significant decrease in fat-free mass, without any significant changes in total body weight early after RTx. Low bone mass was present at all measured skeletal sites already at the time of RTx, and significant bone loss was observed as early as 10–12 weeks post-transplant. Serum osteocalcin and telopeptid in combination with parathyroid hormone were predictors of BMD changes in different skeletal parts; therefore seem to be reasonable choices for routine assessment of bone metabolism in RTx patients. Although, osteoporosis was a prevalent finding in all four SOT groups, lung failure patients consistently had the lowest Z-scores, followed by advanced liver-, kidney- and heart disease patients. Our findings raise awareness of bone disease before and early after transplantation and emphasise that screening and, if necessary, treatment should be initiated timely.

- Dolgos Sz, Hartmann A, Br̃nsnes S, Ueland T, Isaksen GA, Godang K, Pfeffer P, Bollerslev J (2008) Determinants of bone mass in endstage renal failure patients at the time of kidney transplantation. *Clin Transpl* 22: 462–468.
- Dolgos Sz, Hartmann A, Jenssen T, Isaksen GA, Pfeffer P, Bollerslev J (2009) Determinants of short-term changes in body composition following renal transplantation. *Scand J Urol Nephrol* 43: 76–83.
- Dolgos Sz, Hartmann A, Br̃nsnes S, Isaksen GA, Godang K, Ueland T, Pfeffer P, Bollerslev J (2009) Early changes in bone mass, biochemical bone markers and fibroblast growth factor 23 after renal transplantation. *Scand J Clin Lab Inv* 69: 161–167.



## ESZTER PANNA VÁMOS (2009)

### Quality of life and the role of psycho-social factors in the treatment of patients with chronic renal failure

*Supervisor: István Mucsi*

The aspect of quality of life in the treatment of chronic illness has received growing emphasis in recent years and guidelines also mention the improvement of quality of life as a primary goal. While the assessment of quality of life in patients with kidney failure is constantly gaining importance internationally, our country has scarce data on the subject. Very few scales have methodologically well translated and validated Hungarian versions. The questionnaire most widely applied in the chronic kidney disease patient population is called the Kidney Disease Quality of Life Questionnaire (KDQOL-SF™).

The first topic I will elaborate on is the validation of the Hungarian version of the KDQOL questionnaire. The Hungarian version of the KDQOL questionnaire proved to be a reliable and valid tool for quality of life measurements in hemodialysed patients with kidney failure in a psychometric study conducted on a large patient group. With the help of the validated tool we examined relationships between various parts of quality of life and socio-demographic and clinical parameters in patients on hemodialysis. In accordance with data from earlier sources, our results showed that females and elderly patients reported worse quality of life along several dimensions compared to their male and younger counterparts. A strong association was found between the presence of comorbidities, serum albumin level and quality of life. Serum hemoglobin (Hb) levels only showed correlation with physical activity levels, while Kt/V, used to measure dialysis dose, did not show correlation with any of the examined quality of life domains.

The disparities experienced in healthcare can be observed in end stage renal disease therapy also, and access to renal transplantation serves as a good model for this phenomenon. The international medical community is showing a growing interest in examining specific steps and potential obstacles on the road towards transplantation. Data is very scarce about the decisions of patients regarding treatment modalities and the factors influencing their decisions. In this study we examined hemodialysed patients' attitude towards renal transplantation and non-medical factors associated with their decision-making. We have found that, in addition to increasing age, negative patient perceptions about transplantation, negative expectations about health outcomes, the presence of fears about the transplant surgery are associated with unwillingness to consider transplantation as a treatment option. This is important as many of these factors are potentially modifiable with effective and systematic patient education and this would improve the chance for patients to make fully informed treatment decisions about renal replacement therapy.

- Vámos EP, Csepányi G, Zambo M, Molnar MZ, Rethelyi J, Kovacs A, Marton A, Nemeth Z, Novak M, Mucsi I (2009) *Socio-demographic factors and patient perceptions are associated with attitudes to kidney transplantation among hemodialysis patients. Nephrol Dial Transpl* 24: 653–660.
- Barotfi Sz, Molnar MZ, Almasi Cs, Kovacs AZ, Remport A, Szeifert L, Szentkiralyi A, Vámos E, Zoller R, Eremenco S, Novak M, Mucsi I (2006) *Validation of the Kidney Disease Quality of Life-Short Form (KDQOL-SFTM) questionnaire in kidney transplant patients. J Psychosom Res* 60: 495–504.

- Novak M, Mah K, Molnar MZ, Ambrus C, Csepanyi G, Kovacs A, Vamos E, Zambo M, Zoller R, Mucsi I, Devins GM (2005) Factor structure and reliability of the Hungarian version of the Illness Intrusiveness Scale Invariance across North American and Hungarian dialysis patients. *J Psychosom Res* 58: 103–110.

## TAMÁS GÁBOR ZÓBER (2010)

### Positron Emission Tomography Studies on angiotensin II subtype 1 receptor (AT<sub>1</sub>R)

*Supervisors: László Rosivall*

Biodistribution and pharmacology studies on mice, and PET studies on mice, dogs and baboon showed that [<sup>11</sup>C]KR31173 is suitable AT<sub>1</sub>R specific radioligand for PET. High specific binding was confirmed in multiple species, especially in their kidneys and adrenals.

[<sup>11</sup>C]KR31173 showed higher activity and specificity in baboon kidney than previously developed radioligand [<sup>11</sup>C]L-159,884. These findings suggest that [<sup>11</sup>C]KR31173 is suited for future PET imaging in primates and possibly in human studies. We revealed for the first time, that chronic ACEI treatment increases AT<sub>1</sub>R binding in vivo in dog renal cortex under conditions in which the level of circulating angiotensin II (Ang II) is unchanged. Coupled with *ex vivo* measurements, our study indicates that increased renal AT<sub>1</sub>R binding *in vivo* correlates with an upregulation of AT<sub>1</sub>R in the glomeruli. We showed that in experimental 2K, 1C renovascular hypertension the AT<sub>1</sub>R density is increased in the ischemic kidney. The increase in AT<sub>1</sub>R Bmax positively correlated with elevated arterial blood pressure and negatively correlated with reduced perfusion of the ischemic kidney. Following confirmation studies in humans, the PET AT<sub>1</sub>R imaging might become an attractive and valuable diagnostic tool in the diagnosis of RVH.

- Zober TG, Fabucci ME, Zheng W, Brown PR, Seckin E, Mathews WB, Sandberg K, Szabo Z (2008) Chronic ACE inhibitor treatment increases angiotensin type 1 receptor binding in vivo in the dog kidney. *Eur J Nucl Med Mol Imaging* 35: 1109–1116.
- Zober TG, Mathews WB, Seckin E, Yoo SE, Hilton J, Xia J, Sandberg K, Ravert HT, Dannals RF, Szabo Z (2006) PET Imaging of the AT<sub>1</sub> receptor with [<sup>11</sup>C]KR31173. *Nucl Med Biol* 33: 5–13.
- Mathews WB, Yoo SE, Lee SH, Scheffel U, Rauseo PA, Zober TG, Gocco G, Sandberg K, Ravert HT, Dannals RF, Szabo Z (2004) A novel radioligand for imaging the AT<sub>1</sub> angiotensin receptor with PET. *Nucl Med Biol* 31: 571–574.

## ARNOLD SIPOS (2010)

### Advances in renal (patho)physiology using multiphoton microscopy

*Supervisors: János Peti-Peterdi, László Rosivall*

Using two-photon microscopy of the *in vitro* microperfused juxtaglomerular apparatus and intact Munich-Wistar rat kidney, we aimed to determine if MD cells can detect variations in tubular flow per se. Increasing cortical thick ascending limb flow rate from 2 to 20 nl/min (constant 10 mM [NaCl]) produced a significant elevation in  $[Ca^{2+}]_{ic}$  in afferent arteriole (AA) smooth muscle cells (Fluo-4 F/Fo:  $2.08 \pm 0.21$ ). Application of laminar flow directly to the MD apical surface produced the same results even using 10mM salt solutions. Acetylated  $\alpha$ -tubulin immunohistochemistry identified single primary cilia in each MD cells. Under no flow conditions, bending MD cilia directly with a micropipette rapidly caused significant elevations in AA smooth muscle  $[Ca^{2+}]_{ic}$  (Fluo-4 F/Fo:  $1.60 \pm 0.12$ ). Scavenging the superoxide production did not alter the flow-induced tubuloglomerular feedback (TGF), however purinergic blockade (suramin) significantly reduced the response. TGF activation constricted the preglomerular sphincter lumen by  $69.3 \pm 5.9\%$ , as opposed to modest changes in proximal AA segments. Extraglomerular mesangial cells increase  $[Ca^{2+}]_{ic}$ , and contract in concert with the entire intraglomerular mesangium to generate sphincter activity. Suramin, but not the adenosine A1 receptor inhibitor DCPCX, completely abolished the TGF-induced sphincter activation. In conclusion, MD cells are equipped with a tubular flow sensing mechanism which may contribute to MD cell function and TGF. Preglomerular sphincter is the principal effector site of TGF that is controlled by ATP and P2 purinergic receptors. ATP and metabolites are present in the renal tubular and interstitial fluid and are involved in the regulation of salt and water reabsorption. Several connexin (Cx) isoforms form ATP-permeable hemichannels. We localized Cx30 in the apical membrane of renal epithelial cells suggesting a possible release function. The aim of these studies was to test if Cx30 hemichannels release ATP and are involved in the regulation of tubular electrolyte and water transport. WT and Cx30 KO mice cortical collecting ducts were dissected, partially slit-open and microperfused *in vitro*. Using the biosensor technique we showed that ATP release, triggered by an increase in tubular flow, is significantly higher from intercalated cells than from principal cells and were abolished by suramin or in KO tissue. Next, mice were surgically instrumented for clearance and pressure natriuresis measurements. After stepwise increases in BP, urine flow increased 4.2-fold in WT vs. 2.6-fold in KO animals. Fractional urinary  $Na^+$  excretion increased 5.1-fold in WT vs. 2.8-fold in KO animals. This is the first study suggesting that distal tubular Cx30 hemichannels release ATP which inhibits renal salt and water reabsorption causing pressure natriuresis.

- Sipos A, Vargás SL, Toma I, Hanner F, Willecke K, Peti-Peterdi J (2009) Connexin 30 deficiency impairs renal tubular ATP release and pressure natriuresis. *J Am Soc Nephrol* 20: 1724–1732.
- Sipos A, Toma I, Kang JJ, Rosivall L, Peti-Peterdi J (2007) Advances in renal (patho) physiology using multiphoton microscopy. *Kidney Int* 72: 1188–1191.
- Kang JJ, Toma I, Sipos A, McCulloch F, Peti-Peterdi J (2006) Imaging the renin-angiotensin system: an important target of anti-hypertensive therapy. *Adv Drug Deliv Rev* 58: 824–833.

**PROGRAM 1/5.****CLINICAL AND EXPERIMENTAL CARDIOLOGY AND ATHEROSCLEROSIS*****Coordinator:*****Zoltán PROHÁSZKA M.D., Ph.D., D.Sc.**

3rd Department of Internal Medicine

4 Kútvölgyi st, Budapest H-1125

Tel./fax: +36 1 325 1379

E-mail: [prohoz@kut.sote.hu](mailto:prohoz@kut.sote.hu)**Program overview**

The aim of the Program is to carry out experimental and clinical studies on the pathological mechanisms responsible for the cardiac and vascular disorders leading the mortality statistics. Metabolic and immune disturbances, as diabetes and systemic autoimmune diseases are important risk factors in this process—therefore they are used as models. The different approaches are given by the sub-programs.

***Titles of research projects***

Effect of nutrients on atherogenesis and on the formation of the “atherogenic” lipoproteins  
 The role of “new” risk factors and metabolic syndrome in the pathogenesis of the atherosclerosis  
 Lipidology (pathobiochemistry, pathophysiology and clinical investigation of lipid and lipoprotein disorders)  
 Effect of endogenous cardiovascular mediators and drugs on electrophysiological properties of isolated heart preparation  
 Thrombotic aspects of coronary heart disease. Prothrombotic states and their treatment in the clinical practice. The role of endothelial receptors in the atherothrombosis  
 Perilous thrombotic complications in ischaemic heart disease  
 Regulation of circulation in metabolic disease  
 Cellular and molecular genomic alterations of the endothelial metabolism in ischaemic heart disease  
 Endocardial and body surface mapping and their clinical application  
 Electrocardiological diagnostics of ischaemic heart disease in obesity  
 Studies on the role of inflammation in the pathogenesis of cardiovascular and rare vascular diseases  
 Studies on the role of environmental and genetic factors in the atherogenicity of lipoproteins  
 Studies on the role of metabolic and immunological alterations in accelerated atherosclerosis  
 Investigations on genetic, biomolecular and clinical interactions in multifactorial diseases

***Supervisors***

Lajos Szollár  
 Lajos Szollár  
 Lajos Szollár  
 Valéria Kecskeméti  
 Róbert Kiss  
 Róbert Kiss  
 Ákos Koller  
 István Préda  
 István Préda  
 Mihály Medvegy  
 Zoltán Prohászka  
 Zoltán Prohászka  
 Zoltán Prohászka  
 Zoltán Prohászka

Pathomechanism, clinical and therapeutic aspects of different  
types of angioedema  
Endothelial cell physiology and pathophysiology

Henriette Farkas

László Cervenák

### ***Ph.D. students***

Emese Ábrám	pt
Anna Buday	pt
Dorottya Csuka	ft
Petra Gulácsi-Bárdos	ft (a)
Károly Péter Jani	ft
Sarolta Leé	ft
Noori Ebrahim	pt
Zsófia Horváth	pt
Nóra Kiss	pt

### ***Supervisors***

András Végh  
Ákos Koller  
Zoltán Prohászka  
István Préda  
László Cervenák  
Róbert Gábor Kiss  
Róbert Gábor Kiss  
Róbert Gábor Kiss  
István Préda

### ***Ph.D. candidates***

Bernát János Béres	ft
Béla Debreczeni	pt
Krisztina Madách	na
Endre Szűcs	na
Katarina Vargova	ft

### ***Supervisors***

Róbert Gábor Kiss  
Ákos Koller  
Zoltán Prohászka  
Mihály Medvegy  
István Préda

### ***Ph.D. graduates***

Gábor Duray	pt
Balázs Imre Hauser	na
Anita Rác	ft
Gabriella Szabó	na

### ***Supervisors***

István Préda  
Lajos Szollár  
Ákos Koller  
Róbert Gábor Kiss

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

### **GÁBOR DURAY (2009)**

#### **Opportunities and problems of device therapy in the treatment of ventricular arrhythmias and heart failure**

*Supervisor: István Préda*

Implantable cardioverter-defibrillator (ICD) therapy improves survival in patients with structural heart disease and at high risk for life threatening ventricular arrhythmias. Whether elderly patients benefit from device therapy in a similar way as younger patients is largely unknown. We showed in a retrospectively analysis of 375 consecutive

ICD recipients with structural heart disease no significant difference in overall mortality in patients with an age <70 years or ≥70 years. There was no difference in time from device implantation to first adequate ICD therapy and time from first appropriate ICD therapy to death among the two groups. Device associated complications were comparable in both groups. Cardiac resynchronization therapy (CRT) is indicated in patients with heart failure and bundle branch block. It is less clear whether this includes patients with pre-existing right ventricular pacemaker/defibrillator systems, particularly with respect to implantation success and clinical benefit. There is no detailed data concerning anatomical specifications of coronary side branch (CS-SB) anatomy and its relationship to left ventricular (LV) lead implantation in patients undergoing CRT device implantation. Our prospectively designed observational study demonstrates that procedural aspects, implantation success, and clinical response to CRT were comparable for patients undergoing de-novo versus upgrade procedures. Our study is the first that demonstrates that in 70% of the patients with heart failure undergoing CRT device implantation at least 3 potential CS-SB are available for left ventricular lead implantation. The implantation success in the 1st choice CS-SB was 71% without significant differences for any particular side branch region. The main limiting factors of implantation a lead in the selected side branch were: unsuccessful positioning of the lead, lead instability in the selected position, and high stimulation threshold. Lead implantation in an alternative CS-SB results in significantly longer implantation and fluoroscopy times. During the 6-month follow-up, we did not observe a significant difference in the response rate of the patients according to the selected vein regions. We described a possible new mechanism for ICD lead failure: the disintegration of the lead at the level of the tricuspid valve, possibly caused by the mechanical effect of the leaflet motion.

- Duray GZ, Hohnloser SH, Israel CW (2008) *Coronary sinus side branches for cardiac resynchronization therapy: prospective evaluation of availability, implant success, and procedural determinants. J Cardiovasc Electrophysiol* 19: 489–494.
- Duray GZ, Israel CW, Pajitnev D, Hohnloser SH (2008) *Upgrading to biventricular pacing/defibrillation systems in right ventricular paced congestive heart failure patients: prospective assessment of procedural parameters and response rate. Europace* 10: 48–52.
- Duray GZ, Richter S, Manegold J, Israel CW, Gronefeld G, Hohnloser SH (2005) *Efficacy and safety of ICD therapy in a population of elderly patients treated with optimal background medication. J Interv Card Electrophysiol* 14: 169–173.

## BALÁZS IMRE HAUSER (2009)

### Pathomechanisms-based experimental therapies in a porcine model of resuscitated hyperdynamic endotoxemia

Supervisor: Lajos Szollár

In our experiments several factors in the pathomechanisms of septic shock-induced multiple organ failure (MOF), like oxidative stress, vascular hyporeactivity (mediated by activation of KATP channels or vasopressin deficiency), activation of NF-κB or PARP-1 and failure of cellular energy metabolism have been investigated in a clinically relevant porcine model of long-term resuscitated hyperdynamic endotoxemia using a delayed post-treatment approach. In anesthetized, mechanically ventilated and instrumented pigs 24 hrs of endotoxemia with fluid resuscitation (hydroxyethyl starch) resulted in hyperdynamic cir-



culatation and impaired variables of gas exchange, metabolism, NO formation and oxidative stress. The PARP-inhibitor PJ34 improved cardiac output (CO) and attenuated the progressive deterioration of intestinal energy balance. The antioxidant N-acetylcysteine had no major beneficial effects on global or regional hemodynamics, gas exchange, or metabolism and did not improve oxidative damage. The KATP channel-inhibitor HMR 1402 only transiently increased mean arterial pressure (MAP), decreased CO and increased lactate production and lactate/pyruvate ratios suggesting a disturbed cytosolic redox potential. The vasopressin analog terlipressin increased MAP affiliated with decreased CO, and O<sub>2</sub> consumption, restored the hepatic arterial buffer response, and maintained hepatosplanchnic O<sub>2</sub> exchange. It did not deteriorate any variables of hepatosplanchnic metabolism or organ function, and even attenuated hepatosplanchnic acidosis but it was associated with hyperlactatemia originating from extrasplanchnic organs. Ethyl pyruvate administration was associated with stabilization of systemic hemodynamics, improved pulmonary gas exchange, amelioration of systemic and regional venous acidosis, and decreased evidence of oxidative stress and NO formation. The NF-κB inhibitor 15-deoxy-Δ<sup>12,14</sup>-prostaglandin-J2 stabilized MAP, most likely due to improved left ventricular function, but did not affect the other measured parameters of metabolism and organ function. In conclusion, despite many promising results no any approach has reversed most of the deteriorated elements of sepsis-induced MOF within the experimental period, but severe negative side effects have been revealed as well. Further intensive research is needed in clinically relevant long-term models with possible new or combined approaches.

- Hauser B\*, Kick J\*, Asfar P\* (equally contributed), Ehrmann U, Albicini M, Vogt J, Wachter U, Brückner UB, Fink MP, Radermacher P, Bracht H (2005) Ethyl pyruvate improves systemic and hepatosplanchnic hemodynamics and prevents lipid peroxidation in a porcine model of resuscitated hyperdynamic endotoxemia. *Crit Care Med* 33: 2034–2042.
- Asfar P\*, Hauser B\* (equally contributed), Iványi Z, Ehrmann U, Kick J, Albicini M, Vogt J, Wachter U, Brückner UB, Radermacher P, Bracht H (2005) Low-dose terlipressin during long-term hyperdynamic porcine endotoxemia: effects on splanchnic perfusion, oxygen exchange and metabolism. *Crit Care Med* 33: 373–380.
- Vassilev D\*, Hauser B\* (equally contributed), Bracht H, Iványi Z, Schoaff M, Asfar P, Vogt J, Wachter U, Schelzig H, Georgieff M, Brücker UB, Radermacher P, Fröba G (2004) Systemic, pulmonary and hepatosplanchnic effects of N-acetylcysteine during long-term porcine endotoxaemia. *Crit Care Med* 32: 525–532 (Erratum in *Crit Care Med* 32: 1094 [2004]).

## ANITA RÁCZ (2010)

### Mediation of flow dependent responses of venules and its impairment in hyperhomocysteinemia. Role of COX-1 and COX-2 derived thromboxane A2

Supervisor: Ákos Koller

There are several studies regarding the function of large veins, whereas we do not have many knowledge about functions of small veins or venules. Nevertheless, in isolated pre-constricted venules flow-dependent mechanism was showed to be present, the mediation of which is not fully clarified. In addition, precontraction could largely influence the responses of venules. However, flow-dependent responses of venules without precon-



striction and the underlying mechanisms have not been studied yet. We hypothesized that—in addition to nitric oxide (NO) and dilator prostaglandins (PGI<sub>2</sub>/PGE<sub>2</sub>)—thromboxane A<sub>2</sub> (TxA<sub>2</sub>) contributes to the mediation of flow-induced responses of venules. This mechanism can be injured in different pathological conditions such as in venular occlusive diseases and in venous thrombosis. After clinical evidences, in these diseases plasma level of homocysteine (Hcy) is elevated, suggesting that hyperhomocysteinemia (HHcy) has pathological effects on venular functions, the mechanisms of which have not yet been studied. We hypothesized that HHcy leads to the impairment of venular functions due to alterations in the arachidonic acid pathway and due to an enhanced production of reactive oxygen species (ROS). Thus we aimed to study the flow-induced diameter responses of venules isolated from normal and HHcy rats, because these responses indicate the function of endothelium known to be importantly involved in the regulation of venular tone. We have found that increases in intraluminal flow elicited dilations in control, whereas constrictions in HHcy venules. In controls, flow-induced responses were mediated by nitric oxide (NO), dilator and constrictor prostaglandins (PGs), with an overall effect of dilation. Dilator PGs are produced by cyclooxygenase-1 (COX-1), whereas constrictor PGs are produced by cyclooxygenase-2 (COX-2) pathway. In HHcy the flow-induced altered venular response is the result of an altered balance of dilator and constrictor factors. The mediative role of NO and dilator PGs is reduced, whereas the role of constrictor PGs became enhanced. In HHcy, dilator PGs are produced by the COX-1, whereas constrictors both by COX-1 and COX-2 pathway. Moreover, enhanced level of ROS also contributes to the altered vasomotor functions of venules. Because venules determine postcapillary resistance—thus capillary pressure and venous return to the heart—the flow-induced diameter responses of venules may have an important role in the regulation of tissue blood supply, especially in increased flow conditions. We propose that in HHcy, the altered flow-induced venular vasomotor function contributes to the pathological changes of venous circulation and the development of thrombosis and occlusive venular diseases in HHcy.

- Racz A, Veres N, Erdei N, Bagi Z, Koller A (2009) Thromboxane A<sub>2</sub> contributes to the mediation of flow-induced responses of skeletal muscle venules: role of cyclooxygenases 1 and 2. *J Vasc Res* 46: 397–405.
- Racz A, Veres Z, Lotz G, Bagi Z, Koller A (2010) Cyclooxygenase-2 derived thromboxane A<sub>2</sub> and reactive oxygen species mediate flow dependent constrictions of venules in hyperhomocysteinemia. *Atherosclerosis* 208: 43–49.

**GABRIELLA SZABÓ (2009)****Investigations of effects of orally active peptide and glycoside type antithrombotics***Supervisor: Gábor Róbert Szabó*

The thrombembolic diseases are the largest cause of mortality in the Western world. Currently available therapies to reduce the risk of thromboembolic events include vitamin K antagonists (e. g. Warfarin) and low molecular weight heparins (LMWHs), but their use has many limitations. Warfarin is limited by extensive drug and food interactions, LMWHs may provoke bleeding, and because of parenteral application unsuitable for chronic treatment. Our goal was to develop a well-tolerated, novel oral antithrombotic agent with a wide therapeutic window for long term treatment. We examined peptide and glycoside type compounds in rats and in rabbits. The kinetics of the anticoagulant and antiplatelet effects were recorded by measuring clotting parameters (whole blood clotting time; thrombin time; activated partial thromboplastin time, Haptest, sensitive thrombin time, diluted prothrombin time), and platelet aggregation *ex vivo*. The antithrombotic activity of the compounds was studied in various models of experimental thrombosis. The peptides caused dose dependent therapeutic anticoagulant and antiplatelet effect after *per os* administration. The results demonstrated that each peptide induced significant diminution in thrombus weight in a quantitative venous thrombosis model in rats as well as in an extracorporeal arterio-venous shunt model in rabbits and prevented the occlusion of the vessel in arterial thrombosis in rats. The antithrombotic effect of peptides indicated a good correlation with the thrombin time and thrombin induced platelet aggregation. Thioglycosides were orally active antithrombotics possessing only weak anticoagulant effect. The tissue factor pathway inhibitor (TFPI) is a potent inhibitor of the extrinsic coagulation system. The glycosides significantly increased the TFPI level in the plasma, both in rats and rabbits. This effect may contribute to their antithrombotic effect. The pharmacological bioavailability of test materials were investigated in fasted and non-fasted animals. According to these data, the compounds indicated a “structure-dependent species specificity”. From the peptide and glycoside type test materials, the GYKI-66131 (peptide) as well as GYKI-39521-39541 (glycosides) were selected as potent drug candidates for prevention and treatment of thrombembolic disorders. All selected compound resulted in significant protective effect in thrombosis models without any significant side effect.

- Szabó G, Bozó É, Barabás É, Kedves R, Csomor K, Kuszman J (1999) Thioglycoside antithrombotic agents. *Drugs of the future* 24: 1241–1248.
- Szabó G, Barabás É, Kedves R, Csomor K, Kuszman J (2002) Effect of some new thioglycosides on endotoxin-induced disseminated intravascular coagulation in rabbits. *Thromb Res* 107: 357–363.
- Szabó G, Barabás É, Bajusz S (2003) The oral bioavailability and food interaction of thrombin inhibitors. Pp 149–152 in: Coccheri S, Gensini G, Palareti G, Prisco D (eds) *Reports from the 17th International Congress on Thrombosis*.

---

# SCHOOL OF PH.D. STUDIES

---

## 2. CLINICAL MEDICINE

---

### ***Chairman:***

**Zsolt TULASSAY M.D.,**

**member of the Hungarian Academy of Sciences**

2nd Department of Internal Medicine

46 Szentkirályi st, Budapest H-1088

Tel.: +36 1 266 0926

Fax: +36 1 266 0816

E-mail: [tulassay@bel2.sote.hu](mailto:tulassay@bel2.sote.hu)

### **General overview**

The Clinical Medicine Doctoral School has the largest number of programmes among the eight Doctoral Schools of Semmelweis University. The training and research programs offer research projects in a large number of subdisciplines of clinical and applied medicine.

### ***PROGRAM 2/1.***

---

## **OXIDATIVE STRESS AND IMMUNOLOGICAL REACTION IN LIVER DISEASES**

### ***Coordinator***

**János FEHÉR M.D., Ph.D., D.Sc. †**

2nd Department of Internal Medicine

46 Szentkirályi st, Budapest H-1088

Tel./fax: +36 1 317 4548

E-mail: [feher@bel2.sote.hu](mailto:feher@bel2.sote.hu)

### **Program overview**

Evidence accumulates that natural (vitamins, flavonoid type molecules and synthetic butylated hydroxytoluene, dihydro-quinolin-type molecules) antioxidants exert a preventive effect on local oxidative damage in several models *in vitro* and *in vivo*. Therefore, the aim of our Program is to investigate the role of oxidative stress and the shift in pro/antioxidant balance in the pathogenesis of several gastrointestinal and immunological diseases, metabolic disorders and drug side effects by direct and indirect methods. The ongoing experiments focus on steatosis, hepatitis, cirrhosis, hepatocellular carcinoma, gallstone formation, cholestasis, inflammatory bowel diseases, colon neoplasm and metabolic disorders (carbohydrate, lipid) as well as amiodarone toxicity.

***Titles of research projects***

Study of redox homeostasis  
 Neurochemical examination of neural elements innervating the gastrointestinal visceral organs  
 Results of onco-surgical treatment of liver tumors from the aspect of immune system functioning. Prognostics, long term follow up, quality of life  
 Pathogenesis and therapy of non-alcoholic liver disease  
 Food intake, lifestyle and the liver diseases  
 Alcoholic liver disease  
 Up to date treatment in hepatobiliary diseases. The effect of ursodeoxycholic acid and interferon on viral hepatitis and the oxidative stress status  
 Up to date treatment in hepatobiliary diseases. The effect of ursodeoxycholic acid and interferon on viral hepatitis and the oxidative stress status  
 Food intake, lifestyle and the liver diseases  
 The link among carbohydrate and lipid metabolism and free radical reactions and their role in the development of arteriosclerosis  
 Effect of metal complexes on the liver pathobiochemistry

***Supervisors***

Anna Blázovics  
 Erzsébet Fehér  
 Ferenc Jakab  
 János Fehér †  
 János Fehér †  
 János Fehér †  
 János Fehér †  
 Gabriella Lengyel  
 Gabriella Lengyel  
 Anikó Somogyi  
 Klára Szentmihályi

***Ph.D. students***

Rózsa Fehér pt (a)  
 Viktor Zsolt Hegedüs ft  
 Zoltán Mihály pt  
 Barbara Szémán ft  
 Alexandra Wimmer pt

***Supervisors***

János Fehér †  
 Anna Blázovics  
 Anna Blázovics  
 Anikó Somogyi  
 János Fehér †

***Ph.D. candidates***

Géza Nagy ft  
 Éva Bernadett Pongor pt  
 Tímea Varga ft  
 Ildikó Vastagh na

***Supervisors***

Anikó Somogyi  
 Erzsébet Fehér  
 Anikó Somogyi  
 Anikó Somogyi

***Ph.D. graduates***

Márta Kovács pt  
 László Váli ft

***Supervisors***

János Fehér †  
 Anna Blázovics

a, absolutorium; pt, part-time; ft, full-time; na, not affiliated

## *Abstracts of Ph.D. theses successfully defended in 2009*

**MÁRTA KOVÁCS (2009)**

### **The role of capsule endoscopy in obscure gastrointestinal bleeding and portal hypertension**

*Supervisor: János Fehér†*

**Background:** “Wireless” capsule endoscopy (CE) introduced in 2000 revolutionized the diagnostics of the small bowel. We have been carrying out small bowel CE examinations with various indications since 2003. Obscure gastrointestinal bleeding (OGIB) represent 5% of all gastrointestinal bleedings that cannot be established applying traditional endoscopic methods, which represent the most frequent indications of the examinations. The endoscopic appearance in portal hypertension (PH) is well described in the stomach and the colon, but there is a limited number of data available on small bowel changes.

**Aims:** The present retrospective study was aimed to analyze the diagnostic yield, positive and negative predictive values and clinical impact of CE in patients with OGIB. We analyse the effectiveness of surgical therapy based on CE. We evaluate the diagnostic yield and describe the small bowel findings with CE in PH with gastrointestinal bleeding of unknown origin. **Results:** A definitive small bowel bleeding source was detected in 77.4% of the cases studied by CE. The positive and negative predictive values of capsule endoscopy studies were 93.8% and 84.6% respectively. In cases with definitive bleeding sources 71% of patients received therapy in accordance with CE findings. The assessment of the pathological findings of resections justified the definitive bleeding source in 94% of patients who underwent surgery. Lesions originated to PH (angiodysplasias, portal hypertensive enteropathy, varices) were found in all cirrhotic patients; most frequently multiple angiodysplasias (63.6%), while in the control group multiple angiodysplasias were a seldom finding (18.2%). **Conclusions:** Effective therapy may be introduced in accordance with the majority of positive CE results. In case solitary angiodysplasia, tumour, stenosis and actively bleeding diagnosed by CE, the surgical therapy has a high impact. In the course of our examinations the estimated localization of the bleeding source always provided ample support to perform resection. CE is an effective diagnostic method with high diagnostic yield in PH. Multiple angiodysplasias are the most probable findings as the source of small bowel bleeding in these patients.

- Kovács M, Németh A, Pák P, Uhlyarik A, Pák G, Rácz I (2006) *Evaluation of diagnostic yield and clinical impact of capsule endoscopy in patients with obscure bleeding. Orvosi Hetilap* 38: 1827–1833.
- Kovács M, Pák P, Pák G, Fehér J, Rácz I (2009) *Small bowel alterations in portal hypertension: endoscopic study. Hepatogastroenterology* 56: 1069–1073.
- Kovács M, Pák P, Pák G, Oláh A, Rácz I (2008) *The impact of capsule endoscopy on surgical results in patients with obscure gastrointestinal bleeding. Z Gastroenterol* 47: 424–428.

## LÁSZLÓ VÁLI (2009)

### Study of redox homeostasis of different pathologic states of the liver

Supervisor: Anna Blázovics

Changes of the redox homeostasis is of great importance in different pathologic states of the liver, for example during ischaemia-reperfusion or non alcoholic fatty liver disease. One of our aims was to characterise the connections between free radical reactions and changes of element content in *in vivo* animal models. Prevention of these changes were carried out with different antioxidant substances. We examined the liver protecting effects of natural antioxidant table beet (*Beta vulgaris* var. *rubra*), because of its beneficial physiological properties. Metadoxine served as the model of antioxidant medical pretreatment, because this drug is used nowadays in the therapy of hepatitis with different origins. In our study both table beet and metadoxine pretreatment exerted positive effect on redox homeostasis of the liver during hepatic ischaemia-reperfusion. In these experiments beside the lowered free radical levels, pretreatments increased the content of elements, which are essential for the function of antioxidant enzymes of liver. With morphological and molecular biological examination of the liver induction of apoptotic and necrotic reactions were detected. Free radical damage of the duodenum was also determined during operations of the liver. In human studies we measured the oxidative damage of the body due to the presence of primary and metastatic hepatic carcinomas. Besides changes in the redox parameters element content of erythrocytes is different from values of healthy volunteers. Lowered ATP concentration of erythrocytes plays a key role in generation of metastases.

Our results reveal a possible therapeutic strategy for preventing ischaemic-reperfusion damage with antioxidant agents during operations of the liver. However, determination of the optimal duration and dosage of the pretreatment requires careful consideration.

- Váli L, Stefanovits-Bányai É, Szentmihályi K, Fébel H, Sárdi É, Lugasi A, Kocsis I, Blázovics A (2007) Liver-protecting effects of table beet (*Beta vulgaris* var. *rubra*) during ischemia-reperfusion. *Nutrition* 23: 172–178.
- Váli L, Stefanovits-Bányai E, Szentmihályi K, Drahos A, Sárdy M, Fébel H, Fehér E, Bokori E, Kocsis I, Blázovics A (2008) Alterations in the content of metal elements and fatty acids in hepatic ischaemia-reperfusion: induction of apoptotic and necrotic cell death. *Digest Dis Sci* 53: 1325–1333.
- Váli L, Hahn O, Kupcsulik P, Drahos A, Sárváry E, Szentmihályi K, Pallai Z, Kurucz T, Sípó P, Blázovics A (2008) Oxidative stress with altered element content and decreased ATP level of erythrocytes in hepatocellular carcinoma and colorectal liver metastases. *Eur J Gastroent Hepat* 20: 393–398.



**PROGRAM 2/2.****FETAL AND NEONATAL MEDICINE*****Coordinator:*****Zoltán PAPP M.D., Ph.D., D.Sc.**

1st Department of Obstetrics and Gynecology

27 Baross st, Budapest H-1088

Tel.: +36 1 266 0473

E-mail: [pz@noi1.sote.hu](mailto:pz@noi1.sote.hu)**Program overview**

The Ph.D. Program is designed for medical doctors who wish to specialize in prenatal genetics and fetal medicine. Our aims are: to provide medical and science based students with comprehensive knowledge in the field of genetic and fetal medicine, to provide suitable environment for clinical or laboratory based research project, to enable students for the use of laboratory techniques such as PCR, F-PCR, RFLP, blotting techniques, etc. to train students in modern prenatal diagnostic methods, like color-Doppler ultrasound, intrauterine echocardiography, etc.

***Titles of research projects***

Fetal and neonatal developmental disorders of the heart  
Clinical and embryological aspects of assisted reproduction

***Supervisors***

Júlia Hajdú  
János Urbancsek

***Ph.D. students***

Ágnes Flóra Balló

pt

Zsófia Róna

ft

***Supervisors***

Zoltán Papp

Zoltán Papp

***Ph.D. candidates***

Ákos Murber

na

Tibor Várkonyi

ft

***Supervisors***

Zoltán Papp

Bálint Nagy

pt, part-time; ft, full-time, na, not affiliated

---

**PROGRAM 2/3.**

---

**PREVENTION OF CHRONIC DISEASES IN CHILDHOOD*****Coordinator:*****Tivadar TULASSAY M.D.,****member of the Hungarian Academy of Sciences**

1st Department of Paediatrics

53 Bókay J. st, Budapest H-1083

Tel.: +36 1 314 2858; fax: +36 1 313 8212

E-mail: [tulassay.tivadar@med.semmelweis-univ.hu](mailto:tulassay.tivadar@med.semmelweis-univ.hu)**Program overview**

The present research and doctoral program consists of various topics of paediatrics, prevention creates the common basis of the program. No reliable method is available to determine the beginning of a chronic disease. In Hungary the causes of the majority of the leading fatal diseases are to be found already in childhood, although without clinical signs. Their progression gradually leads to a permanent manifest disease with expressed clinical symptoms. A fundamental precondition of preventing the development of chronic diseases is to detect the possibly existing risk factors. Getting to know the cellular and subcellular mechanisms promoting the development of a disease may be of help not only in the prevention, but also in the successful therapy and in eliminating the complications, as well. The doctoral Program is dealing with research fields having outstanding significance in adult cardiovascular morbidity and mortality and where the identification and elimination of risk factors could prevent long-lasting impairments. In the pathogenesis of cardiovascular diseases sodium homeostasis and its cellular regulation are of utmost importance. Within the doctoral Program this question is dealt with in 3 sub-programs. The research work is aimed to study the altered activity, structural changes and genetic regulation of Na/K/ATP-ase enzyme in diseases accompanied by irregular sodium homeostasis. In insulin dependent diabetes mellitus the prevention of late complications: vascular alterations and hypertonia are of cardinal question. Two sub-programs are devoted to study genetic, metabolic and haemodynamic risk factors in animal experiments and clinical physiological examinations. The sub-program dealing with the correlation between chronic renal diseases, cardiovascular alterations as well as uraemia and bone metabolism focuses on the regulatory role of the kidney as regards vascular alterations and bone structure deformities. The pre-term birth and treatments applied involve several late complications and hazardous situations. The harmful side effects of oxygenization can be due to the multiplication of oxidative radicals. This hypothesis has been considered as a possible pathogenetic factor in several other diseases, too. The study of this theory in pre-term babies may provide explanations similarly valid in other systems, in a wider sense, as well. The investigation of postnatal body composition, the hydrodynamic changes accompanied by electrolyte movement may reveal several fundamental regularities. This sub-program offers a completely new approach by using multifrequency bioelectric impedance analysis. The additional four sub-programs seem to be heterogeneous, however, they have one common aspect, namely, all of them are aimed to detect risk factors thereby improving life's quality. The investigation of the connatal urinary tract malformations in neonates and pre-term babies, in addition to the discovery of basic facts, has directly practical significance: to

determine the optimal time and technique of surgical intervention. The number of infantile atopic airway diseases shows an increase proportional with the environmental pollution pointing out the importance of getting to know the natural course and pathomechanism of the disease. The questions of paediatric gastroenterology deal with the immunologic correlation existing between food allergens and intestinal diseases. The deeper knowledge of the pathophysiology of childhood epilepsy syndromes and primary headache disorders may result in a better life quality in adulthood.

### ***Titles of research projects***

### ***Supervisors***

Pediatric gastroenterology	András Arató
Study of inflammatory mediators in pediatric migraine and epilepsy caused by limbic encephalitis	Viktor Farkas
The role of haemodynamic and genetic factors in the pathomechanism of acute and chronic allograft nephropathy	Andrea Fekete
Effect of anesthesia and operative intervention to the immune system	István Kocsis
Role of seasonal and circadian rhythmicity in the development of chronic complication and progression of diabetes mellitus	Anna Körner
Genetic, haemodynamic and metabolic risk factors and molecular pathogenesis of the development of diabetic nephropathy	László Madácsy
Comparative study on the asthmatic children taking part regulary swimming program	György Mezei
Genetics of HELLP syndrome	Bálint Nagy
Diagnosis, prevention and treatment of infections following transplantation	György Reusz
Cardiovascular effects of renal failure and transplantation in childhood	György Reusz
Cardiovascular disorders and renal failure. Possibilities for the preventive options in renal hyperparathyroidism and osteodystrophy in the early phase of renal failure	András Szabó
Studying of pathomechanism, genetic background and therapy of chronic allograft nephropathy	Attila Szabó
Use of hypothermy in the treatment of hypoxic-ischemic encephalopathy of neonates	Miklós Szabó
Pediatric liver diseases. Hereditary metabolic diseases	László Szőnyi
Examinations of factors influencing the morbidity and mortality of pediatric intensive care focus on the carbohydrate metabolism	Péter Tóth-Heyn
Functional immunological studies in pediatric diseases	András Treszl
Functional immunological studies in pediatric diseases	Tivadar Tulassay
New methods in pediatrics	Tivadar Tulassay
Significance of functional genomic examinations in the early and late complications of premature babies	Barna Vásárhelyi
Molecular biological examination of the ischemic injury of the kidney	Ádám Vannay
Role of adaptive immunity (T regulatory cells and lymphocyte markers) in different gastrointestinal disorders (inflammatory bowel disease, celiac disease, allergic colitis)	Gábor Veres

***Ph.D. students***

Márta Bangó	ft
Nóra Fanni Bánki	ft
Tamás Bense	pt
Gábor Dudás	pt
Zoltán Jenőváry	pt
Ambrus Kaposi	ft
Dorottya Kelen	pt
Andrea Kerti	ft
Petra Lakatos	ft
Gábor Marics	pt
Krisztina Molnár	ft
Dorottya Nagy-Szakál	ft
Krisztina Pásti	ft
Ágnes Prókai	ft
Balázs Szalay	ft

***Ph.D. candidates***

Áron Cseh	ft
Orsolya Cseprekál	ft
Ágnes Jermendy	ft
Attila György Kálmán	na
Éva Kis	ft
Krisztina Lukács	ft
Anikó Róka	ft
Monika Sultész	na

***Ph.D. graduates***

Ádám Balogh	ft
Ilona Bányász	ft
Antal Dezsőfi	na
Krisztina Fischer	pt
Krisztina Rusai	ft
Alexandra Szabó	pt
Erna Sziksz	ft
Marina Varga	na

*ft, full-time; pt, part-time; na, not affiliated*

***Supervisors***

Miklós Szabó
Andrea Fekete
András Szabó
István Kocsis
András Szabó
Barna Vásárhelyi
Miklós Szabó
György Reusz
Miklós Szabó
Péter Tóth-Heyn
Gábor Veres
Ádám Vannay
Attila Szabó
Attila Szabó
Barna Vásárhelyi

***Supervisors***

Barna Vásárhelyi
György Reusz
László Madácsy
Tivadar Tulassay
György Reusz
László Madácsy
Miklós Szabó
Györgyi Mezei

***Supervisors***

Barna Vásárhelyi
András Szabó,
Ádám Vannay
András Arató
András Szabó
György Reusz,
Attila Szabó
Endre Cserhádi
András Szabó,
Ádám Vannay
György Reusz

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**ÁDÁM BALOGH (2009)**

### **Measurement of iron-regulatory hepcidin and investigation of its role in perinatal iron homeostasis**

*Supervisor: Barna Vásárhelyi*

Hepcidin is a recently recognized defensin-like peptide, which is considered to be the central regulator of iron metabolism, opening with that new dimension to understand of the pathophysiology of iron-related disorders. The clarification of hepcidin and its role in iron metabolism would provide further explanations to the anemia in inflammation and chronic disease, besides may create new diagnostic and therapeutic opportunities on the field of hemochromatosis and inflammatory anemia. The aim of our study was to develop an easily achievable, reliable quantification method for the determination of urine hepcidin levels in human, in addition to examine a possible association of hepcidin with neonatal iron homeostasis.

After the solid phase synthesis of 9 peptide derivatives according to the sequence of native, 25 aminoacid length human hepcidin, the chemical characterization of peptide derivatives showed, that we produced the desired peptide derivatives in 95% purity. We also performed the functional characterization of peptide derivatives. We proved with the dot blot measurements that the commercially available antibodies are able to recognize the synthetic peptide derivatives. According to the competitive ELISA measurements we can state that our 7 aminoacid length peptide (1-7) might be suitable representatives of the 25-amino-acid form of hepcidin in immune adsorption method. The advantages of 1-7 peptide derivative in contrast to 25-amino-acid form of hepcidin are the easier synthesizability and manageability and the cheaper production.

We described an easy and quick achievable solide-phase extracion method which is suitable to purification of urine and concentration of hepcidin. We proved that the synthetic peptide derivative 1-25 and acetyl-1-25 are properly ionizable in MALDI-TOF MS circumstances. Than we presented a novel MALDI-TOF MS based semi-quantitative, reproducible method for measuring hepcidin concentration in human urine using the synthesized peptide derivative acetyl-1-25 peptide as hepcidin related internal standard.

In our study we have first measured serum prohepcidin and urine hepcidin in healthy human newborns. Serum prohepcidin levels showed no significantly changes, however urine hepcidin levels increased significantly during the first postnatal days. Serum prohepcidin and urine hepcidin levels showed no significant association in healthy human newborns. Associations have been demonstrated between cord blood prohepcidin values and mean corpuscular hemoglobin concentration (MCHC) as well as between urine hepcidin levels and serum iron and total iron binding capacity values. We have demonstrated that neonates with detectable non-protein-bound iron levels in cord blood were presented with lower prohepcidin concentrations.

In summary according to our results it may suggest a possible link between hepcidin and early iron adaptation of newborn's however further investigations should be done to elucidate this issue.

- Balogh Á, Derzbach L, Vásárhelyi B (2004) *Hepcidin, a vasháztartás negatív regulátora. Orv Hetilap* 145: 1549–1552.
- Balogh Á, Bokodi G, Bősze Sz, Kelen D, Prechl J, Szabó M, Vásárhelyi B (2007) *Prohepcidin levels during human perinatal adaptation. Ped Hematol Oncol* 24: 361–368.
- Balogh Á, Horváti K, Mező G, Derzbach L, Szebeni B, Nagy L, Prechl J, Vásárhelyi B, Hudecz F, Bősze Sz (2009) *Synthesis of hepcidin derivatives in order to develop standards for immune adsorption method. J Peptide Sci* 15: 285–295.

## ILONA BÁNYÁSZ (2009)

### The role of the vascular endothelial growth factor and the angiopoietin molecule family in hypoxic diseases

*Supervisors: András Szabó, Ádám Vannay*

Angiogenesis plays a crucial role in several physiological (female reproductive cycle, pregnancy) and pathological (inflammation, wound-healing, tumorigenesis) process occurring in the human organism. Vascular endothelial growth factor (VEGF) and the members of angiopoietin (Ang) molecule-family are main regulating factors of angiogenesis. The aim of our study was to investigate the role of endothelial growth factors in hypoxia-related diseases. Evidences support that beside renal tubular epithelial injury, vascular endothelial injury also plays an important role in development and progression of ischemic acute renal failure (ARF). In our experiments we tested the common alterations of VEGF and its transcription factor, the hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), as well as the Ang/Tie2 system in ischemia/reperfusion (IR) rat kidney model. We found that VEGF and HIF-1 $\alpha$  mRNAs and protein-levels increase after I/R injury. In parallel, we showed the decrease of Ang1 mRNA expression in renal ischemia. Our results support the role of investigated endothelial growth factors in postischemic renal damage. Several experimental and clinical studies have been performed to explain relative protection of female gender during I/R renal injury. We found gender difference in alterations of VEGF/HIF-1 $\alpha$  expressions. Increased VEGF and HIF-1 $\alpha$  mRNA levels were observed in the later reperfusion period when females were compared to males. In females, the increased mRNA level was accompanied with higher VEGF protein level. Several lines of evidence suggest that VEGF has a protective role during acute renal damage. As a result of our study, we suggest that higher VEGF protein level observed in females might contribute for the lower susceptibility of female gender against renal injury. In our human experiments we studied the genetic background of angiogenesis. Abnormal vascular development and consecutive hypoxia predispose to several complications during pregnancy and perinatal life. Impaired vascular remodeling is implicated in the 113 pathogenesis of preeclampsia (PE) which affects 5–8% of pregnant women. The significance of pathological development of vascular system has been recognized in several perinatal diseases. The aim of our human studies was to analyse the functional genetic polymorphisms of VEGF and Ang2 in PE and in complications of low birth weight infants. We showed that carrier state of the VEGF<sub>+405</sub>G allele which predispose to high VEGF producing capability, decrease the risk of severe PE. The carrier state of VEGF<sub>-2578</sub>A allele which predispose to low VEGF producing capability was found to be an independent risk factor during progression of PE. We showed that prevalence of VEGF<sub>+405</sub>C allele was



higher in LBW infants than in healthy term neonates, and carrier state of VEGF C<sub>-2578</sub>A genetic polymorphism has an influence on development of perinatal complications independently from other risk factors. Our results support the role of VEGF and HIF-1 $\alpha$  in pathomechanism of I/R induced ARF, and provide a possible explanation for the protective role of female gender during renal damage. Summarizing our human investigations, we can conclude that genetic polymorphisms which are capable to determine the production of angiogenetic molecules, could be risk factors of development and progression of preeclampsia and perinatal morbidity.

- Bányász I, Bokodi G, Vásárhelyi B, Treszl A, Derzbach L, Szabó A, Tulassay T, Vannay A (2006) Genetic polymorphisms for vascular endothelial growth factor in perinatal complications. *Eur Cytokine Netw* 17: 266–270.
- Bányász I, Bokodi G, Vannay A, Szebeni B, Treszl A, Vásárhelyi B, Tulassay T, Szabó A (2006) Genetic polymorphisms of vascular endothelial growth factor and angiopoietin 2 in retinopathy of prematurity. *Curr Eye Res* 31: 685–690.
- Bányász I, Szabó S, Bokodi G, Vannay A, Vásárhelyi B, Szabó A, Tulassay T, Rigó JJr (2006) Genetic polymorphisms of vascular endothelial growth factor in severe preeclampsia. *Mol Hum Reprod* 12: 233–236.

## ANTAL DEZSŐFI (2010)

### Examination of individual components of innate and adaptive immunity in celiac disease, type-1 diabetes, and the combination of the two diseases

*Supervisor: András Arató*

In our study we screened the prevalence of coeliac disease (CD) in children with type-1 diabetes mellitus (T1DM) and investigated the adaptive and innate immune system in both disorders. We determined Toll like receptor 2 (TLR), TLR3, TLR4 expression in coeliac patients, TLR4, CD14 and TNF- $\alpha$  polymorphisms and HLA-DQ alleles in combination of the two diseases.

According to our results, the frequency of CD in Hungarian children with T1DM is higher than in the control population. We found that coeliac patients detected by screening in a T1DM population had few or no characteristic symptoms at all for this disease. The introduction of gluten free diet improved the somatic development of these children, the need of insuline increased significantly.

We found higher TLR2 and TLR4 mRNA expression and protein levels in the duodenal mucosa of children with treated CD and untreated CD compared with controls. TLR2 and TLR4 mRNA expression and protein levels were even higher in the duodenal mucosa of children with treated CD than in untreated CD. TLR3 mRNA expression was increased in the duodenal mucosa of children with treated CD compared with untreated CD and controls. We were able to detect TLR3 protein only in the biopsy specimens of treated patients with CD. Our finding of elevated TLR2 and TLR4 expression even in treated CD patients with normal villous structure may indicate their potential pathogenetic role in CD. Our results suggest that in patients with T1DM the CD14 -260TT homozygous genotype increases the risk for the development of CD.

Our study indicated that the prevalence of TNF- $\alpha$  <sup>-308</sup>A allele is higher in T1DM than in the healthy population but does not seem to influence the risk of CD in this population.

The prevalence of TNF- $\alpha$  <sup>-238</sup>A allele is higher in T1DM children with CD, but the importance of this finding is still unclear.

The distribution of HLA-DQ genotype is different in children with CD and T1DM. In patients with T1DM and those with CD and T1DM, the occurrence of HLA-DQ2/8 heterozygosity was significantly higher than in children with CD only and in control children. Determination of the HLA-DQ genotype in children with T1DM may help in estimating the risk for the development of CD.

- Dezsőfi A, Szebeni B, Hermann C, Kapitány A, Veres G, Sipka S, Körner A, Madácsy L, Korponay-Szabó I, Rajczy K, Arató A (2008) *Frequencies of genetic polymorphisms of TLR4 and CD14 and of HLA DQ genotypes in children affected by coeliac disease, type I diabetes mellitus, or both. J Pediatr Gastr Nutr* 47: 283–287.
- Arató A, Körner A, Veres G, Dezsőfi A, Ujpál I, Madácsy L (2003) *Frequency of coeliac disease in Hungarian children with type 1 diabetes mellitus. Eur J Pediatr* 162: 1–5.
- Szebeni B, Veres G, Dezsőfi A, Rusai K, Vannay Á, Bokodi G, Vásárhelyi B, Korponay-Szabó IR, Tulassay T, Arató A (2007) *Increased mucosal expression of Toll-like receptor (TLR) 2 and TLR4 in coeliac disease. J Pediatr Gastroenterol Nutr* 45: 187–193.

## KRISZTINA FISCHER (2010)

### Focused ultrasound induced changes in the glomerular ultrafiltration

*Supervisor: András Szabó*

During my Ph.D. work I investigated the potential effects of focused ultrasound and microbubble based ultrasonographic contrast agent on the glomerular ultrafiltration and on the glomerular permeability. In our first set of experiments we found increase in the creatinine clearance, as a measure of the glomerular filtration rate, and in the clearance of the 3,000Da dextran. Furthermore we found increase in the 70,000Da molecular weight dextran clearance, a molecule that can barely cross the glomerular barrier in normal circumstances, which was accompanied with urine production rate enhancement. We observed elevation in the protein to creatinine ratio and in some cases in the FENa%. Each of the mentioned elevation was found to be temporary after the treatment ended returned back to prior treatment level. The histology examination found normal structure at the lower acoustic power levels, although the highest power level tested was associated with focal tubular hemorrhages. Using the cavitation detector we observed bubble activity suggesting either stable or transient cavitation. These cavitation signs can be significant in the understanding of the mechanism by which FUS and the microbubble based ultrasonographic contrast agent modify the glomerular permeability. In later experiments, we found correlation between the applied acoustic power level and the relative creatinine clearance enhancement, the higher acoustic power resulted greater elevation in the relative creatinine clearance. The overall ultrastructure examination showed intact glomerular capillary structure in every examined cases. Using higher magnification we were able to detect significant increase in the podocyte foot process distance. The magnitude of this increase was dependent on the applied power level. It is possible that the increase in the podocyte foot process distance is responsible for the functional changes in the glomerular permeability.

- Fischer K, McDannold NJ, Kardos M, Szabo Ant, Szabo And, Reusz GS, Jolesz F (2009) Renal ultrafiltration changes induced by focused ultrasound. *Radiology* 253: 697–705.
- Fischer K, Gedroyc W, Jolesz F (2010) Focused ultrasound as a local therapy for liver cancer. *Cancer J* 16: 118–124.

## KRISZTINA RUSAI (2009)

### The role of nitric oxide synthase emzymes in the pathogenesis of hypoxia-induced diseases

*Supervisors: György Reusz, Attila Szabó*

The ischemia induced pathological conditions contribute to the mortality and morbidity statistics significantly. Nitric oxide (NO) is a multifunctional signaling molecule playing a central role in the pathophysiology of different hypoxia/ischemia induced diseases. NO is synthesized from L-arginine by the neuronal, inducible and endothelial isoforms of the NO synthases (NOS). The effect of NO depends on its concentration, on the NOS isoform and on the mediators present in the cell environment.

The aim of our present work was to investigate the effect of the NOS isoforms in different hypoxia/ischemia induced diseases.

In the first part of our study we examined the effect of L-arginine, the substrate of NO synthesis and the selective neuronal NOS (nNOS) inhibitor 7-nitroindazole (7-NI) on the ischemia/reperfusion (I/R) injury of the rat kidney.

Our results have demonstrated that L-arginine treatment had no effect on tissue injury and renal function parameters, whereas it increased the expression of all NOS isoforms significantly in the renal tissue.

Treatment with 7-NI did not change either the rate of kidney injury or the renal function parameters. Whereas it increased the expression of the inducible NOS (iNOS) isoform in the postischemic kidney.

Our findings indicate that L-arginine has a significant impact on the postischemic NO system but it has no significant role in the protection from renal ischemic injury. Furthermore, our results suggest that nNOS does not have a central role in renal I/R injury.

Endothelial NOS (eNOS) is one of the main effectors of angiogenesis in mammalian systems. Retinopathy of prematurity (ROP) is a severe perinatal complication affecting mostly low birth weight infants leading to the abnormal neovasculogenesis of the retina.

In the second part of our study we examined if there was any association between the development of ROP and the two genetic polymorphisms of eNOS, 27-bp repeat and T<sup>-786</sup>C polymorphisms, both resulting in decreased NO production.

Our results have demonstrated that the 27-bp repeat polymorphism might contribute to the development of ROP.

- Rusai K, Fekete A, Szebeni B, Vannay A, Bokodi G, Müller V, Viklicky O, Bloudickova S, Rajnoch J, Heemann U, Reusz G, Szabó A, Tulassay T, Szabó AJ (2008) Effect of inhibition of neuronal nitric oxide synthase and L-arginine supplementation on renal ischaemia-reperfusion injury and the renal nitric oxide system. *Clin Exp Pharmacol Physiol* 35: 1183–1189.

- Rusai K, Vannay A, Szebeni B, Fekete A, Borgulya G, Vasarhelyi B, Tulassay T, Szabo AJ (2008) Endothelial nitric oxide synthase gene T-786C and 27-bp repeat gene polymorphisms in retinopathy of prematurity. *Mol Vis* 14: 286–290.
- Rusai K, Huang H, Sayed N, Strobl M, Roos M, Schmaderer C, Heemann U, Lutz J (2008) Administration of interleukin-1 receptor antagonist ameliorates renal ischemia-reperfusion injury. *Transpl Int* 21: 572–580.

## ALEXANDRA SZABÓ (2010)

### Quality of life and psychological status of asthmatic children and their caregivers

*Supervisor: Ende Cserhádi*

In clinical practice quality of life focuses on how the short and long term effects of a disease interferes with the patient's physical well-being, activity, human connections, and mental health. Bronchial asthma through its symptoms interferes strongly with everyday lives of the patients and their caregivers and causes tension, fear and depressive symptoms. In this study I focused on the quality of life and psychological symptoms of adults who were treated with asthma in their childhood, asthmatic children and their caregivers. We used children with chronic renal disease and their caregivers as controls.

Quality of life and psychiatric symptoms were assessed by questionnaires.

We found that 40% of grown up asthmatics still have symptoms, they experienced worse quality of life than the asymptomatic group. Even a couple of asthma attacks a year influences the patients' every day quality of life. Quality of life of the currently asymptomatic patients is also not maximal, due to the allergen avoidance measures.

Asthmatic children experience less depressive score than their healthy peers, but this phenomenon is only significant in pre-school children. Asthmatic children also do not show higher anxiety scores than their healthy peers.

The control group of children with renal diseases does not differ significantly in their depressive symptoms, but their anxiety score tend to be higher than asthmatics.

Asthmatic teenager girls experience the worst quality of life.

Prevalence of psychological symptoms did not depend from the asthma severity score, with this we added arguments to the fact that in asthma subjective and objective symptoms are independent factors even in childhood.

We proved that depression score is higher in both of the asthmatic and renal caregivers than the Hungarian population average. The difference between the score of the two chronic illness group was not significant.

Even up to date care of asthma ends up with quality of life deterioration after years or decades.

Thorough, patient centered and personal asthma care results in no anxiety or depression score elevation.

In Hungary, with the widely accessibility of drugs means better status for patients, and this might lead to a better psychological status.

Due to the higher prevalence of depression amongst asthma caregivers it is also worth to focus on their problems as well during asthma care, and when a diagnosis is suspected offer psychiatric help.

- Szabó A, Cserhádi E (2004) *Bronchial asthma and the short-term quality of life: follow-up study of childhood bronchial asthma in Hungary. Pediatr Allergy Immunol* 15: 539–544.
- Szabó A, Cserhádi E (2006) *Does the actual presence of symptoms influence the long term quality of life in asthma? Int Rev Allergol Clin Immunol* 12: 2.
- Szabó A, Mezei G, Kővári É, Cserhádi E (2010) *Depressive symptoms amongst asthmatic children's caregivers. Pediatr Allergy Immunol* 21: e667–673.

## ERNA SZIKSZ (2010)

### Role of galectin-1, -3, -9 and vascular endothelial growth factor in the pathomechanism of allergic asthma

Supervisors: András Szabó, Ádám Vannay

**Background:** Asthma bronchiale is a complex chronic lung disease characterized by a reversible obstructive ventilatory disturbance, airway inflammation, mucosal enlargement and bronchial hyperreactivity, which molecular mechanisms are not clear. Recently sugar-binding molecules, such as galectins (Gal), came into the focus, which role were described during the adaptive and innate immune responses as well, but their function in allergic asthma is not fully understood.

**Aim:** Using an animal model of asthma our aim was to examine the role of some members of the Gal family, namely Gal-1, Gal-3, Gal-9, and the involvement of vascular endothelial growth factor (VEGF), which is key molecule of angiogenesis and as newly described also of lung remodeling. Since histamine is known as a central mediator of asthma, we also used histidine-decarboxylase gene knock out ( $HDC^{-/-}$ ) mice during our experiments. Furthermore we analyzed the effect of a steroid (dexamethasone, DEX)—often used as anti-inflammatory drug—on Gal-9 expression, which may have eosinophil chemoattractant potential.

**Results:** The protein level of Gal-1,-3,-9 and VEGF was elevated in the asthmatic lung. Using immunohistochemical analysis we found, that alveolar macrophages and lymphocytes are the major source of Gal-1, and their Gal-1 production is enhanced if histamine lacks. Our in vitro experiments on alveolar macrophage cell line show, that histamine can reduce the level of Gal-1. Gal-3 could be detected in all immune cells of the bronchoalveolar lavage (BAL), and 80–90% of the newly immigrated eosinophil and neutrophil granulocytes were Gal-3 positive. There were no differences in the lung Gal-3 protein level of histamine deficient and endogen histamine containing animals. We found that the major sources of Gal-9 in the lung are the BAL lymphocytes, eosinophil and neutrophil granulocytes and alveolar epithelial cells. DEX treatment reduced the level of Gal-9. We found elevated VEGF production in the alveolar macrophages, lymphocytes and eosinophil granulocytes of the BAL after OVA sensitization and challenge. The lack of histamine did not alter the VEGF mRNA expression and protein level.

**Conclusions:** Elevation of Gal-1,-3,-9 and VEGF protein level in the lung suggest that all molecules take part in the pathogenesis of asthma. Based on our results in accordance with data in the literature we hypothesize that Gal-1 and histamine are regulators of each other. We found that histamine has no direct effect on Gal-3 production. Since DEX treatment reduced the amount of eosinophil chemoattractant protein Gal-9, we hypothesize,

that DEX may reduce the immigration of eosinophils into the lung also in this manner and diminish the inflammation.

Based on our results from VEGF experiments we suggest that there might be a histamine independent pathway which also leads to asthma. Our observations would partially explain, why in some cases anti-histamine drugs are inefficient. Therefore VEGF, as a potential mediator of lung remodeling could be a target of researches in the future.

- Sziksz E, Kozma GT, Pállinger É, Komlósi ZI, Ádori C, Kovács L, Szebeni K, Rusai K, Losonczy G, Szabó A, Vannay Á (2010) *Galectin-9 in allergic airway inflammation and hyper-responsiveness in mice. Int Arch Allergy Immunol 151: 308–317.*
- Sziksz E, Kozma GT, Komlósi ZI, Pállinger É, Kardos M, Szebeni B, Losonczy G, Falus A, Szabó A, Tulassay T, Vannay Á (2010) *Increased synthesis of VEGF in allergic airway inflammation in histidine decarboxylase knockout (HDC<sup>+</sup>) mice. Exp Lung Res 36: 420–430.*
- Sziksz E, Kozma GT, Pállinger É, Komlósi ZI, Ádori C, Kovács L, Szebeni B, Rusai K, Losonczy G, Vannay Á, Tulassay T, Szabó A (2009) *Galectin-9 szerepének vizsgálata az allergiás légúti gyulladás és hiperreaktivitás kísérletes egérmodelljében. Gyermekgyógyászat 60: 1.*

## MARINA VARGA (2009)

### Cytomegalovirus infection after kidney transplantation, susceptibility to CMV-infection in association with HLA-genotype

Supervisor: György Reusz

Cytomegalovirus infection is a major infectious complication of transplant recipients, causing significant morbidity and mortality. We can treat this infection effectively only if we know the direct and indirect effects of it, if taking into account the risk-factors, and using sensitive and reliable diagnostic methods for early establishment of diagnosis. In order to avoid severe CMV-infection, profilactic therapy can be introduced. For profilaxis planning it is important to know the CMV seroprevalence of Hungarian population and its specialties. According to our results, the seroprevalence of Hungarian population is high: 86%. CMV seronegative recipients should be transplanted using organs of seronegative donors, however, the chance obtaining the graft from a CMV seronegative donor was shown to be 2% only. In case the seromatching is not feasible, we have to know that the constellation of negative recipient and positive donor is the highest risk-factor. In these cases CMV-infection prophylaxis is essential. In our investigation prophylaxis with ganciclovir is the most effective. It is important to take into consideration that—according to our results—in prophylactic groups the “late-onset” CMV-infection occurs in 1/4 of all cases of CMV-infection, and that there were no signs of seroconversion after the primary CMV-infection in 14% of the patients. Immunocompromised patients require quick diagnosis of acute CMV-infection, the results must be quantitative for monitoring of the antiviral treatment. In our study we have compared conventional methodology (serology and virusisolation-shell vial technique of virusisolation), CMV antigenemia test and quantitative PCR. The last two procedures were found to be the best for the diagnosis of acute CMV-infection in transplanted patients. Considering the advantages and disadvantages of CMV antigenemia test and PCR, we recommend the use of cheaper and less labor-



intensive antigenemia test for kidney transplant patients under the Hungarian circumstances. Genetic variability influences susceptibility to infectious diseases and HLA-molecules are critical for viral antigen uptaking, processing and presenting. Our data suggest that recipients positive for HLA-DQ3 are more susceptible to CMV-infection than a comparable group of patients negative for this HLA-type. The multivariate analysis showed that the HLA-DQ3 positivity is an independent predictor of primary CMV-infection in CMV seronegative recipients with seropositive donor grafts.

- Varga M, Rempert Á, Czebe K, Péter A, Toronyi É, Sárváry E, Fehérvári I, Sulyok B, Járay J (2008) Cytomegalovirus infection after solid-organ transplantation, its risk factors, direct and indirect effects and prevention strategies. *Orv Hetil* 149: 551–558.
- Telkes G, Rajczy K, Varga M, Péter A, Tulassay Zs (2008) Seroprevalence of *Helicobacter pylori* in Central-European uraemic patients and its possible association with presence of HLA-DR12 allele. *Eu J Gastroent Hep* 20: 906–911.
- Varga M, Rajczy K, Telkes G, Hídvégi M, Péter A, Rempert A, Korbonits M, Fazakas J, Toronyi E, Sárváry E, Kóbori L, Járay J (2008) HLA-DQ3 is a probable risk factor for CMV infection in high-risk kidney transplant patients. *Nephrol Dial Transplant* 23: 2673–2678.

## **PROGRAM 2/4.**

---

### **GASTROENTEROLOGY**

#### ***Coordinator:***

**Zsolt TULASSAY M.D.,**

**member of the Hungarian Academy of Sciences**

2nd Department of Internal Medicine

46 Szentkirályi st, Budapest H-1088

Tel.: +36 1 266 4616, fax: +36 1 266 4616

E-mail: [tulassay.zsolt@med.semmelweis-univ.hu](mailto:tulassay.zsolt@med.semmelweis-univ.hu)

### **Program overview**

The importance of gastroenterological diseases is increasing, and because of their frequency and complications, they are considered as one of the most menacing group of diseases. This fact is confirmed by the out-patient and in-patient numeric data, as well as morbidity and mortality indices. Gastroenterology has developed such a discipline that combines the knowledge of different specialities. It includes the pathophysiological data resulted from basic research, the results of clinical research and observations. The research of the gastrointestinal diseases can be done by different approaches and different methodological possibilities. This interdisciplinary topic offers unprecedented opportunities for scientific research. The achievements of last one and half decade resulted relevant changes in several aspects of gastroenterology, and the conventional understanding of development, progression and treatment of diseases had been changed. Despite of the undoubted results several questions need to be answered and new questions are appearing. Gastroenterological diseases can be the topic of wide-spread researches that fit to the scientific basis of public health priorities.

***Titles of research projects***

Intensive care in postoperative and septic conditions associated with gastrointestinal disorders	Katalin Darvas
Examination of proteolytic enzyme systems and cell kinetic parameters in gastrointestinal tumors and digestive diseases	László Herszényi
Immunogenetic risk factors and prognostic factors in colorectal cancer and other malignancies	Judit Kocsis
Novel factors in the development of digestive tract mucosal lesions	László Kopper
Pathogenesis of the <i>Helicobacter pylori</i> related diseases	László Kopper
Pancreas transplantation	Róbert Langer
Examination of peripheral blood mRNA expression markers in patients with colorectal cancer	Béla Molnár
Link among exercise, glucose and lipid metabolism, exercise therapy in diabetes and obesity	Csaba Nyakas
Effect of exercise and food intake on the normal and pathologic brain aging cell physiological processes	Csaba Nyakas
Pathophysiology of the alimentary system	László Rosivall
The role of pattern recognition receptors in non-alcoholic fatty liver disease	Gyöngyi Szabó
Mechanisms of innate immune alterations in HCV infection and its modulation by alcohol use	Gyöngyi Szabó
Toll-like receptor 4-mediated signaling in alcoholic liver disease	Gyöngyi Szabó
Hypoxia-inducible factor in alcoholic liver disease	Gyöngyi Szabó
Extrahepatic complications of chronic liver diseases: hepatic osteodystrophy, autonomic neuropathy. Wilson disease gene mutations, chronic C virus hepatitis and the liver disease associated	Ferenc Szalay
Inflammatory ibowel diseases and the osteopenia	Miklós Szathmáry
Investigation of microcirculation and molecular changes in surgical diseases	Attila Szijártó
Application of video-endoscopic surgical methods in gastroenterology	Tibor Tihanyi
Study of molecular factors deteremining the evolution of bone metastasis	József Tímár
Inflammatory intestine illnesses and osteopenia	Zsolt Tulassay
New factors the digestive system mucosa in the development of laesio	Zsolt Tulassay

***Ph. D. students***

Péter Benkő	ft	Tibor Tihanyi
Evelin Horváth	ft	Ferenc Szalay
Jozilan Hasan Naji Abdullah		Ferenc Szalay
Alexandra Kalmár	ft	Béla Molnár
Katalin Leiszter	ft	Béla Molnár
Katalin Lőrinczy	ft	Pál Miheller
Péter Ónody	pt	Attila Szijártó
Árpád Patai	ft	Béla Molnár
Andrea Schöller	ft	Béla Molnár

***Supervisors***

Júlia Luca Szőcs	ft	Péter Andréka
Kinga Tóth	ft	Béla Molnár
Zsolt Visnyei	pt	Ferenc Szalay

***Ph. D. candidates***

Béla Lombay	pt
János Osztovits	ft
István Pregun	na
Sándor Spisák	ft
Rita Stangl	pt
Sándor Zoltán Szántó	pt
Rita Temesi	pt
Gábor Valcz	ft

***Supervisors***

Ferenc Szalay
Ferenc Szalay
László Herszényi
Béla Molnár
Attila Szijártó
István Pulay
Tibor Tihanyi
Béla Molnár

***Ph. D. graduates***

Ákos Balázs	na
Gabriella Bekő	pt
Gábor Bognár	na
Anikó Folhoffer (Kuczyné)	ft
Levente Ficsór	ft
Imre Kassai	na
Antal Péter	na
Dominika Szőke	ft
Gábor Telkes	na
Viktor Sebestyén Varga	na

***Supervisors***

Péter Kupcsulik
Ferenc Szalay
Lajos Flautner
Ferenc Szalay
Béla Molnár
Péter Andréka
Zsolt Tulassay
Zsolt Tulassay
Zsolt Tulassay
Béla Molnár

ft, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****ÁKOS BALÁZS (2010)****Palliative management of malignant esophageal strictures with endoprosthesis implantation**

*Supervisor: Péter Kupcsulik*

The malignant strictures of the esophagus can be caused by a tumor of the esophagus, a tracheobronchial tumor, or mediastinal tumors. In Hungary the frequency of the occurrence of such a disease is growing. In spite of the improving results of treatment, the percentage of patients in a highly developed stage of this illness is too high, and in these developed cases there is no chance of resection surgery. The inability to swallow and the esophago-respiratory fistulas can cause a life threatening situation. The goal of palliation is to improve the patient's life expectancy and life quality. These goals can be achieved by the method of implanting an endoprosthesis in the esophagus. **Objectives:** The aims of

the study were to examine the methods of endoprosthesis implantation from the aspect of applicability, also to determine the place of the treatment in the system of palliative treatments, the definition of the indications and contraindications for the treatment, and the determinations of the factors that have an influence on the effectiveness of the treatment.

**Methods:** I have gathered and analyzed data about the cases of patients treated at the 1st Department of Surgery of the Semmelweis University with strictures of the esophagus caused by malignant tumors in the time period between 1984 and 2004. Out of 2092 cases the tumor was inoperable in 1604. The endoprosthesis was indicated in 1526 cases. In 41 cases the endoprosthesis was inserted through surgery, and in 824 cases it was implanted by the endoscopic method. In cases in which the prosthesis could not be implanted and/or when complications occurred supportive treatment was administered with gastrostomy in 146, percutaneous endoscopic gastrostomy in 15, catheter jejunostomy in 8 and conservative supportive therapy in 612 cases. The implantation of the prosthesis was combined with radiation treatment in 133 patients and with chemotherapy in 56. In 3 cases respiratory tract stents were implanted, and 2 patients had undertaken laser recanalization. **Results:** In 56.7% of the cases in which the implantation of the prosthesis was indicated the procedure was successful. The implantation of the endoprosthesis solved all the swallowing problems permanently (for a long period of time) in 88.8% of all patients, and in 8.4% it proved to be a temporary solution for the problem. Complications occurred in 29.1% of all cases. The complications were such as: migration of the prosthesis, perforation, haemorrhagia, respiratory compression syndrome, early unexpected death, stent obstruction (food bolus impaction), tumor overgrowth and ingrowth, fistula formation and neoformation, aspiration, reflux. Lethal complications occurred in 2.8% of all cases. The percentage of the complications that occurred after the surgical implantation of the endoprosthesis was 21.9%. The average survival time of patients that have received a prosthesis was 5.4 (0–60) months, with nutritional support including gastrostomy, jejunostomy or PEG 3.6 (0–36) months and with supportive therapy alone 3.2 (0–25) months, respectively. The difference in the survival between the groups treated with prosthesis and other two is significant, while between the latter two is not significant.

**Conclusions:** The implantation of endoprosthesis for palliative treatment in cases of malignant strictures of the esophagus in successful cases is effective. Although the pull-through method has been mostly replaced by less cumbersome push through technique, it still remains a useful procedure in some cases. Prosthesis implantation improves survival and quality of life. Methods used for nutritional support like gastrostomy, jejunostomy and PEG depress the subjective feeling of hunger but not influence survival.

- Balázs Á, Kupcsulik PK, Galambos Z (2008) *Esophago-respiratory fistulas of tumorous origin. Non-operative management of 264 cases in a 20-year period. Eur J Cardio-Thoracic Surg* 34: 1103–1107.
- Balázs Á, Lukovich P, Kokas P, Kupcsulik P (2008) *A nyelőcső stenosisát okozó inoperabilis légúti tumorok palliatív kezeléséről. Medicina Thoracalis* 61: 299–306.
- Balázs Á, Galambos Z, Kupcsulik PK (2009) *Characteristics of esophago-respiratory fistulas resulting from esophageal cancers. A single-center study on 243 cases in a 20-year period. World J Surg* 33: 994–1001.

**GABRIELLA BEKŐ (2010)****Clinical significance of multiplex cytokine measurements in immune-mediated disorders***Supervisor: Ferenc Szalay*

During my Ph. D. work I introduced complex systems into the laboratory that enable us to determinate simultaneously the level of several cytokines. Our measurements indicated that the results obtained by different systems may differ; therefore reference ranges should be established for each systems and one kind of system should be used in one experiment. We performed our studies in disorders with probable involvement of cytokine cascade. In some instances we also determined parameters of free-radical homeostasis and trace element levels that are strongly associated with systemic inflammation. In perinatal disorders we found that proinflammatory cytokines may decrease with postnatal age and hypothermia in postasphyxiated neonates. Systemic cytokine levels determined are also affected by adrenal function, as level of inflammatory cytokines may increase in the presence of low cortisol levels. In another study we found that the diagnosis of perinatal sepsis may be improved by IL-8 determination in addition to procalcitonin and CRP measurements. Our hepatological studies demonstrated that the gender-dependent difference in alcohol induced hepatopathy may be attributed, at least partly, to increased cytokine production after chronic alcohol consumption. Red wine consumption may have an impact on the level of some trace elements and antioxidant defense. In liver cirrhosis we demonstrated that cirrhosis of different origins are characterized by specific cytokine-profiles; i. e. extremely high IL-8 and IL-2 levels are present in PBC and HCV-infection, respectively. In patients with Wilson's disease a systemic inflammation is present, supported by high IL-6 and IL-8 levels. Neurological signs and symptoms are independent of cytokine levels. The levels of a number of trace elements are increased that may contribute to complications. In patients with prostate cancer proinflammatory cytokine levels decrease on administration of a specific antioxidant supplement. The treatment, however, may also increase some growth factors that warrants caution. According to clinical signs and symptoms and IL-6 and TNF- $\alpha$  levels the malnutrition-inflammatory score (MIS) reflects reliably the presence of malnutrition-inflammatory complex syndrome (MICS) in patients after renal transplantation: this finding is of clinical importance when subjects at risk for MICS are to be identified.

Our results indicate that the use of systems suitable for simultaneous detection of cytokine levels, redox homeostasis and trace-element profile may help to identify specific factor in the pathomechanism of immune-mediated disorders and, possibly, may contribute to recognize diagnostic markers and/or therapeutic targets.

- Bekő G, Hagymási K, Szentmihályi K, Bányai ES, Osztoivits J, Fodor J, Fehér J, Blázovics A (2010) Sex-dependent alterations in erythrocyte trace element levels and antioxidant status after a month of moderate daily red wine consumption. *Eur J Gastroenterol Hepatol* 22: 185–191.
- Molnár MZ, Czira M, Ambrus C, Szeifert L, Szentkirályi A, Bekő G, Rosivall L, Remport Á, Novák M, Mucsi I (2007) Anemia is associated with mortality in kidney-transplanted patients—prospective cohort study. *Am J Transplant* 7: 818–824.
- Bekő G (2008) Biochip technika a labordiagnosztikában, citokinmérések. *Orvosképzés* 4: 309–312.

## GÁBOR BOGNÁR (2009)

### Predictive factors for response to neoadjuvant therapy in patients with oesophageal cancer

*Supervisor: Lajos Flautner*

Neoadjuvant chemoradiotherapy (CRT) introduced in the treatment of resectable esophageal cancer produced encouraging results, however overall prognosis remained poor. Twenty-five to thirty percent of the patients achieve a complete pathological response, following neoadjuvant CRT with higher rate of R0-resections and significantly higher survival rates, in comparison to patients without neoadjuvant CRT. On the other hand, patient undergoing neoadjuvant CRT show an increased rate of postoperative complications and mortality. In our study, complete pathological response was achieved in 26.9% of the cases, while 34.6% of the patients proved to be partial responders. Predictive factors for response to neoadjuvant CRT was examined in various pathways and methods (CD34, VEGF, MIB-1, bcl-2: immunohistochemistry and HPV-16, -18 detection with PCR and Southern Blot Hybridization). It is well known that tumours with high proliferative activity respond more to neoadjuvant CRT than those with greater vascularisation (angiogenesis). These two oncological factors were not yet examined in patients with esophageal cancer undergoing neoadjuvant CRT. In this analysis, a relation of 1:5 or less of VEGF expression playing a key role in angiogenesis and MIB-1 indicating proliferative activity was predictive for response to neoadjuvant CRT. In addition, multivariate analysis revealed that a proliferative activity higher than 40% results in significantly higher survival. The bcl-2 apoptosis-factor was not found to be predictive.

The role of certain types of human papilloma virus (HPV) in the pathogenesis of esophageal cancer is known, with an average infection rate of 20–30%. The E6 protein of HPV-16 and 18 causes degradation of p53 tumoursuppression-protein resulting inhibition of apoptosis. HPV is responsible for inhibition, but not for mutation of p53 leading to hyperexpression of VEGF amongst other unfavourable prognostic factors. This is one of the paradox enigmas of the oncopreventive and oncogenetic impacts of HPV. It was reported that certain highly HPV-infected cancers show more than 90% complete pathological response to neoadjuvant CRT but the potential correlation between HPV infection and response to CRT was not yet investigated. We identified the two most common malignant type of HPV (HPV-16,-18) by PCR and Southern Blot Technique. Results were compared with immunohistochemical findings and clinico-pathologic parameters. HPV-infection was only detected in responder cases showing remission to neoadjuvant CRT. We presume that HPV-infection contribute via indirect mechanisms (inhibition of VEGF hyperexpression) to a better prognosis. Our hypothesis was confirmed by survival statistics. Multivariate analysis revealed MIB-1 expression, remission to neoadjuvant CRT and R0 resection as independent, significant prognostic factors. In terms of remission to neoadjuvant CRT, MIB-1 and pN proved to be independent prognostic factors.

- Imdahl A, Bognar G, Schulte-Mönting J, Schöffel U, Farthmann EH, Ihling Ch (2002) Predictive factors for response to neoadjuvant therapy in patients with oesophageal cancer. *Eur J Card Thor Surg* 21: 657–663.
- Bognar G, Imdahl A, Ledniczky Gy, Ondrejka P (2008) Possible role of human papilloma-virus infection in response to neoadjuvant therapy in patients with esophageal cancer. *Hepato-Gastroenterology* 55: 93–97.



- *Bognar G, Istvan G, Bereczky B, Ondrejka P (2008) Detection of human papillomavirus type 16 in squamous cell carcinoma of colon and its lymph node metastases with polymerase chain reaction and Southern Blot hybridisation. Pathology Oncology Research 14: 93–96.*

## LEVENTE FICSÓR (2010)

### Digital microscopy the diagnosis of gastrointestinal histological samples

Supervisor: Béla Molnár

Digital microscope technology has the potential to become a very important tool for pathologists who analyze patient samples for the presence, or absence, of serious diseases, including cancer. An important benefit of the growth of digital pathology is that the transfer of slides between individuals no longer needs to happen physically thanks to the presence of digital slide based telepathology systems where the image information are stored on internet connected servers. Integration of image analysis solutions with digital pathology platforms is the next major step in the evolution of this field.

My objective was to evaluate digital microscopy as diagnostic platform and to develop new techniques which improve the diagnostic efficiency of gastrointestinal samples. My thesis examines three major questions: whether the digitally represented histological images are adequate for diagnosis purposes, whether it is feasible to develop image analysis algorithms which can support the evaluation of gastric and colon biopsies, and finally what are the possibilities and importance of three-dimensional reconstruction of digital histological images from diagnostic point of view.

The preliminary results state that histological slide evaluation of gastrointestinal samples is more reliable by digital microscopy based teleconsultation than the earlier used static image based methods, since the whole slide is accessible for the pathologist and there is reduced possibility for sampling error. My studies also showed that the diagnosis and decision making can be further supported by digital microscopy enabled automated image analysis. Higher order structures like glands can be segmented and analyzed by the aid of newly developed algorithms like the “cellweb” method. The tissue cytometric features play critical role in the classification of different type of diseases among colon and gastric samples but further development required to enhance the classification accuracy and to recognize more and more diseases. The automatic detection of certain histological structures could enhance the effectiveness of the three-dimensional reconstruction but we found that even the manual uses of the reconstruction software can reduce the evaluation time and increases the evaluation accuracy in certain type of diseases like polyps or inflammatory bowel disease. Altogether we can state that the recently developed digital microscopy based solutions give a new level of efficiency to the pathology science.

- *Wu ML, Varga VS, Kamaras V, Ficsor L, Tagscherer A, Tulassay Z, Molnar B (2005) Three-dimensional virtual microscopy of colorectal biopsies. Arch Pathol Lab Med 129: 507–510.*
- *Ficsor L, Varga V, Berczi L, Miheller P, Tagscherer A, Wu ML, Tulassay Z, Molnar B (2006) Automated virtual microscopy of gastric biopsies. Cytometry B Clin Cytom 70: 423–431.*

- Molnár B, Berczi L, Ficsor L, Varga VS, Tagscherer A, Tulassay Z, Kumar S, Dunn B (2009) *Digital slide and virtual microscopy-based routine and telepathology evaluation of gastrointestinal biopsy specimen*. Pp 5–18 in: Kumar BE, Dunn BE (eds) *Telepathology*, Springer.

## IMRE KASSAI (2010)

### Innovative techniques in cardia surgery using the apex of the heart

*Supervisor: Péter Andréka*

The apex is one of the most useful parts of the heart in surgical treatment although it does not often show pathological changes requiring surgical intervention. It has played an indispensable role in the appearance of the truly innovative surgical techniques and keeps it at present as well. These methods mean recovery and convalescence for millions of people with heart diseases. Some of the methods made the already existing techniques safer like the LV vent or the LV arterial cannula in dissections and last but not least the apical suction devices in OPCAB surgery. Some others have served as basis for new kinds of treatments which could have never appeared without the use of the cardiac apex. These are the alternative treatment options for aortic valve disease, the preferable implantation method in left ventricle assist device therapy, and the transapical lead implantation for completing CRT systems.

The author of this thesis is well-trained and skilled in this field, only two of the above mentioned procedures are without his personal experience, one of them not being presented in Hungary at all. The OPCAB method without as well as—after its availability—with the apical suction device (Kassai *et al.* [1995] Alternative method for coronary revascularization: surgery without cardiopulmonary bypass. *Cardiol Hung* 24(3): 14–16), and the arterial cannula through the LV apex for surgical treatment of acute proximal aortic dissection have been applied for the first time in Hungary by him, based on and proved by his detailed scientific research about surgery for coronary artery disease and aortic dissection. The first successful bridge-to-transplant long-term mechanical circulatory support in Hungary belongs to the team of the Gottsegen Hungarian Institute of Cardiology where he is in charge of this project. One of the most important parts of the applied Berlin Heart® BiVad system is the LV apical cannula. With close cooperation of his cardiologist colleagues, the author developed a fundamentally new approach for alternative LV lead implantation for CRT, and applied it with success first in the world (Kassai *et al.* [2008] New method for cardiac resynchronization therapy: transapical endocardial lead implantation for left ventricular free wall pacing. *Europace* 10(7): 882–883; Kassai *et al.* [2009] Alternative method for cardiac resynchronization: transapical lead implantation. *Ann Thorac Surg* 87: 650–652). This method allows significantly more patients to be responders of this increasingly important treatment of heart failure.

The scientific cardiac surgical works of the author detailed in this thesis prove the importance of the permanent requirement and implementation for innovative techniques in cardiac surgery despite the nowadays so frequent negative opinions about the general nightfall of this field of medicine.

- Kassai I, Varga A, Weber D, Damenija G, Koltai Cs, Haan A, Meszaros M (1998) *Effects of crystalloid versus blood cardioplegia on myocardial protection during cardiopulmonary bypass*. *Cardiol Hung Suppl*: 87.

- Kassai I, Haan A, Soltesz B, Revesz E, Dekany P, Gyorgy M, Szedo F (1995) *Alternative method for coronary revascularization: surgery without cardiopulmonary bypass. Cardiol Hung* 24: 14–16.
- Vigh A, Kassai I, Rashed A, Gombocz K, Simon J, Alotti N, Lupkovics G (2000) *Experience gained during coronary artery bypass grafting without cardiopulmonary bypass. Cardiol Hung Suppl*: 65.

## ANIKÓ FOLHOFFER (KUCZYŃÉ) (2009)

### Genetic and clinical characteristics of Wilson disease in patients from Hungary

*Supervisor: Ferenc Szalay*

The number of known mutations within the ATP7B exceeds the 350 according to the recent data of Wilson disease (WD) mutation of the Human Genom database. In Hungary similarly to patients with WD from other Central European countries the H1069Q point mutation is the most frequent one, as we reported earlier. To assess the impact of genetic testing for diagnosis of WD the mutations of ATP7B gene were studied in an internationally large number of patients (n=142), completing the genetic map of Europe. Altogether 35 different mutations were found. H1069Q was the most frequent mutation in Hungary, detected in 100 patients (70%). Thirty-four further mutations were found by sequencing, including ten new, hitherto undescribed and not listed in the international WD mutation database: L517-fs (c1549-1559del11), N676I, S693Y, Y715H, M769L, W939C, V1001G, V1039F, P1273S and G1281D. In 51/142 patients (36%) the diagnosis of WD was established by adding mutational analysis, making further diagnostic tests unnecessary. Our results confirmed the general view of impact of genetic testing in patients presenting with hepatic disease or asymptomatic siblings of an index case, as we documented in several case reports. Examination of index patients' siblings is especially important, since in case of two disease-causing mutations beginning of the therapy can be prophylactic in the development of clinical symptoms in preclinical stage. The occurrence of acute hepatic failure was relatively frequent among the Hungarian Wilson patients and seemed to be associated with H1069Q heterozygous genotype.

- Folhoffer A, Horváth A, Hegedűs D, Firneisz G, Dunkel K, Willheim C, Ferenci P, Lakatos PL, Szalay F (2003) *ATP7B génmutációk magyarországi Wilson kóros betegekben; esetismertetések a betegség változatos klinikai megjelenésére. Orv Hetil* 144: 2509–2515.
- Firneisz G, Szonyi L, Ferenci P, Willheim C, Horvath A, Folhoffer A, Tulassay Z, Szalay F (2004) *The other mutation is found: follow-up of an exceptional family with Wilson disease. Am J Gastroenterol* 99: 2504–2505.
- Nagy J, Folhoffer A, Horváth A, Csák T, Taba G, Szentmihályi K, Szalay F, Zekó R (2005) *Kinetic study of zinc sulphate release from lipophilic matrices prepared for the therapy of Wilson's disease. Pharmazie* 60: 524–526.

**ANTAL PÉTER (2010)****Experiences obtained with upper panendoscopy in organ transplant recipients with special regard to cytomegaloviral infections of the upper gastrointestinal tract***Supervisor: Béla Molnár*

Gastrointestinal complications of solid organ transplant patients are frequent; they may be even life-threatening. Less serious complaints mean considerable deterioration in quality of life of patients indeed. The reasons of these complications are generally complex related to the original disease, organ transplant operation, graft function, and immunosuppression. Gastrointestinal complications of immunosuppression are result of the side effects of immunosuppressive therapy or consequences of different infections. Cytomegalovirus is a major pathogen for transplant patients even in the gastrointestinal tract. Considerable number of transplanted patients is followed up in our clinic. The basic diagnostic method of gastrointestinal complications is endoscopy with endoscopic biopsy. In this study our experiences of upper endoscopies on symptomatic solid organ transplant patients were reviewed. Symptoms, complaints, endoscopical and histological findings of patients were analyzed. Findings on kidney and liver transplant patients were compared: gastric ulcers, *Helicobacter pylori* and candida infections were more frequent on kidney transplanted patients. Conventional histology proved less suitable for the diagnosis of cytomegalovirus infection from biopsy samples, qualitative polymerase chain reaction method was used for detection of viral DNA. With this method in 48 % of investigated transplant patients CMV infection was proved; in about half of these patients gastrointestinal CMV disease was supposed. Criteria of antiviral treatment were determined. The proportion of urgent endoscopies for upper gastrointestinal bleeding proved significantly higher in liver transplant patients, the most frequent source of bleeding were esophageal varices. However in kidney transplant patients bleeding gastro-duodenal ulcers were found more frequently. Diagnostic and therapeutic urgent upper endoscopies on organ transplant patients are suggested to perform by the same way as by endoscopies on non-transplanted patients. Further investigations could be performed to analyze the immunosuppressive drug induced mucosal lesions, and to evaluate the detection of CMV replication in the gastrointestinal tract.

- Péter A, Telkes G, Varga M, Sárváry E, Kovalszky I (2004) Endoscopic diagnosis of cytomegalovirus infection of upper gastrointestinal tract in solid organ transplant recipients: Hungarian single-center experience. *Clin Transplant* 18: 580–584.
- Varga M, Rajczy K, Telkes G, Hídvégi M, Péter A, Rempert Á, Korbonits M, Fazakas J, Toronyi É, Sárváry E, Kóbori L, Járny J (2008) HLA-DQ-3 is a probable risk factor for CMV infection in high risk kidney transplant patients. *Nephrol Dial Transplant* 23: 2673–2678.
- Péter A, Telkes G, Varga M, Járny J (2008) A gastrointestinalis traktus cytomegalovírus fertőzése szervtranszplantált betegekben. *Orv Hetil* 149: 2463–2470.

**DOMINIKA SZÓKE (2009)****Genetic factors related to the histological and macroscopic lesions of the stomach***Supervisor: Zsolt Tulassay*

Certain premalignant conditions of gastric cancer are related to particular genetic alterations. Polymorphisms of the IL-8, TNF- $\alpha$ , and p53 genes—which play a significant role—can be identified in patients suffering from gastritis and IM. *H. pylori* infection is another important factor, which influences the effects of the investigated genes and polymorphisms. With the application of high-capacity screening methods such as resequencing arrays also offering the additional advantage of an expedited diagnosis, further genetic factors can be identified. Various methods for polymorphism detection are available for identifying sequence alterations. Unfortunately, the GeneChip p53 Probe Array is apparently not reliable enough for use as a stand-alone DNA resequencing method. Based on my research results certain types of resequencing microarray may prove a sensitive and reliable method for genetic screening for diagnostic purposes. Nevertheless, CS is always recommended for verification.

For a better understanding of the role of polymorphisms or mutations in the etiology of gastric disease, laser-microdissection of the affected cells, identified by IHC, is proposed. This could afford early recognition of patients at risk of developing micro- or macroscopic, pathological lesions as well as the introduction of preventive measures.

- Szoke D, Molnar B, Solymosi N, Racz K, Gergics P, Blasko B, Vasarhelyi B, Vannay A, Mandy Y, Klausz G, Gyulai Z, Galamb O, Spisak S, Hutkai B, Somogyi A, Berta K, Szabo A, Tulassay T, Tulassay Z (2009) Polymorphisms of the ApoE, HSD3B1, IL-1beta and p53 genes are associated with the development of early uremic complications in diabetic patients: results of a DNA resequencing array study. *Int J Mol Med* 23: 217–227.
- Szoke D, Molnar B, Solymosi N, Sipos F, Galamb O, Gyorffy A, Tulassay Zs (2009) The RR genotype of codon 72 of p53 gene reduces the development of intestinal metaplasia. *Dig Liver Dis* 41: 179–184.
- Szoke D, Molnar B, Solymosi N, Klausz G, Gyulai Zs, Toth B, Mandy Y, Tulassay Zs (2008) On the T-251A polymorphism of IL-8 relating to the development of histological gastritis and on the G-308A polymorphism of TNF- $\alpha$  relating to the development of macroscopic erosion. *Eur J Gastroen Hepatol* 20: 191–195.

**GÁBOR TELKES (2009)*****Helicobacter pylori* before and after the kidney transplantation***Supervisor: Zsolt Tulassay*

Peptic ulcer and related pathologies, such as gastritis and duodenitis, are known to occur with increased frequency (20–60%) and severity in chronic renal failure and in renal transplant recipients. The frequency of severe complications is about 10% and 10% of those might prove fatal. Gastrointestinal complications might play a role in the outcome of kidney transplantation, as they are associated with an increased risk of graft loss.

Between 2001 and 2006, serologic testing for *H. pylori* revealed a 49.3% of positivity rate of uraemic recipients of subsequent kidney transplantation. *H. pylori* infection rate and its decrease with year of birth was the same in the uraemic patients and in the normal population. The carrier status of HLA-DR12 increases 2.76 fold the risk of *H. pylori* infection. My work is the first observation of any possible relationship between *H. pylori* and HLA-DR12.

From 1994 to 2007, 672 upper GI endoscopies were done in 543 cases of 439 kidney transplant patients. 20–25% of all kidney recipients needed gastroscopy in their “posttransplant life”; the rate of clinically significant endoscopic findings was 84%. 17% of patients required the examination in the first three months, 27% of all patients required it in the first year. In 17% of cases ulcer was detected; 29.3% of ulcers developed in the first three months, 45% of them in the first year. The presence of *Helicobacter pylori* was verified in 20.9% and did not change in time, suggesting, that eradication happens at the early peri-operative period. MMF increased the risk of erosive lesions, and the risk of harbouring CMV in the gastro-duodenal mucosa, while *H. pylori* and CMV were not associated with any specific endoscopic findings. The rate of observed endoscopic alterations did not differ in the H2RA and in the PPI group, indicating the equivalence of these two groups of AST in this setting. Adopting a low threshold for endoscopy in a specialised centre revealed very frequent abnormalities that required medical intervention.

- Telkes G, Rajczy K, Varga M, Péter A, Tulassay Zs (2008) Seroprevalence of *Helicobacter pylori* in Central-European uraemic patients and its possible association with presence of HLA-DR12 allele. *Eur J Gastroenterol Hepatol* 20: 906–911.
- Varga M, Rajczy K, Telkes G, Hidvégi M, Péter A, Rempert Á, Korbonits M, Fazakas J, Toronyi É, Sárváry E, Kóbori L, Járny J (2008) HLA-DQ3 is a probable risk factor for CMV infection in high-risk kidney transplant patients. *Nephrol Dial Transplant* 23: 2673–2678.
- Péter A, Telkes G, Varga M, Sárváry E, Kovalszky I (2004) Endoscopic diagnosis of cytomegalovirus infection of upper gastrointestinal tract in solid organ transplant recipients —Hungarian single centre experience. *Clin Transplant* 18: 580–584.

## VIKTOR SEBESTYÉN VARGA (2010)

### Detection of circulating tumor cells in samples from colorectal cancer patients using digital fluorescent microscopy and image cytometry

Supervisor: Béla Molnár

Recently enhanced focus is set on the rare cells of the human organ (circulating tumor cells, physiologic and pathologic stem cells, fetal cells). Circulating tumor cells can be detected in peripheral blood samples by cytometry. Slide based image cytometers provide similar data as flow cytometry by digitally processing the cell images. For the detection of circulating tumor cells, they have several advantages over FCM. In the recent years brightfield whole slide imaging became commercially available and starts to be established in pathology.

We created an imaging cytometer system called Scanning Fluorescent Microscope (SFM) by using a motorized fluorescent microscope, special scanning and image processing algorithms and a fluorescently stained compensation slide. We showed that this system



is capable of quantitative and stoichiometric measurements and it provides comparable results to other well established cytometry solutions on large cell populations. We showed that SFM detects rare cells more reliably as flow or laser scanning cytometry.

We extended the commercially available MIRAX MIDI whole slide imager with fluorescent scanning capability and used it with the SFM methodology. We showed that the fluorescent whole slide imaging is possible and using the SFM methodology, it provides even better results as the SFM system due to the technical advancements in illumination and camera technology.

The automated slide loading, sample detection and scanning capabilities of whole slide imaging systems combined with image cytometer software make possible the clinical screening for colorectal cancer patients.

- Varga VS, Bocsi J, Sipos F, Csendes G, Tulassay Z, Molnár B (2004) *Scanning fluorescent microscopy is an alternative for quantitative fluorescent cell analysis. Cytometry A 60: 53–62.*
- Bocsi J, Lenz D, Mittag A, Varga VS, Molnar B, Tulassay Z, Sack U, Tarnok A (2006) *Automated four-color analysis of leukocytes by scanning fluorescence microscopy using quantum dots. Cytometry A 69: 131–134.*
- Bocsi J, Varga VS, Molnár B, Sipos F, Tulassay Z, Tarnok A (2004) *Scanning fluorescent microscopy analysis is applicable for absolute and relative cell frequency determinations. Cytometry A 61: 1–8.*

---

## ***PROGRAM 2/5.***

---

### **DENTAL RESEARCH**

#### ***Coordinator:***

**Gábor VARGA M.Sc., Ph.D., D.Sc.**

Institute of Oral Biology

4 Nagyváradi sq, Budapest H-1089

Tel.: +36 1 210 4415, fax: +36 1 210 4421

E-mail: [varga-g@net.sote.hu](mailto:varga-g@net.sote.hu)

#### **Program overview**

The aim of this Ph.D. program is to select open minded, self-supporting applicants who are able to acquire the knowledge of complex understanding of theoretical and clinical state of the art in the field, and are able to use this knowledge in education and research. Therefore, the Program prepares for the two different but not unconnected directions: it focuses on the better understanding of physiological and pathological processes in the oral cavity, as well as on the development and application of new therapeutic methods in all branches of dentistry.

***Titles of research projects******Supervisors***

Preventive program monitoring, risk factor evaluation using laboratory and clinical epidemiological methods	Jolán Bánóczy
Examination of oral cavity manifestations using clinical and laboratory methods	Jolán Bánóczy
Biological and clinical study and development of maxillofacial soft and hard tissue surgical techniques in rehabilitation	József Barabás
Theory-designed experiments in the characterisation of dental materials at the molecular level	Gregory A. Chass
Computational design of molecules and materials	G. Imre Csizmadia
Comparison of imaging techniques	Csaba Dobó Nagy
Characterisation of dental materials for the purpose of clinical use	Csaba Dobó Nagy
Effects of psychological, electromagnetic and heat stimulation in the expression and secretion of Hsp70 type stress in the salivary glands	Tibor Károly Fábíán
Role of vascular endothelium in the regulation of the oral circulation	Árpád Fazekas
Role of sensory and autonomic nerve fibers in the development of inflammation in the parodontium and the pulp	Árpád Fazekas
Driven parodontal tissue regeneration	István Gera
The importance of screening of the oral human papillomavirus (HPV), and the role of the extracellular matrix and the tight junction structure in the pathogenesis of tumours in the oral cavity	Péter Hermann
Evaluation of visual and instrumental tooth shade determination techniques	Péter Hermann
Diagnostic and therapeutic aspects in dentistry supporting the prevention and rehabilitation in diseases of the craniofacial region	Melinda Madléna
Assessment of xerostomia with associated sicca symptoms and evaluation of the minor salivary gland flow rates in different conditions and diseases	Krisztina Márton
Stomatological aspects of immunopathologic disorders	Gábor Nagy
Congenital dental anomalies and their orthodontic implication.	Noémi Katinka Rózsa
Root resorption during orthodontic treatment	
Study of the clinical progression and examination of extracellular matrix (ECM) components in oral squamous epithelial cancers and in conditions preceding oral cancers	Zsuzsanna Suba
Histological integration of bone replacement and implanted materials in the oral region	Zsuzsanna Suba
Permanent teeth injuries and care	Ildikó Tarján
Correlations of oral tumors and glucose metabolism disorders	Márta Ujpál
Progenitor cells of oral origin, signals and scaffolds for foundation of tissue engineering applications	Gábor Varga
Study of the molecular regulation of epithelial ion and fluid secretion	Gábor Varga
A human salivary gland model to study the molecular mechanisms of epithelial differentiation and for the development of gene implantation techniques	Gábor Varga
Investigation of pediatric disease related orofacial disorders	András Végh

Laser assisted decontamination of the implant surface in the surgical treatment of peri-implantitis  
Salivary gland research

Péter Windisch

Tivadar Zelles

### ***Ph. D. students***

Emese Ábrám	pt (a)
Anita Beck	ft
Erzsébet Bori	ft
Tamás Chikány	ft
Krisztina Cséplő	pt
Edina Erőss	pt
Laura Ferenczi	ft
Márta Fülöp Papp	ft
Milán Gyurkovics	ft
Attila Horváth	ft (a)
Máté Jász	pt
Bence Kádár	ft
Karola Kálló	ft
Márton Kivovics	pt
Ferenc Koppány	pt (a)
Imre Zoltán Kun	pt
Szabolcs Lányi	pt
Mercedes Linninger	pt
Ádám Lőrincz	ft
Szilvia Mihályi	ft
Bálint Nemes	pt
Katalin Perczel-Kovács	ft
Mortazavi Reza	it
Kata Ágnes Sasvári	pt
László György Schmideg	pt
Zsófia Somogyi	pt
Péter Stiedl	ft
Örs Lehel Tollas	pt
Mihály Vaszilkó	pt

### ***Ph. D. candidates***

Sándor Bogdán	na
Judit Borbély	pt
Gabriella Borsos	na
Bálint Molnár	pt
Krisztián Nagy	na
Gabriella Óvári	ft
Attila Zsolt Szűcs	na

### ***Supervisors***

András Végh
Tibor Károly Fábíán
Gábor Varga
Péter Windisch
György Szabó
András Végh
András Végh
Zsuzsanna Suba
Zsolt Lohinai
István Gera
Gábor Varga
György Szabó
Gábor Varga
György Szabó
György Szabó
Zsuzsanna Szabó
Ida Nyárasdy
József Barabás
Zsuzsanna Suba
András Végh
Gábor Varga
Zsuzsanna Suba
József Barabás
Péter Hermann
István Gera
Tivadar Zelles
Zsuzsanna Suba

### ***Supervisors***

József Barabás
Péter Hermann
András Végh
Gábor Varga
Gábor Varga
István Gera
József Barabás

***Ph.D. graduates***

József Blazsek	na
Irma Demeter	ft
Marianna Kátay-Király	ft
Beáta Kerémi	ft
Miklós Kovács	na
Edit Maka	na
Ferenc Oberna	na
Emil Segatto	pt
Ákos Szűcs	pt

***Supervisors***

Gábor Varga
Gábor Varga
Gábor Varga
Árpád Fazekas
Csaba Dobó Nagy
József Barabás
György Szabó
András Végh
Gábor Varga

a, absolutorium; ft, full-time; pt, prt-time, it, international; na, not affiliated

***Abstracts of Ph.D. theses successfully defended in 2009 and 2010*****JÓZSEF BLAZSEK (2009)****Experimental study of tissue regeneration**

*Supervisor: Gábor Varga*

On the role of salivary glands in the control of integrity and regeneration of oral mucosa. One of the life's principium is the faculty of self-defence. Complementary to the skin, the mucosa which composed of tightly sealed epithelial cells play a basic defensive role, too. Epidermal growth factor (EGF) plays a major role in the post-traumatic regeneration of the epithelial layer. (1) Our investigations confirmed that salivary glands secrete a wide range of EGF. Partial ablation of the salivary glands (sialoadenectomy) or chronic gustatoric stimulation via citric acid both induce salivary gland hypertrophy. Ablation of the salivary gland induces an increase in mass of the glandula parotis. On the other hand, following ablation, an increased gustatoric stimulus decreases the EGF concentration by 90% in the gl. parotis, while the salivary EGF level raises by 20%. (2) A basic recognition confers that the survival time of experimental rats was more than 90 days following removal of grand salivary glands, strongly arguing that the grand salivary glands are not vital organs. (3) Upon pilocarpin stimulus the contribution of small salivary glands to total protein secretion was 15% while only 1 to 2 % for the amylase secretion. Small salivary glands are under vegetative control in rat. (4) Following ablation of grand salivary glands, the level of salivary proteins, amylase and EGF raised slowly and modestly. Our results document the persistence of residual EGF in saliva. The results support the idea that following ablation of the grand salivary glands the dispersed small salivary glands compensate the EGF secretion. (5) The results suggest, that our new method may provide a useful tool for the investigation of biofilm physiology in the oral cavity, with particular respect to small salivary glands in the absence of grand salivary glands. Our data, together, shed light on novel, hitherto not well established aspects of the adaptive faculty of small salivary glands. On one hand, we have identified the amylase enzyme and EGF in the biofilm covering the mucosa and tooth. On the other hand, we show that pilocarpin stimulated the secretion of both, which could be inhibited by

propranolol. It can be hypothesized that in humans too, in case of accidents, tumour or other causes resulting loss of gland salivary gland function, the small salivary glands may substitute for EGF secretion. Analysis of bone regeneration using a novel implantation model in the rat. The clinical application of data, obtained on the field of experimental tissue-engineering is conditioned by the availability of rapid and kvantitative evaluation systems. Modeling in dental implantology is still hampered by the lack of such practical methodology, therefore we have searched for and elaborated a novel system applying the rat tail spongy vertebrae, as a model. (1) Using the rat tail model the kinetics of bone regeneration and osseointegration of implants can be evaluated quantitatively. This technique makes it possible, for the first time, to simultaneously measure the qualitative morphometric and quantitative biomechanical changes of newly-formed bone on the effect of systemic or local therapeutical treatments. (2) We have tested the usefulness of our implantation method by measuring the effects of amino-bisphosphonate (Zometa®) on bone neogenesis and consolidation of titanium implant. The results provide evidence that Zometa significantly increased bone neogenesis and inforced the integration of titanium. The results provide new information on the beneficial effects of Zometa in the case of bone regeneration or using titanium implants. On the other hand the method has opened up the way how to analyse novel regulatory factors and substitution materials. (3) These investigations uncovered, surprisingly, that the complete haematopoietic bone marrow is absent in all 25 caudal vertebrae, despite the presence of osteoblasts and osteoclasts there. This discovery provides a guiding cue in understanding the genetic, cellular and molecular control of HSC seeding in BM and the involvement of endosteal osteoblasts in forming the haematopoietic stem cell niche. Literature of Summary: (1) Cohen S, Elliott GA (1963) The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. *J Invest Dermatol* 40: 1–5. (2) Sarosiek J, Feng T, McCallum RW (1991) The interrelationship between salivary epidermal growth factor and the functional integrity of the esophageal mucosal barrier in the rat. *Am J Med Sci* 302(6): 359–363. (3) Blazsek J, Offenmüller K, Burghardt B, Kisfalvi I, Birki K, Wenczl M, Varga G, Zelles T (1996) Possible compensation in epidermal growth factor production by saliva in rat. *Inflammopharmacology* 4:279–295. (4) Blazsek J, Varga G (1999) Secretion from minor salivary glands following ablation of the major salivary glands in rats. *Arch Oral Biol* 44(1): S45–48. (5) Amant N, McDonald M, Godfrey C, Bilston L, Little D (2007) Optimal timing of a single dose of zoledronic acid to increase strength in rat fracture repair. *J Bone Miner Res* 22: 867–876. (6) Blazsek J, Dobó Nagy Cs, Blazsek I, Varga R, Vecsei B, Fejérdy P, Varga G (2008) Aminobisphosphonate stimulates bone regeneration and enforces consolidation of titanium implant into a new rat caudal vertebrae model. *Pathology and Oncology Research* 15(4): 567–577. Short THESIS Partial ablation of the grand salivary gland. (1) The EGF secretion is under noradrenalin regulation, in grand salivary glands. (2) Ablation of the submandibulary and sublingual salivary glands does not result in the total disparition of EGF from the secreted saliva. (3) The amount of EGF measured in saliva exceeds the production by one gland. (4) The quantity of EGF in salive can be increased by the stimulation of the salivary gland. (5) The Parotis is capable of high protein and EGF secretion even in case of low tissue pool. (6) Salivary glands constitute a unique tissue system, provided by the fact that the function of affected glands are compensated by the complementary, remaining glands. Total ablation of the grand salivary gland. (7) Following total ablation of salivary glands the average survival time exceeds 90 days, indicating that these glands are not of vital importance. (8) Small salivary glands in rat are under vegetative regulation,

and following pilocarpin stimulus they produce 15% of the whole proteins while only 1–2% of the whole amylase. (9) Small salivary glands have compensatory regenerative potentials at the level of protein and EGF production, following removal of the grand salivary glands. Modelling of bone regeneration and physio-pathological studies. (10) Our results revealed, that caudal vertebrae in rat provide an ideal host environment to carry out preclinical investigations on bone modifying substances. Remarkably, interference with fully active bone marrow is less compromistic here, due to the paucity in bone marrow parenchyme at this anatomical location. Therefore, we propose that the tail vertebra model represents a more realistic system for large scale comparative studies. (11) Despite the aplastic characters of bone marrow parenchyma in caudal vertebrae the osteoblast and osteoclast compartments are highly active. The cellular and molecular mechanisms responsible for this unusual tissue composition are not known. This situation provides a physiological tool to discover some basic regulatory mechanisms operating between normal haematopoietic stem cells and osteoblasts. (12) Previous publications and our ongoing works indicate that new informations on the aplastic tail vertebrae might provide some basic knowledge to better understand the function of bone marrow microenvironment and the disfunctions in the case of myelodysplasia, preleukaemia and acute myeloid leukaemia.

- Blazsek J, Offenmüller K, Burghardt B, Kisfalvi I, Birki K, Wenczl M, Varga G, Zelles T (2006) Possible compensation in epidermal growth factor production by saliva in rat. *Inflammopharmacology* 4: 279–295.
- Blazsek J, Varga G (1999) Secretion from minor salivary glands following ablation of the major salivary glands in rats. *Arch Oral Biol* 44: 45–48.
- Blazsek J, Dobó Nagy Cs, Blazsek I, Varga R, Vecsei B, Fejérdy P, Varga G (2009) Amino-bisphosphonate stimulates bone regeneration and enforces consolidation of titanium implant into a new rat caudal vertebrae model. *Pathol Oncol Res* 15: 567–577.

## IRMA DEMETER (2010)

### Examination of ion secretion in cells of epithelial origin

Supervisor: Gábor Varga

We investigated the  $\text{HCO}_3^-$  secretion of the parotid acinar Par-C10 and pancreatic ductal HPAF cell line, and the  $\text{Ca}^{2+}$  clearance in Par-C10 cells. The cells seeded onto Transwell-Clear membranes form confluent monolayers. In short circuit current ( $I_{\text{sc}}$ ) measurements anion secretion is stimulated by ATP in both cell lines, and by forskolin in Par-C10 cells. In Par-C10 cells bumetanide, an inhibitor of  $\text{Na}^+\text{-K}^+\text{-2Cl}^-$  cotransporter-1 (NKCC1), prevented the effect of forskolin in  $\text{HCO}_3^-$ -free solution. Measuring the intracellular pH ( $\text{pH}_i$ ), the recovery from acid load is  $\text{Na}^+$ -dependent and sensitive to EIPA, an inhibitor of  $\text{Na}^+\text{/H}^+$  exchanger-1 (NHE1). In  $\text{HCO}_3^-$  solution basal EIPA and  $\text{H}_2\text{DIDS}$ , inhibitor of  $\text{Na}^+\text{-HCO}_3^-$  cotransporter-1 (NBC1), inhibits the  $\text{pH}_i$  recovery from acid load. Basal application of the inhibitors results in a  $\text{pH}_i$  drop, which can be enhanced by ATP or forskolin. CFTRinh-172 prevents the effect of forskolin only apically. Withdrawal of  $\text{Cl}^-$  from the basal side results in an ATP- and forskolin-insensitive  $\text{pH}_i$  elevation that can be inhibited by DNDS, inhibitor of anion exchanger-2 (AE2). Basal carbachol or apical ATP application results in an elevation of intra-cellular  $\text{Ca}^{2+}$  level.  $\text{Ca}^{2+}$  clearance is only enhanced by



forskolin apically. The clearance in the presence of  $\text{La}^{3+}$  cannot be further inhibited either by Ru360, an inhibitor of mitochondrial  $\text{Ca}^{2+}$  uptake, or by  $\text{Na}^+$ -free solution; only the plasma membrane  $\text{Ca}^{2+}$  ATP-ase (PMCA) selective carboxyeosin is effective. In HPAF cells bumetanide inhibits the effect of ATP on ISC only in  $\text{HCO}_3^-$ -free solution. The pH recovery from acid load is  $\text{Na}^+$ -dependent, and sensitive to a different degree on the two sides to EIPA. In  $\text{HCO}_3^-$  solution application of  $\text{H}_2\text{DIDS}$  and EIPA inhibits the pH recovery. The inhibition of  $\text{HCO}_3^-$  influx and  $\text{H}^+$  efflux results in a pH drop that can be enhanced by ATP but not by forskolin. Our conclusion is that both cell lines secrete  $\text{HCO}_3^-$ . In Par C10 cells NHE1, NBC1, NKCC1 and AE2 are located on the basal, and CFTR on the apical side.  $\text{HCO}_3^-$  secretion is enhanced by intracellular cAMP- and  $\text{Ca}^{2+}$ . mAChR is expressed on the basal, and purinergic receptors on the apical side. The  $\text{Ca}^{2+}$  clearance is handled through PMCA transporters on both sides, but only the apical clearance is stimulated by protein kinase A. HPAF cells express NHE, NBC, and NKCC1 on the basal, and NHE on the apical side, thus HPAF cells are suggested to originate from large ducts. The anion secretion is stimulated by ATP but not by forskolin.

- Demeter I, Hegyesi O, Nagy Á, Case RM, Steward MC, Varga G, Burghardt B (2009) Bicarbonate transport by the human pancreatic ductal cell line HPAF. *Pancreas* 38: 913–920.
- Demeter I, Szűcs Á, Hegyesi O, Földes A, Rácz GZ, Burghardt B, Steward MC, Varga G (2009) Vectorial bicarbonate transport by Par-C10 salivary cells—a model for epithelial electrolyte secretion. *J Physiol Pharmacol* 60(Suppl 7): 197–204.
- Baggaley E, McLarnon S, Demeter I, Varga G, Bruce JIE (2007) Differential regulation of the apical plasma membrane  $\text{Ca}(2+)\text{-ATPase}$  by protein kinase A in parotid acinar cells. *J Biol Chem* 282: 37678–37693.

## MARIANNA KÁTAY-KIRÁLY (2010)

### Neural differentiation of dental pulp stem cells

Supervisor: Gábor Varga

The plasticity of human dental pulp stem cells (DPSCs) has been demonstrated by several studies showing that they appear to self-maintain through several passages, giving rise to a variety of tissues, making them an attractive donor source for neuronal tissue replacement. Using already described standard protocols to differentiate them into mature neural cells, DPSC cultures exhibited only transient differentiation followed by reformation of fibroblasts or massive cell death. Our new three-step protocol, consisting of (1) epigenetic reprogramming, (2) simultaneous PKC/PKA activation, followed by (3) neural maturation, resulted in robust differentiation of DPSCs shown by cell morphology, immunocytochemistry, RT-PCR, and real time PCR for neural specific markers NES, N-tub, NGN2, Mash1, NSE, NF-M and GFAP. Moreover, patch clamp measurements showed the appearance of TTX sensitive sodium currents ( $\text{I}_{\text{Na}}$ ) and TEA sensitive delayed rectifier potassium currents ( $\text{I}_{\text{KDR}}$ ) in these cells.

To study their survival and differentiation *in vivo*, fluorescently labeled, neurally induced DPSCs were transplanted into the cerebrospinal fluid of 3-days-old male Wistar rats. In some experiments, a cortical cold lesion of the forelimb motor cortex of the rats was performed 1 week after the cell transplantation. To study the functional aspect of

neural cell formation, the labeled DPSCs harbored by 300  $\mu\text{m}$ -thick horizontal brain slices, specifically prepared for electrophysiological recordings were investigated. DPSCs implanted into the cerebrospinal fluid of the lateral ventricles of newborn rats migrated into a variety of brain regions. Most of the cells were localized in the progenitor zones of the brain: in the subventricular zone (SVZ), subgranular zone (SGZ) and subcallosal zone (SCZ). Immunohistochemical analysis revealed that the transplanted DPSCs expressed some of the above mentioned early neuronal and glial specific markers. Moreover, the cells displayed  $I_{\text{Na}}$  and  $K_{\text{DR}}$  currents. Four weeks after the injury, the cells migrated towards the lesion, and expressed these markers in a much higher proportion. Their measured  $I_{\text{Na}}$  and  $K_{\text{DR}}$  currents significantly increased.

Our results demonstrate that DPSC cells show evidence for neural differentiation and development both *in vitro* and *in vivo*, indicating that the dental pulp contains a cell population that is capable of neural commitment by our three step protocol. Therefore these cells can be considered as a stem cell source for regenerative therapy.

- Kádár K, Porcsalmy B, Király M, Molnár B, Jobbágy-Ovári G, Somogyi E, Hermann P, Gera I, Varga G (2009) Humán fogbél eredetű őssejtek izolálása, tenyésztése és jellemzése. *Fogorv Sz* 102: 175–181.
- Kádár K, Király M, Porcsalmy B, Molnár B, Rácz GZ, Blazsek J, Kálló K, Szabó EL, Gera I, Gerber G, Varga G (2009) Differentiation potential of stem cells from human dental origin —promise for tissue engineering. *J Physiol Pharmacol* 60(Suppl 7): 167–175.
- Király M, Porcsalmy B, Pataki A, Kádár K, Jelítai M, Molnár B, Hermann P, Gera I, Grimm WD, Ganss B, Zsembergy A, Varga G (2009) Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. *Neurochem Int* 55: 323–332.

## BEÁTA KERÉMI (2010)

### Functional investigation of oral tissues in health and disease

*Supervisor: Árpád Fazekas*

We used the non-invasive laser Doppler flowmetry (LDF) in order to determine the gingival (G) blood flow (BF) at different teeth in humans. GBF was recorded bilaterally, positioned the flow probe on three sites 1 mm above the marginal gingiva of the upper and lower central incisors and at six permanent teeth on the right side (11–16, FDI notation). No differences were observed between the mean GBF values at the left and right central incisors. The six upper right teeth also showed close similarity. There was a significant GBF value elevation at lower incisors as compared to that in the upper jaw. Our results indicate that there is homogenous marginal blood perfusion in both the maxillary and mandibula healthy gingiva.

The BF of the submandibular gland (SMG) measured by LDF was homogenous. The vasoregulatory role of nitric oxide (NO) in the circulation of the SMG of rats was studied. The NO synthase blocker N $\omega$ -nitro-L-arginine methyl ester (L-NAME) significantly increased vascular resistance (VR) in the SMG. The possible involvement of NO for the preservation of BF to the rat SMG after uni- or bilateral occlusion of the common carotid was studied.

In normal rats, carotid occlusion resulted in an immediate decrease in ipsilateral SMGBF. After the cessation of carotid occlusion, hyperaemia was observed in the SMG. Effect of L-NAME pretreatment, were identical between the control groups, though the magnitude of the alterations was significantly less. The SMGBF was well-maintained. NO had only a restrained effect. We investigated the effects of the pentadecapeptide BPC157 (1) on the vasculature of healthy and inflamed gingiva, (2) we determined the degree of inflammatory bone resorption around the whole tooth, and (3) we investigated the differences in the changes of bone structure. The GBF was measured by LDF in the upper central papilla before and after systemic BPC157 application, did not influence neither the general (mean blood pressure and heart rate) nor the local (GBF and GVR) haemodynamic parameters. The gingival capillary permeability was studied by the Evans-blue technique on the 13th day of ip. injection of BPC157 in ligature-induced periodontitis model. Ligature-induced experimental periodontitis resulted in increased gingival extravasation which was reduced by the daily administration of 10µg/bmkg and not by 100ng/bmkg BPC 157. There were no differences between the control sides of the groups. The alveolar bone morphometric parameters were studied by microCT. BPC157 significantly reduced the ligature-induced bone loss in the mesial, buccal, mesiolingual and lingual side. Chronic application of BPC157 has anti-inflammatory effect in experimental periodontitis.

- Vág J, Hably Cs, Kerémi B, Kovács E, Bartha J, Fazekas Á (2001) *Role of nitric oxide in the regulation of blood flow in the rat submandibular gland during carotid artery occlusion.* Arch Oral Biol 46: 261–267.
- Vág J, Kerémi B, Hably Cs, Bartha J, Fazekas Á (2002) *Angiotensin II type 1 (AT1) receptor blockade enhances the L-NAME induced vasoconstriction in rat submandibular gland.* Exp Physiol 87: 327–333.
- Kerémi B, Lohinai Zs, Komora P, Duhaj Sz, Borsi K, Jobbágy-Óvári G, Kálló K, Székely AD, Fazekas Á, Dobó-Nagy Cs, Sikiric P, Varga G (2009) *Antiinflammatory effect of BPC 157 on experimental periodontitis in rats.* J Physiol Pharmacol 60(Suppl 7): 115–122.

## MIKLÓS KOVÁCS (2010)

### Superficial and in-depth analysis of thin films considering the role of artefacts

Supervisor: Csaba Dobó Nagy

Thin films have a broad range of applications in electronics, in optics, in medicine, etc. The results of my dissertation contribute to this rapidly growing field. First, experimental results are presented for the excimer laser ablation of highly oriented pyrolytic graphite at 248 nm. The morphology and the depth of the ablated pits are monitored by atomic force microscopy, while the material characterization is performed by micro-Raman spectroscopy. Single shot ablation threshold was found to be 2,23 J/cm<sup>2</sup> laser fluence. Raman spectra indicate the formation of amorphous carbon layers as a result of laser irradiation. A several hundred nanometers-high ring-like structure can be observed around the ablated pits. The diameter of this structure increases with laser fluence. Ion microbeam analysis is an important technique in thin film characterization, however, the evaluation of its elemental maps are hindered by low counts/pixel statistics available within limited acquisition time, mainly at low elemental concentrations. I presented a method based on a non-linear iterative deconvolution scheme, designed to recover step-like profiles with forcibly

low counts. To demonstrate its performance and effectiveness, the algorithm was applied to one-dimensional profiles of elemental maps with a micrometer resolution, generated by micro Rutherford Backscattering Spectrometry (mRBS). A simulation-based method was also described, with which the degrading effect of micro-CT artefacts on certain parameters of the observed structure could be determined. Simulations were carried out with polychromatic and monochromatic X-ray source and a linearization method with a second-order polynomial fit algorithm was used in specific cases to correct the beam hardening artefact. This method was tested on a virtual half crown of a tooth with an artificial caries lesion. With the adjustment simulated in this study, micro-CT having polychromatic X-ray source resulted in the same level of error, as monochromatic one, if the linearization method to correct the beam hardening was used. The presented simulation based method is a useful way to determine artefacts caused distortions for other studies testing objects with different material and geometry.

- Kovács M, Kántor Z, Simon A (2003) *Reconstruction of low-count step-like signals in ion microbeam analysis. Vacuum 71: 53–57.*
- Kovács M, Danyi R, Erdélyi M, Fejérdy P, Dobó-Nagy Cs (2009) *Distortional effect of beam-hardening artefacts on micro-CT: a simulation study based on an in vitro caries model. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108: 591–599.*
- Kovács M, Fejérdy P, Dobó Nagy Cs: *A fém műtermék a fejnyaki cone-beam CT (CBCT) képeken. Fogorvosi Szemle 101: 171–178.*

## FERENC OBERNA (2009)

### Treatment of the malignant tumors of the oral cavity and the oropharynx with microsurgical reconstructive methods

*Supervisor: György Szabó*

90% of the tumors of the oral cavity and the oropharynx is planocellular carcinoma, which is the 6th most frequently occurring malignant tumor both worldwide and in Hungary. Patients often receive treatment only at an advanced stage of the disease, which is a combined oncotherapy, the most important part of which is the radical surgical intervention. The complex function of the oral cavity, the appearance of the patients, and their future role in society all change as a result of surgery. Primary free tissue transplantation may provide not only a cure but also an acceptable quality of life for the patient.

The objective of my work was to analyze the feasibility of microsurgical free tissue transplantation in the maxillofacial region, with emphasis on the treatment of oral cavity and oropharynx malignancies. We carried out 151 free tissue transplantations on 142 patients in the maxillofacial region between August 2003 and July 2008. The indication for free tissue transplantation was malignant tumor in 87% of the cases, the remaining 13% was other disease. 100 patients with oral cavity or oropharynx propagation received complex oncologic treatment. In 80 cases primary surgery, microsurgical tissue transplantation was carried out, along with adjuvant postoperative irradiation of 94% of the patients. Secondary surgery and microsurgical free tissue transplantation was carried out in 20 patients because of recurrence. The quality of life questionnaire was filled out by 50 surgically cured patients, and 22 patients receiving radiotherapy to retain function for base of

tongue carcinoma with exterior beam radiation and auxiliary interstitial high dose rate brachytherapy (HDR-BT).

I observed that in reconstructive surgery for soft tissue replacement (96%) the radial fasciocutaneous flap (RFF: 83%), the musculocutaneous latissimus dorsi flap (LD: 8.5%), for hard tissue or composite tissue replacement (4%) the fibula bone or bone-skin flap (FF: 8.5%) is suitable even with the low risk of complete (RFF: 6.6%, LD: 0% and FF: 0%) and partial flap necrosis (LD+FF: 1%). Surgical loupes provide adequate precision for microsurgical suturing of the vascular structures of these flaps. The success rate is 100% in the treatment of benign disease. It was only necessary to reoperate 50% of the intraorally transplanted radial free flaps after flap necrosis. Acute revision was carried out due to bleeding or flap circulation deficiency in 13% of the cases. Flap revascularization was successful in only in the case of early arterial occlusion, in 5% of the cases a second free tissue transfer was necessary. The second free flap was the latissimus dorsi myocutaneous flap, which has a take rate of 100%. Preoperative irradiation did not influence the success rate of the tissue transplantation. We recorded postoperative complications in 42% of the cases, out of this the healing complication of the forearm donor site (19.8%) can be highlighted. Complications necessitating newer surgical intervention occurred in 4.6% of the cases.

I found that the total survival rate of the patients with primary surgery and postoperative irradiation (OS: 69%) and the tumor specific survival (DSS 71%; ST I–IV 100–100–68–65%) is better than the rates achieved in the recurrent group (OS: 49%, DSS 51%; ST I–IV: 100–100–73–25%). In the group undergoing primary surgery, the tumor specific survival (DSS) is negatively influenced by the advanced tumor size (T1–3 vs. T4  $p=0.0331$ ), lymph node involvement (pN0/pN+  $p=0.0134$ ), the regional localization of the lymph node (one region lymph node involvement/multi region lymph node involvement  $p=0.0268$ ), and perineural tumor advancement ( $p=0.0498$ ). In the group of patients who were reoperated due to recurrence, negative predictors of the total and tumorspecific survival were lymph node metastasis (OS  $p=0.0124$ , DSS  $p=0.0045$ ), the involvement of more than one lymph node (OS  $p=0.0542$ , DSS  $p=0.0306$ ), extracapsular tumor advancement (OS  $p=0.016$ , DSS  $p=0.0058$ ) and multi level lymph node metastasis (OS  $p=0.0595$ , DSS  $p=0.032$ ).

I can establish from the evaluation of the quality of life of the treated and cured patients that the functional values significantly decreased in the primary surgical treatment group, and the extent of this was higher compared to the tongue radix tumor patients receiving radiotherapy alone. It can be shown, that reestablishing the continuity of the mandible did not influence the quality of life compared to those receiving only soft tissue replacement. The occurrence rate of decreased sense of taste and xerostomy following irradiation is similar, but the group undergoing surgery reported significantly less pain. In conclusion, I established that free tissue transplantation in the maxillofacial region is a safe and reliable, and is an invaluable surgical tool in the treatment of patients presenting with advanced oral cavity malignancies or tumor recurrences. The radical surgical intervention combined with free tissue transplantation and postoperative irradiation provide a realistic chance of curing this disease demanding so many casualties, while ensuring an acceptable quality of life.

• Takácsi-Nagy Z, Polgár Cs, Oberna F, Somogyi A, Major T, Remenár É, Fodor J, Kásler M, Németh Gy (2004) *Interstitial high-dose-rate brachytherapy in the treatment of base of tongue carcinoma. Strahlenther Onkol* 180: 768–775.



- Oberna F, Takácsi-Nagy Z, Réthy Á, Pólus K, Kásler M (2005) Buccal mucosal transposition flap for reconstruction of oropharyngeal-oral cavity defects: an analysis of 6 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 99: 550–553.
- Kásler M, Takácsi Nagy Z, Oberna F, Fodor J (2008) Salvage surgery for lokoregional failure definitive radiotherapy for base of tongue cancer. *In Vivo* 22: 803–806.

## EMIL SEGATTO (2010)

### Examination of orofacial orthopaedic disorders associated with spinal deformities

Supervisor: András Végh

There have been many publications on the relationship between the spinal deformities responsible for the development of postural disorders and the various dentofacial deviations. The majority of them deal with the dentofacial relevance of the scoliosis, some of them are on the orofacial orthopaedic deformities associated with the hyperkyphosis. This publication provides the results of an overall research series that determines the dentofacial character specific not only to the scoliotic deviation but also to the other most frequent spinal deformity—M. Scheuermann—and even to the poor body posture of different directions showing no spinal deformities yet.

The first part of the publication presents the orthopaedic features characteristic to the examination groups as well as the examination methods thereof. The former research results are provided in details; they give a description of the dentofacial features going together with the orthopaedic deviations.

The correlations between the individual orthopaedic parameters and the dentofacial deviations described in the former publications are mainly justified, but many new correlations are explored, too. The majority of these are based on examination methods that have not been used in the case of such a patient group so far. A group of the new examination methods used in the early phase of the research has not been used later due to the poor scientific value of the their outcome. The other group of the new examination methods provided such outcomes that significantly contributed to the development of the dentofacial character of the examined patient groups. The ponderous use of the mentioned methods in the digital image diagnostics made the modernisation of them necessary. It led to the development of such a radiograms analysing software that is capable of making an overall mandibular asymmetry examination due to its easy handling and very broad data analysing capacity.

The last part of the publication provides a detailed description of the outcomes of the former scientific researches serving as the basis of the mentioned examination software as well as the practical steps of the operation thereof.

The aim of the presentation of the published examination results and of the asymmetry examination software (that is intended to be a useful tool also for the interdisciplinary early recognition) is to provide a wider—and mutually useful—knowledge for the specialists of the two fields, orthopaedics and orthodontics in order to prevent and totally cure the orthopaedic and dentofacial deviations developing at the same age.

- Segatto E, Jianu R, Marschalko P, Vegh A (2006) Dentofacial features of children diagnosed with scoliosis and Scheuermann's disease. *TMJ* 56: 259–264.



- Segatto E, Lippold C, Vegh A (2008) Craniofacial features of children with spinal deformities. *BMC Musculoskelet Disord* 9: 169.
- Lippold C, Segatto E, Vegh A, Drerup B, Moiseenko T, Danesh G (2010) Sagittal back contour and craniofacial morphology in preadolescents. *Eur Spine J* 19: 427–434.

## ÁKOS SZÚCS (2009)

### Proliferation and exocrine transport mechanisms in epithelial glands

Supervisor: Gábor Varga

**Background:** Our aim was to determine the  $\text{HCO}_3^-$  transport mechanisms in salivary gland and human pancreatic cell line (Par-C10 and Capan-1), to study the mechanism of PKC and ERK activation on proliferation in Panc-1C cells and to investigate the role of regulatory peptide in the processes mentioned above. **Methods:** Intracellular pH was measured by microfluorometry. Epithelial polarization was assessed by immunolocalization of occludin. Expression of mRNA for key electrolyte transporters and receptors was evaluated by RT-PCR. DNA synthesis was studied by measuring  $[3\text{H}]$  thymidine incorporation, PKC and ERK cascade activation was detected with immunoblotting. Results: Par-C10 and Capan-1 cells grown on permeable supports formed confluent, polarized monolayers with well developed tight junctions. In Capan-1 cells the recovery of pH<sub>i</sub> from an acid load can be blocked by and H2DIDS. In Capan-1 cells dose-dependent increases in  $\text{HCO}_3^-$  secretion were observed in response to stimulation of both secretin and VPAC receptors. ATP and UTP applied to the apical membrane stimulated  $\text{HCO}_3^-$  secretion but were inhibitory when applied to the basolateral membrane in Capan-1 cells. However in Par-C10 cells application of ATP to the luminal and basolateral membrane resulted in a same accelerated fall in pH<sub>i</sub> caused by the inhibitors, UTP stimulated  $\text{HCO}_3^-$  secretion when applied to the luminal side, while basolateral administration was without effect. In Panc-1C cells PMA and CCK inhibited, NT stimulated DNA synthesis. These effects were inhibited by Ro-32-0432 but not by Gö6983. PMA, CCK and NT caused an ERK activation that was inhibited by Ro-32-0432. PMA, CCK and NT all activated cyclin D1, while p21CIP1 expression was increased by only PMA and CCK, but not by NT; each of these effects was inhibited by PD98059. Conclusion: In Capan-1 cells, at the basolateral membrane, an  $\text{Na}^+/\text{H}^+$  exchanger (probably NHE1) and an  $\text{Na}^+-\text{HCO}_3^-$  cotransporter (probably pNBC1) contribute to the cellular accumulation of  $\text{HCO}_3^-$ . There is also an  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransporter (probably NKCC1) which may generate a driving force for  $\text{Cl}^-$  secretion. Secretin, VIP and forskolin all stimulate  $\text{HCO}_3^-$  secretion across the apical membrane in Par-C10 and Capan-1 cells. We have also shown that both apical and basolateral purinoceptors regulate  $\text{HCO}_3^-$  secretion. Our results provide evidence for PKCε-mediated differential ERK activation and growth regulation in Panc-1C cells. Identification of the mechanisms by which these key signaling pathways are modulated could provide a basis for the development of novel therapeutic interventions to treat pancreatic cancer.

- Szucs A, Demeter I, Burghardt B, Ovari G, Case RM, Steward MC, Varga G (2006) Vectorial bicarbonate transport by Capan-1 cells: a model for human pancreatic ductal secretion. *Cell Physiol Biochem* 18: 253–264.

- Racz GZ, Szucs A, Szlavik V, Vag J, Burghardt B, Elliott AC, Varga G (2006) Possible role of duration of PKC-induced ERK activation in the effects of agonists and phorbol esters on DNA synthesis in Panc-1 cells. *J Cell Biochem* 98: 1667–1680.
- Demeter I, Szűcs Á, Óvári G, Varga G (2005) Capan-1 sejtek HCO<sub>3</sub> szekréciójában szerepet játszó transzportfolyamatok vizsgálata. *Orvostudományi Értesítő* 78: 56–60.

## PROGRAM 2/6.

### CLINICAL HAEMATOLOGY

#### **Coordinator:**

**Lídia SRÉTER M.D., Ph.D., D.Sc.**

2nd Department of Internal Medicine

46 Szentkirályi st, Budapest H-1088

Tel.: +36 1 266 0926, fax: +36 1 266 0816

E-mail: [sreter.lidia@med.semmelweis-univ.hu](mailto:sreter.lidia@med.semmelweis-univ.hu)

#### **Program overview**

Studies about the prognostic factors and complex therapies of haematologic disorders can contribute to more efficient treatment of this patient group. The role of infectious agents and environmental factors in the etiology of malignant lymphomas is a very interesting and up to date field of research (e.g. post-transplantation). We will study the diagnostic use of the methods of modern molecular biology and its efficiency in the follow-up control of our patients, too. The pathogenesis of the thrombosis in malignancies and the frequency of the cytokine gene-polymorphism and p53 mutations and their therapeutic importance. The pathophysiological bases of the plasma cell-dyscrasies and their therapeutic implications are also part of our research. The bone marrow transplantation was a breakthrough in the therapy of the malignant diseases. The hemopoietic stem cell transplantation can be a model of the immunotherapy, and it may be studied well. The connection of the specific immunological state after transplantation with the complications of the transplantation, with the relapse of the disease and with the survival is still subject of investigation even nowadays. The course of the development of the immunological tolerance after allogeneic transplantation is still unknown either. The investigation of the haematologic disorders can be the subject of the research, which belongs to the scientific bases of the public health priorities because of the high frequency of oncohaematological disorders.

#### ***Titles of research projects***

Clinical and molecular haemostasis research

Role of cytokine DNA polymorphisms in the pathogenesis of malignant haematological diseases

Myeloproliferative disorders and their familial aspects

Immunohaematological aspects of malignant lymphomas

Role of growth factors and their receptors in the regulation of the development and destruction of lymphoma cells

#### ***Supervisors***

Imre Bodó

Judit Demeter

Judit Demeter

Judit Demeter

László Kopper

Possibilities of individualized therapy in pediatric malignancies	Gábor Kovács
The possible role of neutrophil granulocytes in thrombosis associated to neoplastic diseases	Raymund Machovich
Study of liver injury following allogenic and autologous bone marrow transplantation: role of toxic and immunological factors	Tamás Masszi
Prognosis and treatment of malignant haematologic diseases	Tamás Masszi
Clinical administration of haematopoietic stem cells	Tamás Masszi
Human stem cell-membranetransport proteins and their alterations during cell differentiation	Balázs Sarkadi
Study of structure-function linkage in human ABC membrane-transport proteins	Balázs Sarkadi
Role of growth factors and their receptors in the regulation of the development and destruction of lymphoma cells	Anna Sebestyén
Role of infectious agents and environmental factors in malignant lymphoid tumors	Lídia Sréter
Role of mesenchymal stem cells in the regulation of immune processes	Ferenc Uher
Mesenchymal stem cells and regenerative medicine-stem cell therapy in type 1 diabetes	Ferenc Uher

### ***Ph. D. students***

Katalin Csordás	ft
Olivér Tamás Eipel	ft
Anikó Fodor	ft
Beáta Hegyi	ft
Karolina Nemes	ft
Péter Prekopp	pt
Bernadett Sági	ft
Márta Zubreczki-Hegyi	ft

### ***Ph. D. candidates***

László Gopcsa	na
Adrienn Mohl	na
Péter Pál Reményi	na

### ***Ph. D. graduates***

Valéria Dudics	pt
Krisztina Heltai	na
Veronika Urbán (Suhajda)	pt

ft, full-time; pt, part-time; na, not affiliated

### ***Supervisors***

Gábor Kovács
Gábor Kovács
Judit Demeter
Ferenc Uher
Monika Csóka
Lídia Sréter
Ferenc Uher
Gábor Kovács

### ***Supervisors***

Tamás Masszi
Imre Bodó
Tamás Masszi

### ***Supervisors***

Ferenc Uher
István Vályi Nagy
Ferenc Uher

## ***Abstracts of Ph.D. theses successfully defended in 2009***

**VALÉRIA DUDICS (2009)**

### **Mesenchymal stem cells as potential source for musculoskeletal diseases mainly for cartilage repair**

*Supervisor: Ferenc Uher*

Because of the lack of vascularity and paucity of cellularity, articular cartilage damaged by disease or trauma has a limited capacity for regeneration. Mesenchymal stem cells (MSCs) have the potential to differentiate into distinct mesenchymal tissues; including cartilage and bone hence they can be attractive cell source for cartilage tissue engineering approaches. Marrow samples were removed during bone surgery and adherent cell cultures were established. The cells were then passed into a newly developed microaggregate culture system in a medium containing transforming growth factor- $\beta$ 3, insulin, dexamethasone, and/or demineralized bone matrix. *In vitro* chondrogenic activity was measured as metabolic sulfate incorporation in pellet cultures. Cell aggregates were also analyzed by histology and by weight of newly synthesized cartilage. Our findings show that DBM possess all the necessary conductive features of a carrier, serving at the same time as a natural source of inductive chondrogenic factors.

One of our objective here was to compare the *in vitro* chondrogenic potential of MSCs isolated from patients with rheumatoid arthritis (RA) and osteoarthritis (OA) with cells from normal donors. Culture-expanded MSCs from RA and from OA patients did not differ significantly from the normal population with respect to their chondrogenic potential *in vitro*, therefore these cells in these patients may serve a potential new prospect in the cartilage replacement therapy as well.

Galectin-1 stimulated the chondrogenic differentiation of mesenchymal cells in low concentration. We have evaluated the effect of recombinant human galectin-1 on the proliferation and survival of murine and human hematopoietic stem and progenitor cells. We show that low amount of galectin-1 increases the formation of granulocyte-macrophage and erythroid colonies. Gal-1 blocked BM progenitor cell migration induced by CY/G-CSF treatment, indicating a novel anti-inflammatory function of the lectin. We have found that low and moderate amount of galectin-1 stimulate the chondrogenic differentiation of MSCs. Therefore, recombinant galectin-1 might be used during cellular therapy of inflammatory joint and bone diseases as an anti-inflammatory agent.

- Vas V, Monostori É, Fajka-Boja R, Dudics V, Uher F (2005) *Biphasic effect of recombinant galectin-1 on the growth and death of early hematopoietic cells. Stem Cells* 23: 279–287.
- Kiss J, Kunstár A, Fajka-Boja R, Dudics V, Tóvári J, Légrádi Á, Monostori É, Uher F (2007) *A novel anti-inflammatory function of human galectin-1: inhibition of hematopoietic progenitor cell mobilisation. Exp Hematol* 35: 305–313.
- Dudics V, Kunstár A, Kovács J, Lakatos T, Géher P, Gömör B, Monostori É, Uher F (2009) *Chondrogenic potential of mesenchymal stem cells from patients with rheumatoid arthritis and osteoarthritis—measurements in a microculture system. Cells Tissues Organs* 189: 307–316.

**KRISZTINA HELTAI (2009)****New risk factors for atherosclerosis**

*Supervisor: István Vályi Nagy*

Nowadays, inflammation and autoimmune processes are of crucial importance to the development and progression of atherosclerosis, their role is comparable to the significance of the conventional risk factors.

They produce cytokines, adhesive molecules, CRP, antibodies, both autoimmune and anti-pathogen ones, that have an effect on every molecular mechanism known till now causing endothelial injury and accelerating atherosclerosis. Thus there is clear evidence now that they contribute to the deterioration of atherosclerosis.

For two decades, the histological examination of both human and animal organs, animal experiments reproducing the disease and much seroepidemiologic data have confirmed that chronic infection and the presence of all pathogens lead to the progression of the atherosclerotic progress.

One of our studies also proved that anti-*Chlamydia pneumoniae* IgG level increased in consequence of chronic *C. pneumoniae* infection has a positive correlation with the prevalence of coronary artery diseases and elevated cholesterol level, too, indicating that chronic infection is a risk factor of coronary artery diseases.

Another study focused on the joint impact of the chronic infection and autoimmune processes from the aspect of the risks of stable angina pectoris and myocardial infarction. We revealed (in accordance with the international results) that, among the pathogens we studied, chronic *C. pneumoniae* infection is related to coronary artery diseases, whereas CMV and HSV ones are not. Patients with infarction have significantly higher anti-*Chlamydia pneumoniae* IgG levels compared to the control group. When both anti-*Chlamydia pneumoniae* IgG level was positive in high dilution and anti-HSP antibody level characteristic of autoimmune processes rose, there was a remarkably increased risk of both stable and acute coronary illnesses. The greatest (15.5 times high) increase in risk of myocardial infarction was found with elevated anti-*Chlamydia pneumoniae* IgG and anti-hHSP60 levels, while, in case of stable angina pectoris, it was 8 times higher with elevated anti-*Chlamydia pneumoniae* IgG and anti-mHSP65 levels. These results also confirm, that the joint presence of chronic infection and autoimmune processes increase the probability of the development of atherosclerotic vascular diseases, including coronary artery diseases, too.

Many trials were carried out to reveal the role which the pathogens and inflammatory processes adventitiously reactivated by percutaneous intervention has among the now only partly known contributors of restenosis. Our study showed (as we know, for the first time), that pathogen DNA can be detected in a higher proportion from the blood samples after the percutaneous intervention, than before it, and IL-6 and CRP levels were also remarkably higher. According to these results, percutaneous intervention can activate pathogens in the plaques and inflammation, however, a trial with a greater case number is needed to clarify the relationship between these processes and restenosis.

CRP level, as an aspecific inflammatory marker, is widely used in clinical practice. Nowadays, it is an accepted opinion that CRP is a predictive and independent risk factor of the atherosclerotic vascular diseases. Its role in increasing risk is as strong as high HDL cholesterol level, diabetes, and elevated troponin level in acute coronary syndrome.

According to our results, CRP level measured in ST-segment elevation myocardial infarction has a strong relation with the extent of the coronary artery disease, the higher the CRP level is, the more of significant stenoses are on the great coronary arteries.

- Heltai K, Marton A, Ludwig E, Szabóki F (2000) *Chlamydia pneumoniae és a coronaria betegség. Cardiol Hung* 1: 5–9.
- Heltai K, Kis Z, Burian K, Endresz V, Veres A, Ludwig E, Gönczöl É, Vályi-Nagy I (2004) *Elevated anti-Chlamydia pneumoniae, anti-HSP60 and anti-HSP65 antibody levels are independent risk factors in myocardial infarction and ischemic heart disease. Atherosclerosis* 173: 339–346.
- Petrovay F\*, Heltai K\*, Kis Z, Tresó B, Gonczol E, Burian K, Endresz V, Vályi-Nagy I\* (2007) *Chronic infections and histamine, CRP and IL-6 levels after percutaneous transluminal coronary angioplasty. Inflamm Res* 56: 362–367 (\*contributed equally to the work).

## VERONIKA URBÁN (SUHAJDA) (2009)

### A possible stem cell therapy for diabetes mellitus

Supervisor: Ferenc Uher

**Introduction:** Recent studies have suggested that the adult bone marrow harbours cells that can influence  $\beta$ -cell regeneration in diabetic animals. Other reports, however, contradicted these findings. Our aim was to elucidate this question. **Materials and Methods:** To address this issue, we used an animal model with type 1 diabetes in which the disease was induced with streptozotocin (STZ) in female C57Bl/6 mice. Freshly prepared sex-mismatched bone marrow cells (BMC) and syngeneic or allogeneic mesenchymal stem cells (MSC) were concomitantly administrated into sublethally (250 cGy) irradiated diabetic mice. **Results:** Blood glucose and serum insulin concentrations rapidly returned to normal levels accompanied with efficient tissue regeneration after a single injection of a mixture of 106 BMC/105 MSCs. Successful treatment of diabetic animals was not due to the reconstitution of the damaged islet cells from the transplant since no donor-derived  $\beta$ -cells were found in the recovered animals, indicating a graft initiated endogenous repair process. Moreover, MSC injection caused the disappearance of  $\beta$ -cell-specific T lymphocytes from diabetic pancreas. **Conclusion:** We suggest that two aspects of this successful treatment regimen operate parallelly in our model. First, BMCs and MSCs induce the regeneration of recipient-derived pancreatic insulin-secreting cells. Second, MSCs inhibit T-cell-mediated immune responses against newly formed  $\beta$ -cells. Thus, the application of this therapy in human patients suffering from diabetes may be feasible. human patients suffering from diabetes may be feasible.

- Kertész Z, Vas V, Kiss J, Urbán VS, Pozsonyi É, Kozma A, Pálóczi K, Uher F (2006) *In vitro expansion of long-term repopulating hematopoietic stem cells in the presence of immobilized Jagged-1 and early acting cytokines. Cell Biol Internat* 30: 401–405.
- Urbán VS, Kiss J, Kovács J, Gócza E, Vas V, Monostori É, Uher F (2008) *Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. Stem Cells* 26: 244–253.



**PROGRAM 2/8.****PHYSIOLOGY AND PATHOLOGY OF THE MUSCULO-SKELETAL SYSTEM*****Coordinator:*****Miklós SZENDRŐI M.D., Ph.D., D.Sc.**

Department of Orthopaedics

27 Karolina st, Budapest H-1113

Tel.: +36 1 466 6611

E-mail: [szenmik@orto.sote.hu](mailto:szenmik@orto.sote.hu)**Program overview**

The Ph.D. Program is designed for medical doctors who wish to be specialized in basic science and clinical research of musculoskeletal medicine, orthopaedics, trauma surgery and rheumatology. Our aims are: to provide medical and science based students with comprehensive knowledge in the field of orthopaedics and trauma surgery, and rheumatology, and surgery of the bone and soft tissue tumors, to provide suitable environment for clinical or biomechanical laboratory based research projects, to enable students for the use of laboratory techniques such as classical histology, immunohistochemistry, collagen typisation and to train students in modern biomechanical laboratory techniques, as gait analysis.

***Titles of research projects***

Articular sport injuries of the knee

Development of assistive products for persons with mobility impairments

Measurement in rehabilitation medicine

Application of high technology in rehabilitation

Role of psychosocial factors in the rehabilitation of patients with bone fracture due to osteoporosis

Clinical and experimental examination of treatment options in injuries of the articular loaded surface

Effect of orthopedic abnormalities and injuries on movement—Orthopedic movement analysis

Migration of total joint replacement investigated by using radio-stereophotogrammetric analysis

Importance of secondary conditions in the physical and rehabilitation medicine process

Infection and autoimmunity in inflammatory diseases of the joints

Examination of synovial sarcoma SYT-SSX fusion gene products in tissue cultures and xenografts

Clinical oncology in bone and soft tissue tumors

Non-surgical treatments of juvenile and aneurysmal bone cysts: sclerotization, cyst modelling and examination of cyst remodelling

Quality of life after the complex therapy of bone tumors

***Supervisors***

István Berkes

Péter Cserhádi

Zoltán Dénes

Gábor Fazekas

Gábor Fazekas

László Hangody

Rita M. Kiss

Jenő Kiss

Lajos Kullmann

Gyula Poór

Zoltán Sági

Miklós Szendrői

Miklós Szendrői

Miklós Szendrői

Occurrence and treatment (minimal surgical interventions) of bone metastases	Miklós Szendrői
Recidival tumor forming ability, malignization in borderline bone tumors	Miklós Szendrői
Examination of prognostic factors in certain bone tumors	Miklós Szendrői
Replacement options in extensive bone defects, comparative study of massive osteochondral homografts, autografts, endoporthesis	Miklós Szendrői
Biomechanical effect of percutaneous vertebroplasty in osteoporotic vertebral fractures on the neighbouring vertebrae: clinical and laboratory investigations	István Szikora
Histological and kinetic alterations in diseases and developmental disorders of the locomotor apparatus	György Szőke

***Ph.D. students***

Szabolcs Benis	pt (a)
István Béla Bors	pt (a)
Dóra Mihola (Dombayné)	pt
Zsuzsa Fekete	pt (a)
Ágnes Fincziczki	ft
László Fónyad	pt (a)
György Hangody	ft
László Rudolf Hangody	ft
Gergely Holnapy	pt
Lőrinc Ijjas	ft
Ákos Kecskeméti	pt (a)
Bernadett Német (Kertész)	pt
Mariann Kiss-Polauf	pt
Zsuzsa Márialigeti	pt
Dorottya Judit Nagy	ft
Lívia Nagy	pt
Zoltán Oláh	pt
Orsolya Péter	pt
Kálmán Rábai	pt
Károly Schandl	pt
Gábor Skaliczki	pt
Mária Takács	pt
Szilvia Zsilák	pt (a)

***Supervisors***

Tamás Farkas
Rita M. Kiss
Gyula Poór
Gyula Poór
Géza Bálint
Zoltán Sági
György Szőke
György Szőke
Rita M. Kiss
György Szőke
János Hamar
György Szőke
Jenő Kiss
Géza Bálint
Gyula Poór
Lajos Kullmann
Miklós Szendrői
Gábor Fazekas
Péter Cserhádi
Zsombor Lacza
Miklós Szendrői
Rita M. Kiss
Gyula Poór

***Ph.D. candidates***

Kristóf Andrónyi	pt
István Baráth	pt
Gyula Domos	ft
Tamás Lőrincz	na
György Tibay	pt

***Supervisors***

Gábor Krakovits
Tibor Mózes
György Szőke
Miklós Szendrői
László Hangody

***Ph. D. graduates***

Lajos Bartha	pt
Zoltán Bejek	pt
István József Kádas	pt
Sándor Kiss	na
Ákos Péter Kynsburg	ft
Károly Pap	ft
Borbála Pazár	pt
Judit Ildikó Pulai	na
Zsolt Vendégh	pt

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

***Supervisor***

László Hangody
Miklós Szendrői
Zoltán Magyari
György Szőke
Miklós Szendrői
György Szőke
Gyula Poór
Gyula Poór
János Hamar

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****LAJOS BARTHA (2009)****Data related to the problems of biodegradable materials in the treatment of articular cartilage defects**

*Supervisor: László Hangody*

The biomechanically solid hyalin cartilage is unable to regenerate in adults. The treatment of full thick defects of the joint surfaces is one of the most significant challenges of the orthopedic practice. In the last decades especially intensive research is ongoing to find the methods to fill the full thick defects of the joint surfaces with hyalin quality substance to prevent OA. In my work the actual surface composition procedures, the recent results of surface creation are presented along with my own animal and clinical experiments. In animal experiments I investigated six different biodegradable substances used in filling up the donor areas, to prevent a rare potential complication, the postoperative joint effusion. My objective was to find a substance inhibiting the bleeding of the donor site and to form an implant having similar biological, physicochemical characteristics which enhances the renovation of the joint surfaces. My endeavor was to include such materials to fill up the donor areas in the animal experiments, which have no license yet for human procedures. I examined six different biodegradable filling materials. Between tested materials the collagen implants easily penetrated by blood, permitting invasion of mesenchymal cells. They provide good base the create fibrous cartilage on the articular surface and do not limit vascular and tissue integration in the deeper layers. The substances used in animal tests all blocked postoperative bleeding. Between investigated materials only the compressed collagen however fulfilled the other important criteria and promoted impregnation of blood, allowing invasion of stem cells and cellular and vascular invasion. A further criterion was to allow creation of acceptable fibrous cartilage on the surface. According to the histological results, between tested materials, only the compressed bovine collagen correspond to this conditions.

However in 2005 we pioneered in the World filling up the donor site in human mosaic-plasty procedures with PolyActive-B! This material has been approved by the FDA in the

USA, this fact was also important in our choice. Based on advantageous animal tests and on routine oral surgical practice the assumption was made, that this material will not purely replace bone, but it creates fibrous cartilage on the surface, fulfilling our expectations. In the our clinical tests the PolyActive-B plugs implanted in human donor areas prevented the postoperative bleeding of the joint, at the same time they did not hinder the healing of the donor channels. Conclusion based on our histological analysis prove that the PolyActive-B plugs got impregnated with blood, facilitating such a way the invasion of mesenchymal stem cells. They formed an appropriate scaffold structure to ensure a good quality base for the reparation of the osteochondral defect. Healing the defect resulted in good quality filling. Inside the PolyActive-B plugs excellent vascularization and cellular integration was observed. No inflammation, arthrofibrosis or foreign body reaction was detected, proving the biocompatibility of the PolyActive-B plugs. The most significant result of my work is to discover, that both substances used in my animal and clinical tests blocked the unwanted postoperative joint bleeding.

New result is in my work to prove, that both the compressed collagen used in my animal tests and the PolyActive-B used in human experiments fills up with blood according to our expectations. Our tests proved that these materials can provide the planned and suspected mesenchymal cellular invasion.

The second and equally important result of my work is to prove the creation of congruent surfaces using PolyActive-B in the human tests. Histology proved the degradation of this substance with local new bone formation. Surface of the PolyActive-B plugs enhance creation of fibrous cartilage. Based on research and theoretical considerations the PolyActive-B could be the basis to find further cartilage surface creative techniques.

- Hangody L, Vásárhelyi L, Hangody LR, Sükösd Z, Tibay Gy, Bartha L, Bodó L (2008) *Autologous osteochondral grafting—technique and long-term results*, *Injury* 39: 32–39.
- Pehlivan M, Bartha L, Duska Zs, Hangody L (2003) *Autologous osteochondral mosaic-plasty—rationale and clinical practice*. *Artroplasti Artroszkopik Cerrahi/J Arthroplasty Arthroscopic Surgery* 14: 59–66.
- Bartha L, Hangody L, Kaposi Novák P, Vajda A (2007) *The role of biodegradable materials in the treatment of articular cartilage defects*. *EKLEM Hastalıkları ve Cerrahisi/Joint Diseases & Related Surgery* 18: 101–107.

## ZOLTÁN BEJEK (2010)

### The use of computer navigation for total knee arthroplasty—a follow up of rehabilitation by gait analysis

*Supervisor: Miklós Szendrői*

The primary objective of the research was to examine the influence of computer navigation on total knee arthroplasty. The other goal was the follow up of the postoperative period and the rehabilitation. Since we used the device for minimal invasive technique operations as well, we focused on the effects of computer navigation on this surgical approach also. It could be determined that the device increased the positioning accuracy of the components in both groups. The biomechanical problems due to malpositioning only emerge later on, therefore we compared the direct postoperative results of the minimal invasive group to the outcomes of patients operated by the conventional method.

We found that there is less postoperative blood loss as well as faster improvement in knee movement in the minimal invasive surgery group.

We used the ZEBRIS gait analysis device for long term follow up, which proved to be informative for our examination when used at 2 kilometers/hour walking pace.

With the help of the device, we were able to conduct the one year follow up examinations for the three surgical groups, and we recorded and compared the gait parameters preoperatively, then 3, 6, 9, and 12 months after the operation. With each parameter analyzed, we found characteristic values, which dynamically changed with time in each of the three groups. It was observed that the rate of rehabilitation did not differ between the conventional and computer navigated surgery groups. However, the rate of rehabilitation in the minimal invasive surgery group proved to be faster. Even three months after the operation, the difference could be observed to the benefit of the minimal invasive group. The EMG tests further proved this fact. The recuperation of the extensor apparatus was faster in the minimal invasive group also.

Based on the previous mentioned, computer navigation improves the positioning accuracy of the prosthesis components, especially in the minimal invasive surgical approach, where visibility is reduced due to the technique itself.

The ZEBRIS gait analysis system is competent for the one year rehabilitation follow up of patients who underwent total knee replacement surgery. With its help, it was proven that the rate of rehabilitation in the minimal invasive group is faster, not just in the early postoperative period. Even three months after the operation, a difference was observed to the benefit of the minimal invasive group.

- Bejek Z, Paróczai R, Illyes A, Kiss RM (2006) *The influence of walking speed on gait parameters in healthy people and in patients with osteoarthritis. Knee Surg Sports Traumatol Arthrosc* 14: 612–622.
- Bejek Z, Sólyom L, Szendrői M (2007) *Experiences with computer navigated total knee arthroplasty. Int Orthop* 31: 617–622.
- Bejek Z, Paróczai R, Illyés Á, Kocsis L, Kiss RM (2006) *Gait parameters of patients with osteoarthritis of the knee joint. Facta Univ: Ser Phys Educ Sport* 4: 9–16.

## ISTVÁN JÓZSEF KÁDAS (2009)

### Pathomechanism of open tibial fractures and comparative analysis of the treatment strategies

Supervisor: Zoltán Magyar

**Introduction:** Treatment of tibial fractures always requires a great effort. In case of bone fractures, the internal static support of the human body gets injured. If an internal fixation would result in adverse effects, the external fixator is applied as an external static frame. In terms of static considerations, this external frame is different from the internal one: it provides less stability but its impact on the fracture is variable both in space and time. In case of open tibial fractures also severe soft tissue damage coexists. Its treatment requires application of individual protocols. There is no unique way of treatment, since no stable internal fixation can be performed in the infected environment, and, in turn, instability increases the risk of septic complications. A self-generating vicious circle develops. We tried to find a solution for this problem.

**Material and methods:** We experienced high ratio of septic complications after monotherapies (external fixator, plate, intramedullary nail). Combined therapies had favorable influence on the final outcome. Synergy of advantages of the applied methods was successful, thus we used these ways of treatment consistently. 658 patients were treated for open tibial fractures at the National Institute of Traumatology and Emergency Medicine during the course of 15 years. In the initial phase (Group A) we applied monotherapies, such as plating and later an external fixator and intramedullary nailing. The option of combined treatment, i.e. changing the treatment method was introduced in the last 10 years (Groups B and C). We performed 352 monotherapies during the course of the investigated period of time (15 years): Plate: 74, E. F.: 112, reamed IM nail: 104, UTN: 62 cases. 270 cases were treated in a combined therapy manner. E. F. + reamed IM nail: 100, E.F + UTN: 57, E. F. + plate: 30, whilst E. F. + brace: 83. Other ways of treatment were applied in case of 36 patients.

**Results:** As a favorable result of management technique, the rate of primary soft tissue consolidation has gradually increased. Group A: 66%, Group B: 75.7% and Group C: 80.9%. Also the ratio of septic complications has decreased accordingly. A: 15.5 %, B: 9.4%, C: 6.6%. Application of recently developed stabilizing devices resulted in increasing ratio of bony consolidation. The ratio of bone healing disturbances has decreased: A: 31.6%, B: 18.0%, C: 8.7%. The rate of amputations has considerably decreased: A: 4.9%, B: 2.3%, C: 0.7%. These highlighted data indicate that healing expectancies of open tibial fractures are directly improved by elaboration, introduction and consistent application of treatment protocols.

**Conclusions:** Favorable effect can be achieved via combination of advantages and reasonable elimination of disadvantages of the different methods. On the basis of the followup data, after primary advantageous extra-focal application of an external fixator, open tibial fractures can be optimally treated via the change of treatment prior to onset of septic complications.

- Kádas I, Magyari Z, Vendégh Z, Gloviczki B (2009) *Changing the treatment to reduce complication rate in open tibial fractures. Int Orthop* 33: 1725–1731.
- Vendégh Z, Melly A, Tóth B, Wolf K, Farkas T, Józán J, Hamar J, Kádas I (2009) *Effects of neuropeptides and vasoactive substances on microcirculation of the callus after tibial osteotomy in rabbits. Acta Vet Hung* 57: 427–439.

## SÁNDOR KISS (2010)

### Special use of fixateur externe in the treatment of developmental musculoskeletal disorders and bone tumours

Supervisor: György Szőke

The development of the external fixators has enabled the spread of limb lengthening. Even with the recent technical advances several open questions have still remained in the practice. (1) An important one of them is the final leg length discrepancy at maturity. In fibular hemimelia, which is the most frequent dysplasia of the leg there is a late in maturity at younger age. However, later the maturity of the bones is accelerated, and these patients reach the skeletal maturity sooner than the unaffected children. Consequently the use of Moseley charts for prediction of leg length discrepancy in fibular



hemimelia gives an incorrect result. We have constructed a new chart based on our series of data of skeletal age which is more accurate at prediction of leg length discrepancy in this disease. (2) The contracture of joints is one of the most frequent and most serious complications of the leg lengthening. Therefore we examined the range of motion of the malleolar joint during leg lengthening at different rates of distraction at animal model. We have demonstrated that the rise of the daily rate causes exponentially elevated danger of restriction of motion. The normal daily rate in the group of young animals did not cause any restriction of motion, while in the group of mature animals contracture is already observed. Doubled daily rate in the group of young animals caused a moderate loss in motion, while in the group of mature animals this led to the serious restriction of the ankle motion. The rise of the extent of lengthening with normal daily rate caused a moderate loss of motion in the group of young animals. (3) In human practice we have proved that the unilateral device for humeral lengthening was suitable, we were able to leave the fixator in the humerus until total bony reconstruction, so there was no need for plate fixation or bone transplantation. Significant, 50 percent humeral shortening could be corrected entirely by this method. (4) Excision of the exostosis of the ulna did not effect the deformity of the forearm and the ulnar instability of the radiocarpal joint in the disease of multiple hereditary exostosis with Masada type I. and II/b ulnar shortening. The slip of carpal bones could be treated effectively by the lengthening of the ulna. The lengthening was performed easily by linear unilateral device. (5) We have demonstrated a new method for the treatment of malignant bone tumours affecting the proximal metaphysic region of the tibia. We could save the proximal epiphysis and use free fibular autologous graft for restoring the integrity of the tibia, and used an Ilizarov ring fixator for fixation. We have achieved a tumour free status and a good loadable extremity.

- Kiss S, Pap K, Vizkelety T, Terebessy T, Balla M, Szőke Gy (2008) *The humerus is the best place for bone lengthening. Int Orthop 32: 385–388.*
- Pap K, Berki S, Shisha T, Kiss S, Szőke Gy (2009) *Structural changes in the lengthened rabbit muscle. Int Orthop 33: 561–566.*
- Kiss S, Domos Gy, Berki S, Terebessy T, Szőke Gy (2009) *A bokaízület mozgásterjedelmének változása lábszárhosszabbítás során, állatkísérletes modellen. Magyar Traumat 52: 159–163.*

## ÁKOS PÉTER KYNSBURG (2009)

### The role of proprioceptive training in the prevention of lateral ankle ligament injuries

*Supervisor: Miklós Szendrői*

Main goal of my study was to prove the positive proprioceptive effect of a preventively applied neuromuscular training in competitive athletes of a high-risk sport—with an epidemiologically already proven preventive effect on ankle ligament injuries.

First, in order to identify the sport with the highest injury-risk, I defined the sports-specific incidence of ankle injuries based on comparable literature data. Processing 119 relevant papers in full-text, published until February 2002, only 61 provided comparable exposition-based, sports- and ankle-specific incidence data. Based on these data, contact team sports feature the highest injury-risk, with handball at the top of the list.

Applying the neuromuscular training in a therapeutic fashion for 6 weeks on young athletes with chronic lateral talocrural instability I observed, that the proprioceptive sensory function of the ankle plantarflexors—represented by the posterior slopes—on the injured side did not differ significantly from healthy controls before the start of the programme; at follow-up this function improved vastly and became significantly better, compared to controls. This is an indirect sign on the preventive effect of the training. In accordance with the epidemiological data I examined the proprioceptive sensory effect of preventively applied neuromuscular training on twenty ankles of ten elite-level female handball players. As a result proprioceptive sensory function of the ankle improved in all directions as well as in every single directions strongly significant, also in comparison to healthy controls. This also justifies the name “proprioceptive training” for this preventive method.

Regarding improvement between single directions, and also between dominant and non-dominant sides, there were no significant differences. In comparison to the therapeutic group it can be stated, that in patients with chronic lateral talocrural instability proprioceptive training should be continued on long-term at a lower intensity after it was successfully applied therapeutically.

While applying proprioceptive drills in a preventive fashion, incidence changes have been similar to those of earlier epidemiological studies, investigating larger samples.

As conclusion I consider the incorporation of proprioceptive drills into the regular training regimen in contact team sports indispensable, in other sports recommended for the prevention of ankle injuries.

- Halasi T, Kynsburg Á, Tállay A, Berkes I (2004) *Development of a new activity score for the evaluation of ankle instability. Am J Sports Med* 32: 899–908.
- Halasi T, Kynsburg Á, Tállay A, Berkes I (2005) *Changes in joint position sense after surgically treated chronic lateral ankle instability. Br J Sports Med* 39: 818–824.
- Kynsburg Á, Halasi T, Tállay A, Berkes I (2006) *Changes in joint position sense after conservatively treated chronic lateral ankle instability. Knee Surg Sports Traumatol Arthrosc* 14: 1299–1306.

## KÁROLY PAP (2009)

### The response of striated muscle to extremity lengthening

*Supervisor: György Szőke*

The response of the muscle is critical in determining the functional outcome of the limb lengthening. My thesis consists of two parts: basic and clinical research. In the first part we evaluated the histological and immunohistochemical changes in the muscle tissue after limb lengthening in skeletally mature and immature rabbits. 23 male New-Zeland white rabbits, divided into six groups, were operated on and different lengthening protocols were used in mature and immature rabbits. The histopathologic changes were analyzed by a semiquantitative method according to the scoring system of Lee *et al* (1994). The changes of proliferative activity of muscle progenitor satellite cells were measured by bromodeoxyuridine immunostaining. After evaluation of the five main degenerative parameters and the changes of the dividing activity of satellite cells it is evident that the adults lengthened at a rate of 1.6 mm/day showed more degenerative changes than those

lengthened at 0.8 mm/day. The adult 1.6 mm/day lengthened group presented significantly higher damage in the muscle and lower regenerative signs compared with the young 1.6 mm/day lengthened group, according to the summarized degenerative scores.

The aim of our clinical research was to examine the muscle response after lengthening the humerus in children and young adults. Between 1984 and 2005, 11 humeri were elongated with Wagner fixator at the Orthopaedic Department of Semmelweis University. The average age of patients at the time of surgery was 17.8 years (12–31). The lengthening protocol was 1 mm distraction daily (callotaxis) after 7 days latency period. The average rate of lengthening was 6.2 (4.5–10.5) cm, the achieved lengthening was 27% (16–44%). One patient suffered from temporary radial paresis, and temporary flexion contracture of the elbow was observed as complication after placing the fixator. According to our results, the muscle associated complications were less frequent than in the lower limb at same lengthening rate. The muscle function during the lengthening was good, and did not inhibit their daily life. We could leave the fixator in the humerus until total bony reconstruction so there was no need for plate fixation or bone transplantation, and this led a fast functional recovery of the upper extremity after the fixator removed.

- Pap K, Berki S, Shisha T, Kiss S, Szőke G (2009) *Structural changes in the lengthened rabbit muscle. Int Orthop* 33: 561–566.
- Kiss S, Pap K, Vizkelety T, Terebessy T, Balla M, Szőke G (2008) *The humerus is the best place for lengthening. Int Orthop* 32: 385–388.
- Shisha T, Kiss S, Pap K, Simpson H, Szoke G (2006) *Relative ability of young and mature muscles to respond to limb lengthening. J Bone Joint Surg Br* 88B: 1666–1669.

## **BORBÁLA PAZÁR (2010)**

### **Role of non-MHC genes in the pathogenesis of immune-mediated rheumatic diseases**

*Supervisor: Gyula Poór*

Genetic factors play important role in the pathogenesis of immune-mediated rheumatic diseases such as rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS). In all three diseases well-known MHC associations have already been described. Recent studies show evidence of non-MHC genes as susceptibility factors of the immune-mediated diseases as well. We examined the role of HLA-DRB1 alleles in susceptibility to JIA and the role of the HLA-B27 subtypes in susceptibility to AS in Hungarian population samples. Our results concerning the HLA-DRB1 associations of JIA and the HLA-B27 associations of AS in Hungarians are similar to previously reported data in other Caucasian populations. Among the non-MHC genes we examined the TLR4 Asp299Gly and Thr399Ile polymorphisms in AS and reactive arthritis (ReA), the TLR2 intron 2 GT microsatellite polymorphism in RA, AS and JIA. The above mentioned TLR4 polymorphisms are not associated with AS or ReA in Hungarians. The TLR2 intron 2 GT microsatellite polymorphism confer susceptibility to development of chronic inflammation in RA and AS via increasing TLR2 expression. We determined the MBL2 polymorphisms and serum MBL level in Hungarian JIA patients. Low MBL levels were characteristic for both polyarticular and oligoarticular JIA together with the presence of low MBL2 genotypes. Low MBL serum level could predispose to JIA by increasing suscepti-

bility to microbial infections. We determined the prevalence of the PTPN22 C+1858T SNP in Hungarian JIA patients. In our population we found no association between the PTPN22 C+1858T SNP and JIA. Comparing our results with studies on Finnish and Japanese populations we assume that the PTPN22 association contributes to the development of JIA only in certain population. We examined the recently identified 5 ARTS1 SNPs and 9 IL23R SNPs in AS in Hungarians. In our population sample, the variant allele frequencies of the ARTS1 and IL23R do not differ significantly from the previously observed frequencies in US and UK cohorts. Thus we confirm that non-MHC genes also have remarkable effect on disease development in AS.

- Pazár B, Gergely P Jr, Nagy ZB, Gombos T, Pozsonyi E, Rajczy K, Balogh Z, Sevcic K, Orbán I, Szodoray P, Poór G (2008) Role of HLA-DRB1 and PTPN22 genes in susceptibility to juvenile idiopathic arthritis in Hungarian patients. *Clin Exp Rheumatol* 26: 1146–1152.
- Sáfrány E, Pazár B, Csöngéi V, Járomi L, Polgár N, Sipeky Cs, Horváth IF, Zeher M, Poór G, Melegh B (2009) Variants of the IL23R gene are associated with ankylosing spondylitis but not with Sjögren syndrome in Hungarian population samples. *Scand J Immunol* 70: 68–74.
- Gergely P Jr, Pazár B, Nagy ZB, Gombos T, Rajczy K, Balogh Z, Orbán I, Sevcic K, Poór G (2009) Structural polymorphisms in the mannose-binding lectin gene are associated with juvenile idiopathic arthritis. *J Rheumatol* 36: 843–847.

## JUDIT PULAI (2010)

### The role of the fibronectin binding $\alpha 5\beta 1$ integrins in cartilage degradation, new insights into the pathogenesis of osteoarthritis

Supervisor: Gyula Poór

Signals arriving from the ECM through integrins play pivotal role in several biological functions of the chondrocytes. My aim was to study the role of  $\alpha 5\beta 1$  integrins in chondrocyte survival and in cartilage destruction by identifying matrix metalloproteases and potential cytokine mediators induced by fibronectin fragments (FNF), furthermore to investigate the mechanism of their stimulation. Human articular chondrocytes isolated from normal or osteoarthritic tissue were cultured. Cell survival was evaluated by fluorescent dye. FNF-induced MMP-13 production was demonstrated by gelatin zymography, the inflammatory mediators were investigated by cytokine cDNA and protein arrays. Inhibitor studies were used to evaluate the regulation of cell signaling and were visualized by immunoblot. The gene expression was analysed by RT-PCR, the protein synthesis by immunoblot and ELISA. Chondrocyte treatment with  $\alpha 5\beta 1$  specific integrin blocking antibodies resulted in apoptotic cell death providing evidence that  $\alpha 5\beta 1$  integrins transmits survival signals in serum free media. FNF treatment resulted in increased MMP-13 expression, which was partly dependent on the IL-1 signal transduction pathway. Stimulation of the chondrocytes by FNF resulted in a >2-fold increase in IL-6, IL-8, MCP-1, and GRO- $\beta$ . Inhibitor studies revealed that chondrocyte chemokine expression was dependent on NF- $\kappa$ B activity, but independent of IL-1 autocrine signaling. Stimulation of the  $\alpha 5\beta 1$  integrins by FNF resulted in MAPK activation and subsequent increased MMP-13 and cytokine and chemokine expression. The ability of FNF to stimulate MMP-

13 production and expression of multiple cytokines and chemokines suggests that damage to the cartilage matrix is capable of inducing a pro-inflammatory state responsible for further progressive matrix degradation through chemoattraction. Targeting the signaling pathways activated by FNF may be an effective means of inhibiting production of multiple mediators of cartilage destruction, thus could play an important role in the treatment of degenerative and inflammatory arthritis.

- Pulai JL, Chen H, Im Hee-Jeong, Kumar S, Hanning C, Hegde PS, Loeser RF (2005) *NF- $\kappa$ B mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments. J Immunol 174: 5781–5788.*
- Pulai JL, Del Carlo M, Loeser RF (2002) *The  $\alpha$ 5 $\beta$ 1 integrin provides matrix survival signals for normal and osteoarthritic human articular chondrocytes in vitro. Arthritis and Rheumatism 46: 1528–1535.*
- Forsyth C, Pulai JL, Loeser RF (2002) *Fibronectin fragments and blocking antibodies to  $\alpha$ 2 $\beta$ 1 and  $\alpha$ 5 $\beta$ 1 integrins stimulate Mitogen Activated Protein Kinase signaling and increase collagenase-3 (MMP-13) production by human articular chondrocytes. Arthritis and Rheumatism 46: 2368–2376.*

## ZSOLT VENDÉGH (2009)

### Effects of neuropeptides and vasoactive substances on the microcirculation of the developing callus—a gap-osteotomy model on rabbit tibiae

Supervisor: János Hamar

**Basic experiments:** The knowledge of the regulation of bone marrow blood flow is of high clinical importance. However, relatively little is known about the regulation of the microcirculation in the bone marrow and developing callus. In our present study we have measured the changes of bone marrow microcirculation at two different ipsilateral sites (femur and tibia) of the rabbit with laser-Doppler flowmetry. Intra-arterial bolus injections of neuropeptides and vasoactive substances were given to elicit reactions of the microcirculation. Changes in flow, vascular resistance, and duration of action of the substances were measured and calculated. However, the basal blood flow velocities of the both bones were considerably different, but no statistically significant difference could be revealed between the local microvascular reactions of the femur and the ipsilateral tibia, thus the femur can be used as a reference site in our future investigations of the microcirculation of the developing callus in the gap osteotomy of the tibia.

**Gap osteotomy model:** Bone healing is regulated by a number of humoral factors, inflammatory mediators, growth factors and by other biologically active substances, such as hormones and neuropeptides. This process is accompanied by angiogenesis and neural ingrowth requiring satisfactory substrate- and oxygen-supply. The regulation of tissue blood flow during the course of callus development is extremely limited. We investigated the effects of intraarterially applied neuropeptides and vasoactive substances on the microcirculation of the developing callus in rabbit tibiae 10 and 15 days after osteotomy. The changes of blood flow reactions of the developing callus and the ipsilateral femoral bone marrow were detected by laser-Doppler flowmetry. Active substances can elicit vasoactive reactions early following bone injury, possibly because reactive neural structures grow into the developing callus parallel with blood vessels and at a very early stage

of callus formation. Differences in the neutralizing or re-uptake mechanisms could be responsible for the observed variations in the recovery times.

- Vendégh Z, Melly A, Toth B, Wolf K, Farkas IT, Kádas I, Hamar J (2009) *Effects of neuropeptides and vasoactive substances on the microcirculation of the callus after tibia-osteotomy in rabbits. Acta Vet Hung* 57: 427–439.
- Vendégh Z, Hamar J, Wolf K, Melly A (2005) *Reaktionen diaphysärer Kallusgefäße des Kaninchens auf vasoaktive Substanzen nach Spaltosteotomie der Tibia: eine Laser-Doppler-Flowmetrie-Studie. European Surgery Acta Chirurgica Austriaca* 37 (Suppl 203): 109.
- Vendégh Z, Hamar J, Wolf K, Melly A (2005) *Reaktionen diaphysärer Kallusgefäße des Kaninchens auf vasoaktive Substanzen nach Spaltosteotomie der Tibia: eine Laser-Doppler-Flowmetrie-Studie. European Surgery Acta Chirurgica Austriaca* 37 (Suppl 203): 131.

---

## **PROGRAM 2/9.**

### **PULMONOLOGY**

#### **Coordinator:**

**György LOSONCZY M.D., Ph.D., D.Sc.**

Department of Pulmonology

1/C Diósárok st, Budapest H-1125

Tel.: +36 1 355 9733, fax: +36 1 214 2498

E-mail: [losonczy@pulm.sote.hu](mailto:losonczy@pulm.sote.hu)

#### **Program overview**

The subject of pulmonology comprises diseases of major public health importance, i. e. chronic obstructive lung disease (affecting 3–5% of the adult population), bronchial asthma (200,000 patients in Hungary), lung cancer and tuberculosis. This Program offers research opportunities and advanced training for physicians interested in pulmonological science. The 8 subprograms cover the major areas of respiratory research and students will acquire specialized clinical skills, physiology, microbiology, biochemistry and molecular biology laboratory experience. In addition, the Program offers comprehensive courses in clinical pulmonology and basic science.



***Titles of research projects***

Non-invasive investigation of airway inflammation in pulmonary diseases		<b><i>Supervisors</i></b> Balázs Antus
The role of TNFX promoter polymorphism and anti Hsp 70 antibody in the development and prognosis of lung cancer		Zoltán Bártfai
Mechanism and clinical significance of angiogenesis and lymph-angiogenesis in lung cancer		Balázs Döme
Molecular immunologic characterization of BAL monocytes		András Falus
Animal experimental modelling of emphysema		Zoltán Hantos
Study of pulmonary mechanics		Zoltán Hantos
Pharmacology and role of inhalational drugs in the treatment of airway inflammatory diseases		Gábor Horváth
Non-invasive investigation of airway inflammation in pulmonary diseases		Ildikó Horváth
The activity, role and the interaction of enzyme systems in allergic bronchospasm		Márk Kollai
Lung tumors		László Kopper
Natural killer (NK) T lymphocytes in airway inflammation		György Losonczy
Examination of novel tyrosine kinase inhibitory molecules with selective antitumoral activity, modelling of relations between structure and biological action		György Mészáros
Prognostic and predictive factors in lung cancer		Judit Moldvay
Lower respiratory tract infections		Ferenc Rozgonyi
Application of molecular epidemiological methods in the clinical practice of tuberculosis		Ákos Somoskövi
Diagnosis and therapy of endobronchial diseases. Interventional pulmonology		János Strausz

***Ph. D. students***

András Bikov	ft	<b><i>Supervisors</i></b> Ildikó Horváth
Krisztina Gál	pt	Veronika Müller
András Kállai	pt	Zoltán Hantos

***Ph. D. candidates***

Anikó Bohács	na	<b><i>Supervisors</i></b> György Losonczy
György Lang	na	Balázs Döme
Zsófia Lázár	ft	Ildikó Horváth
Zsuzsanna Orosz	na	Zoltán Bártfai
Tamás Tompos	pt	György Losonczy

***Ph. D. graduates***

Krisztina Bogos	na	<b><i>Supervisors</i></b> Balázs Döme
Krisztina Czebe	na	Balázs Antus
András Lörx	pt	Zoltán Hantos
Judit Lukács	ft	Ákos Somoskövi
Ferenc Rényi-Vámos	na	Balázs Döme

ft, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**KRISZTINA BOGOS (2009)**

### **The role of bone marrow-derived circulating vascular and lymphatic endothelial progenitor cells in lung cancer**

*Supervisor: Balázs Döme*

Lung cancer is the most common malignancy in terms of both incidence and mortality in the western world. Although modest survival benefit has been observed with surgery and chemo (radio)therapy, even in surgically resectable cases, more than 50% of patients develop metastases within 5 years and an efficacy plateau has been reached. Given the still dismal survival rates, attention over recent years has focused on novel molecular targeted therapies with different mechanisms of action and better toxicity profile. The tumor induced angiogenesis is one of the most exciting research areas in this field. Until recently, it was generally accepted that vascularization and lymphangiogenesis of tumors arises exclusively from endothelial sprouting. The bone marrow derived endothelial progenitor cells (EPCs) and the newly identified bone marrow-derived cell population, called lymphatic/vascular endothelial progenitor cells (LVEPCs) have been shown to contribute to vascular and to lymph capillary growth in experimental tumor systems. The circulating endothelial progenitor cells and the mature exfoliated endothelial cells are potential biomarkers for monitoring the efficacy of antiangiogenic therapies in the future. **Aims:** The aim of the study was to identify the role of the EPC cells in non-small cell lung cancer (NSCLC). Further aim was to studying the role of the circulating LVEPC cells in small cell lung cancer (SCLC) and the relationship with the diseases progression. **Methods:** EPCs labeled with CD34, CD133, and vascular endothelial growth factor receptor-2 (VEGFR2) antibodies were counted by flow cytometry in the peripheral blood of 53 NSCLC patients. Furthermore, by means of a relative quantitative reverse transcription real time-PCR approach, we measured VEGFR2, CD133, CD34, and VE-cadherin mRNA in the peripheral blood samples of the same patient population. EPCs in tumor samples were identified by confocal microscopy using CD31, CD34, CD133, and VEGFR2 antibodies. To measure the number of circulating LVEPC, peripheral blood samples were collected from 88 patients with limited-disease SCLC before therapy. The control group was also included 32 individuals. There were determined the numbers of CD34-positive/VEGFR3-positive double-positive LVEPC in the peripheral blood of 32 control subjects and 88 SCLC patients by flow cytometry. The levels of VEGF-C in the peripheral blood of controls and patients with SCLC were carried out by using ELISA method. **Results:** Although immunofluorescent labeling of microvessels made clear that incorporation of EPCs is a rare phenomenon in NSCLC tissue (9 of 22 cases), circulating EPC levels before therapeutic intervention were increased in NSCLC patients ( $p < 0.002$ , versus healthy controls), and high pretreatment circulating EPC numbers correlated with poor overall survival ( $p < 0.001$ ). Furthermore, in the subgroup of responders to treatment, the post-treatment EPC numbers in the peripheral blood were significantly lower compared with nonresponding patients. Interestingly, pretreatment mRNA levels of CD133, VE-cadherin, and CD34 were not significantly increased in NSCLC patients, whereas VEGFR2 expression was increased by 80-fold. Moreover, posttreatment VEGFR2 mRNA level in

the peripheral blood was significantly higher in the subgroup of nonresponding patients when compared with posttreatment level of patients responding to antitumor therapy. CD34-positive/VEGFR3-positive LVEPC levels were significantly increased in patients (versus controls;  $p < 0.01$ ), and there was also a significant relationship between LVEPC counts and lymph node metastasis ( $p < 0.01$ ). High pretreatment circulating LVEPC numbers correlated with poor overall survival ( $p < 0.01$ ). Although we observed significantly elevated VEGF-C concentrations in patients (versus controls;  $p < 0.01$ ), there was no significant correlation between VEGF-C and LVEPC levels. Moreover, no significant differences in peripheral blood VEGF-C levels were seen between patients subgrouped by clinicopathologic variables including tumor and lymph node stages and survival. **Conclusion:** Circulating levels of bone marrow-derived EPCs are significantly increased in NSCLC patients and correlate with clinical behavior. Peripheral blood levels of bone marrow-derived LVEPCs are significantly increased in patients with SCLC and correlated with lymphatic involvement and poor prognosis.

- Bogos K, Renyi-Vamos F, Dobos J, Kenessey I, Magyar M, Tovari J, Timar J, Strausz J, Ostoros G, Klepetko W, Ankersmit HJ, Lang G, Hoda MA, Nierlich P, Dome B (2009) High VEGFR-3 positive circulating lymphatic/vascular endothelial progenitor cell level is associated with poor prognosis in human small cell lung cancer. *Clin Cancer Res* 15: 1741–1746.
- Dome B, Timar J, Ladanyi A, Paku S, Renyi-Vamos F, Klepetko W, Lang G, Dome P, Bogos K, Tovari J (2009) Circulating endothelial cells, bone marrow-derived endothelial progenitor cells and proangiogenic haematopoietic cells in cancer: from biology to therapy. *Crit Rev Oncol Hematol* 69: 108–124.
- Dome B, Timar J, Dobos J, Meszaros L, Raso E, Paku S, Kenessey I, Ostoros G, Magyar M, Ladanyi A, Bogos K, Tovari J (2006) Identification and clinical significance of circulating endothelial progenitor cells in human non-small cell lung cancer. *Cancer Res* 66: 7341–7347.

## KRISZTINA CZEBE (2009)

### Posttransplant care of lung transplant recipients using invasive and non-invasive methods

Supervisor: Balázs Antus

In recent years lung transplantation became an accepted therapeutic modality for end-stage disease of the lungs and the pulmonary circulation. Pulmonary complications including infections and acute or chronic rejections are the most important factors, which may worsen allograft outcomes. Although bronchoscopy and bronchoalveolar lavage are helpful in the diagnostics of the complications, they are invasive, expensive and relatively time-consuming techniques. Moreover, the use of surveillance bronchoscopies is still debated, and more evidence is needed to include them into the regular protocol of transplant patients. On the other hand there is increasing interest in non-invasive methods such as the fractional exhaled nitric oxide (FENO) measurement and exhaled breath condensate (EBC) analysis, which could be potentially useful in the assessment of pulmonary complications in lung transplant patients.

The aims of our studies were: first, to assess the importance of the use of bronchoscopy in the diagnostic procedures in the follow up of lung transplant recipients; second, to test whether pulmonary infections influence FENO levels in otherwise clinically-stable lung transplant recipients, and third, to investigate the temporal variability of EBC pH in stable lung transplant patients.

Our retrospective study confirmed that many patients have silent rejection or subclinical infection in the first post-transplant year, and the routine surveillance bronchoscopies allowed detection and targeted treatment of these complications. Thus, surveillance bronchoscopies are of high clinical importance. Our further prospective studies demonstrated that pulmonary infections are associated with increased FENO levels in patients with lung allograft, and that treatment decreases FENO values to baseline after recovery. Unfortunately, the measurement of FENO by itself as a screening tool for infections seems to be limited by its low sensitivity. Looking at temporal changes in EBC pH we showed that the variability of EBC pH in stable lung transplant recipient is small, and it is similar to that in healthy non-transplant subjects. Further longitudinal studies are needed to assess the potential role of EBC pH measurement in the detection of pulmonary complication of lung transplant recipients.

- Antus B, Csiszér E, Czebe K, Horváth I (2005) *Pulmonary infections increase exhaled nitric oxide in lung transplant recipients: a longitudinal study. Clin Transplant 19: 377–382.*
- Czebe K, Kullmann T, Csiszér E, Barát E, Horváth I, Antus B (2008) *Variability of exhaled breath condensate pH in lung transplant recipients. Respiration 75: 339–344.*
- Czebe K, Barta I, Antus B, Valyon M, Horváth I, Kullmann T (2008) *Influence of condensing equipment and temperature on exhaled breath condensate pH, total protein and leukotriene concentrations. Respir Med 102: 720–725.*

## ANDRÁS LORX (2010)

### Respiratory mechanics in ventilated patients studied with low-frequency forced oscillations

*Supervisor: Zoltán Hantos*

We studied 44 intubated and mechanically ventilated patients during short apneic intervals at different levels of PEEP, with small-amplitude forced oscillations between 0.4 and 4.8 Hz. Depending on the underlying disease and its severity 4 groups were formed: COPD, AECOPD, pneumonia (group 1) and severe pneumonia (group 2). Based on the LIS value pneumonia patients with a score greater than 2.5 were considered into group 2. In 26 patients, measurements were made before and after inhalation of fenoterol hydrobromide plus ipratropium bromide (Berodual). Newtonian resistance (RN), and coefficients of tissue resistance (G) and elastance (H) were estimated from the impedance data (Zrs) by fitting with different models, including the constant-phase model, two extended CP models with distributed elastance or resistance, and in the AECOPD/COPD group all models with a lumped central shunt compliance. The distributed models resulted in a minor improvement in fitting error, at a price of involving an extra parameter, and less reliable parameter estimation. RN and the G/H ratio in the AECOPD/COPD group, and all parameters in group 2 showed a negative PEEP dependency, reflecting the volume dependence of the airway calibre and the improved homogeneity of the lungs in the

AECOPD/COPD patients, and beyond, recruitment in the pneumonia group. H were markedly higher in group 2 compared to group 1. The bronchodilation (Berodual) was also associated with simultaneous decreases in G and H, indicating recruitment of lung unit in AECOPD/COPD, and a decrease in G as a sign of improvement in heterogeneity in the pneumonia group. In conclusion, the measurement of low-frequency Zrs can be accomplished in ventilated ICU patients during short apneic periods, and offers valuable and unique information on the mechanical status of the airways and tissues.

- Lorx A, Szabó B, Hercsuth M, Péntzes I, Hantos Z (2009) Low-frequency assessment of airway and tissue mechanics in ventilated COPD patients. *J Appl Physiol* 107: 1884–1892.
- Lorx A, Suki B, Hercsuth M, Szabó B, Boda K, Péntzes I, Hantos Z (2010) Airway and tissue mechanics in ventilated patients with pneumonia. *Respir Physiol Neurobiol* 171: 101–109.

## JUDIT LUKÁCS (2009)

### The molecular epidemiological relations of tuberculosis in Hungary

Supervisor: Ákos Somoskövi

During 2002 in Hungary the incidence of tuberculosis among homeless was 676 cases per 100.000 people. The situation is particularly alarming in Budapest, where already in 2006 every third patient with tuberculosis was homeless. Based on these epidemiological observations, a retrospective population-based study has been carried out that aimed at identifying the means of transmission among 66 homeless patients from Budapest, with the help of analysis of *M. tuberculosis* strains with IS6110 DNA fingerprint method. According to the results of our study 47 (71.2%) isolates could be classified into 11 different clusters. The high rate of cases that could be clustered indicates that the vast majority of the tuberculosis among the homeless resulted from recent transmission. 14.9% of all the clustered patients were staying at the same three homeless shelters at the time of diagnosing the disease. Our results call attention to the fact that the screening and treatment of homeless people should be taken more seriously and the controlling of homeless shelters should be reinforced. During further analysis of the strains derived from the homeless we identified a locally originating microepidemy, in a district of Budapest with the highest tuberculosis incidence, caused by a *M. tuberculosis* genotype that according to the international databases has not been detected in any other part of the world yet.

In another study a retrospective population-based molecular epidemiologic examination was carried out in order to provide a better knowing of transmission dynamics of drug resistant tuberculosis in Hungary, during which we examined 68 resistant *M. tuberculosis* strains with IS6110 DNA fingerprint and spoligotyping techniques. 54.4% of the resistant strains and 75.0% of the multi-drug resistant strains could be clustered. The fact that 81.1 % of the clustered cases was new infection indicates that such a high rate of resistant diseases are the consequence of a freshly and actively spreading infection and do not result from reactivation. According to our results the incidence of drug resistant tuberculosis in Hungary is underestimated as well as therapy and control of the resistant diseases are not adequate.

In our further examinations we examined a *M. tuberculosis* strain isolated from a woman with Mongolian origin, with IS6110 and spoligotyping methods. The examination con-

firmed the aetiological role of the *M. tuberculosis* belonging to the so-called Beijing genotype, which has never been before detected in Hungary.

- Lukacs J, Tubak V, Mester J, David S, Bartfai Z, Kubica T, Niemann S, Somoskovi A (2004) *Conventional and molecular epidemiology of tuberculosis in homeless patients in Budapest, Hungary. J Clin Microbiol* 42: 5931–5934.
- Ködmön C, Lukács J, Niemann S, Dávid S, Vadász I, Somoskői A (2007) *Új Mycobacterium tuberculosis törzs okozta mikroepidémia budapesti hajléktalanokban. Orvosi Hetil* 8: 339–342.
- Kodmon C, Niemann S, Guitierrez MC, Sola C, Rastogi N, Lukacs J, Somoskovi A (2007) *Molecular clues of a microepidemy among homeless tuberculosis patients in Budapest due to new and local Mycobacterium tuberculosis clade. Infect Genet Evol* 7: 632–635.

## FERENC RÉNYI-VÁMOS (2009)

### The investigation of the mechanisms of lymphangiogenesis and the role of the lymphatic vessels in lung cancer

*Supervisor: Balázs Döme*

To this day we have significantly less information on the lymphatic system formation of tumors, of tumor-induced lymphangiogenesis (TILA) than tumor induced angiogenesis (TIA).

Tumor lymphatic system analysis was not possible due to the lack of specific markers until recently. The discovery of LYVE-1, D2-40 and podoplanin lymphatic markers provided tools for the observation of tumor-associated lymphatics, the comparison of the biological behaviour of angiogenic and non-angiogenic phenotypes, and the description of the roles of circulating progenitor (vascular and lymphatic) cells.

Experimental studies suggest that tumors are able to induce the formation of new lymphatic capillaries, primarily by the production of cytokines such as the members of the VEGF (Vascular Endothelial Growth Factor) family and that TILA activity correlates with the number of lymph node metastasis and that lymphovasculogenesis (LVEPCs) might play a role in the lymphatic system formation of lung cancer.

The aim of our study is to draw a comparison between the lymphangiogenesis of angiogenic and non-angiogenic phenotyped non-small-cell lung cancers and examine the correlation between peripheral blood LVEPC (Lymphatic Vascular Endothelial Cell) cell numbers and VEGF-C serum level, and the impacts of these on the development and survival of lymphatic metastasis in SCLC (Small Cell Lung Cancer).

We have analyzed how NSCLC (Non Small Cell Lung Cancer) acquires its lymphatic network and investigated whether the extent of lymphangiogenesis may be related to the angiogenic phenotype (angiogenic vs. non-angiogenic) and/or to the risk of lymph node metastasis and to patient survival.

Our study demonstrates, for the first time, that lymphangiogenesis occurs exclusively in the angiogenic growth type of human NSCLCs, and that LVD (Lymphatic Vessel Density) is correlated to clinical behavior and to lymph node status only in this growth type of NSCLCs. However, it also provides the first evidence that the risk of lymph node metastasis as well as a shorter survival was more likely to occur in the patient population with non-angiogenic tumors, and that these tumors mainly co-opt host tissue lymphatics



during their growth, in contrast to most of the angiogenic ones, which expand with concomitant lymphangiogenesis. The current study demonstrates, for the first time, that the circulating numbers of bone marrow-derived LVEPCs are significantly increased in SCLC patients and that these numbers correlate with the extent of tumor spread to regional lymph nodes and with patients' survival.

- Renyi-Vamos F, Tovari J, Fillinger J, Timar J, Paku S, Kenessey I, Ostoros G, Agocs L, Soltesz I, Dome B (2005) Lymphangiogenesis correlates with lymph node metastasis, prognosis, and angiogenic phenotype in human non-small cell lung cancer. *Clin Cancer Res* 11: 7344–7353.
- Bogos K, Renyi-Vamos F, Dobos J, Kenessey I, Tovari J, Timar J, Strausz J, Ostoros G, Klepetko W, Ankersmit HJ, Lang G, Hoda MA, Nierlich P, Dome B (2009) High VEGFR-3-positive circulating lymphatic/vascular endothelial progenitor cell level is associated with poor prognosis in human small cell lung cancer. *Clin Cancer Res* 15: 1741–1746.
- Dome B, Timar J, Ladanyi A, Paku S, Renyi-Vamos F, Klepetko W, Lang G, Dome P, Bogos K, Tovari J (2009) Circulating endothelial cells, bone marrow-derived endothelial progenitor cells and proangiogenic hematopoietic cells in cancer: from biology to therapy. *Crit Rev Oncol Hematol* 69: 108–124.

---

## PROGRAM 2/10.

---

## OPHTHALMOLOGY

### **Coordinator:**

**Ildikó SÜVEGES M.D., Ph.D., D.Sc.**

Department of Ophthalmology

25–29 Tömő st, Budapest H-1083

Tel.: +36 1 210 0280/ext. 51612

E-mail: [suveges.ildiko@med.semmelweis-univ.hu](mailto:suveges.ildiko@med.semmelweis-univ.hu)

### **Program overview**

Vision is essential for human life, but not endangered by several frequent eye diseases, the treatment of which has not been completely established. The Ph.D. Program in ophthalmology addresses the pathophysiology and pathology of the most important eye diseases. The research projects cover the diseases of the light refractive media of the eye, the biochemical and histological alterations important for vision, and the experimental models of the ocular alterations. Ocular blood flow and aqueous humour dynamics are especially important topics of the Ph.D. school. Glaucoma, one of the blinding eye diseases with the highest prevalence is investigated with special emphasis.

***Titles of research projects***

Physiology and pathophysiology of the neuroretina in certain diseases, especially in hereditary retinal diseases  
 Clinical—biological—imaging examinations of retinal ganglion cell apoptosis in glaucoma  
 Wound healing of the cornea especially in refractive surgical procedures  
 Biomechanical examination of the anterior lens capsule  
 Physiology and pathophysiology of vision. The titles of research projects include the examination of the refractive layers of the bulb in pathologic conditions and the follow-up of lesions occurring in diseases of the eye  
*In vitro* culturing of corneal limbal stem cells and examination of their potential in clinical application

***Supervisors***

Ágnes Farkas  
 Gábor Holló  
 Zoltán Zsolt Nagy  
 Zoltán Zsolt Nagy  
 Ildikó Süveges  
 Nóra Szentmáry

***Ph.D. students***

Zsuzsanna Antus pt  
 Anna Énzsöly ft  
 Eszter Fodor pt  
 Anita Garas ft  
 Ágnes Ildikó Takács ft (a)  
 Erika Tátrai ft

***Supervisors***

Ildikó Süveges  
 János Németh  
 János Németh  
 Gábor Holló  
 Zoltán Zsolt Nagy  
 György Salacz

***Ph.D. candidates***

László Imre na  
 Krisztina Hagyó-Kosina ft  
 Ákos Kusnyerik ft  
 Olga Lukáts na  
 Kata Miháلتz na  
 Attila Nagymihály na  
 Miklós Schneider na  
 Mónika Ecsedy (Szabó) na  
 Krisztina Knézy (Szépkúti) na  
 Rita Vámos pt

***Supervisors***

Ildikó Süveges  
 János Németh  
 Ildikó Süveges  
 Zoltán Zsolt Nagy  
 János Németh  
 Ildikó Süveges  
 János Németh  
 János Németh  
 Ágnes Farkas

***Ph.D. graduates***

Balázs Lesch ft  
 Mónika Popper ft  
 Ferenc Balázs Salló na

***Supervisors***

Ágnes Farkas  
 Ildikó Süveges  
 György Salacz

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2010***

**BALÁZS LESCH (2010)**

### **Clinical and genetic examinations of X-linked juvenile retinoschisis**

*Supervisor: Ágnes Farkas*

X-linked juvenile retinoschisis is one of the most common X-linked recessively inherited, incurable, bilateral, progressive vitreoretinal dystrophies limited almost exclusively to males. XLRS is caused by a functional damage of the retina-specific RS1 protein due to a mutation of the RS1 gene. Damage of its physiologic tissue adhesive function leads to a characteristic disruption of retinal layers called retinoschisis primarily affecting the macular area and causing a decline of central visual acuity.

Morphological (fundus, OCT), functional (visual acuity, ERG) and molecular genetic examination of 24 families suffering from XLRS was carried out and the time course of the disease was modelled by the help of Hungarian ophthalmologic centres. Patients were divided into two groups: one group with cystic macular changes (Group I) and to another one having atrophic macula (Group II) for a more correct assessment of the influence of morphological changes (from cystic to atrophic stage).

A small improvement of decreased visual acuity of patients was observed until 13 years of age, while until 45 years a slow, later an accelerating decline was observable.

We extended the international classification scheme of XLRS and the longitudinal and transversal changes of intraretinal cystoid spaces observed by age were described in detail. The cut-off value, belonging to the transition from the cystic (CFT ↑) to the atrophic (CFT ↓) stage was 28 years of age.

Pronounced reduction of scotopic maximal b-wave amplitudes characteristic of the disease were confirmed by GfERG. The mfERG response densities of P1- (b) waves of both patient groups were significantly decreased in all rings and the implicit times were significantly delayed in rings 3–5.

This is the first study to estimate the genetic background of patients with XLRS in Hungary and 3 novel mutations were detected. In case the novel c.78+1G>C splice site mutation the two possible alternative splicing mechanisms were first published in humans by our group. Although the degree of spatial changes of the tertiary structure of mutant RS1 protein was not correlated with the severity of the disease, obvious genotype-phenotype correlation was found in some mutations.

Autosomal recessive isolated foveal retinoschisis, rarely described in the literature, was confirmed by clinical and genetic examinations in a female patient, who initially was thought to suffer in XLRS by her fundus picture.

- Lesch B, Szabó V, Kánya M, Somfai GM, Vámos R, Varsányi B, Pámer Zs, Knézy K, Salacz Gy, Janáky M, Ferencz M, Hargitai J, Papp A, Farkas Á (2008) Clinical and genetic findings in Hungarian patients with X-linked juvenile retinoschisis. *Mol Vis* 14: 2321–2332.
- Lesch B, Szabó V, Kánya M, Varsányi B, Somfai GM, Hargitai J, Vámos R, Fiedler O, Farkas Á (2008) Truncation of retinoschisin protein associated with a novel splice site mutation in the RS1 gene. *Mol Vis* 14: 1549–1458.

- Lesch B, Szabó V, Salacz Gy, Varsányi B, Somfai GM, Farkas Á (2006) Az X-kromoszómához kötött öröklődő juvenilis retinoschisis korszerű komplex vizsgálata. Szemészet 143: 165–174.

## MÓNKA POPPER (2010)

### *In vivo* corneal confocal microscopy in diabetes

*Supervisor: Ildikó Süveges*

The transparent cornea is one of the most interesting and most remarkable tissues in the human body. The maintenance of corneal transparency depends on the delicate balance of the components of its highly complex structure, also including one of the densest innervations in the human body.

Diabetes, especially its most prevalent type, type-2 diabetes is endemic in developed nations. Nowadays, with the increasing importance of the disease, the detection of diabetic keratopathy is also gaining importance in early detection, and objective quantification of disease progression, and evaluation of the risk of complications. Diabetic keratopathy causes impaired corneal wound healing, and is believed to be related to peripheral diabetic neuropathy.

*In vivo* confocal microscopy is an ideal approach for the investigation of the cornea *in vivo*. It can visualize the structure, layers, and cells of the cornea with high resolution, in real time, and non-invasively.

We developed and validated a non-invasive, fast, reliable and reproducible method for *in vivo* quantitative (cell counting) measurements of corneal cell layers using a commercially available scanning-slit confocal microscope. We have proven the reliability and reproducibility of the method by validation. We performed *in vivo* cell density estimation and comparison in six layers along the optical axis of the cornea of type-2 diabetic patients and healthy controls using the non-invasive cell counting method we developed and validated. We found significant difference between cell densities of diabetic patients and healthy controls in the basal epithelial layer (the cell densities of diabetics being lower) but not in the other layers. By *in vivo* confocal microscopic evaluation of the sub-basal nerve layer, we demonstrated that corneal innervation is impaired in diabetes already at an early stage of the disease. The number of subbasal nerve fiber bundles is significantly decreased in type-2 diabetic patients, when compared to healthy controls, likewise the basal epithelial cell density. Furthermore, we observed very highly reflective cells immediately beneath the basal epithelial cells' layer, typically in close vicinity of subbasal nerves. We consider these most probably Langerhans- or dendritic cells. According to our knowledge we were the first to investigate and describe corneal stromal nerves in diabetes using *in vivo* corneal confocal microscopy. We observed degenerated or pathologically regenerating stromal nerves. In the corneal stroma, structures consisting of a highly reflective head and a vague tail were also found. The origin of these structures is yet unknown, nevertheless we observed that they are significantly less prevalent in healthy controls than in diabetics. From the ophthalmological complications of diabetes, we contributed to the body of clinical and scientific knowledge regarding diabetic keratopathy by showing that changes occurring already in the early stages of disease can be visualized and evaluated non-invasively using *in vivo* confocal microscopy. Taken

together, we showed that *in vivo* confocal microscopy can evaluate diabetes-related changes in the cornea. The future clinical potential of the technique, besides follow-up of disease progression and treatment efficacy, lies in its possible prognostic value for the evaluation of diabetic population before interventions affecting the cornea.

- Popper M, Morgado AM, Quadrado MJ, Van Best JA (2004) *Corneal cell density measurement in vivo by scanning slit confocal microscopy: method and validation*. *Ophthalmic Res* 36: 270–276.
- Quadrado MJ, Popper M, Morgado AM, Murta JN, Van Best JA (2006) *Diabetes and corneal cell densities in humans by in-vivo confocal microscopy*. *Cornea* 25: 761–768.
- Popper M, Quadrado MJ, Morgado AM, Murta JN, Van Best JA, Müller LJ (2005) *Subbasal nerves and highly reflective cells in corneas of diabetic patients: in vivo evaluation by confocal microscopy*. *Invest Ophthalmol Vis Sci* 46: ARVO E-Abstract 879.

## FERENC BALÁZS SALLÓ (2010)

### The role of vascular interfaces in age-related macular degeneration

*Supervisor: György Salacz*

The aim of our studies was to investigate the role of the outer vascular interface of the retina in age-related macular degeneration (AMD). In our clinical study we aimed to probe the functional implications of macular soft drusen regression in AMD eyes. Of 960 patients screened, soft drusen regression was detected in 34 cases, 14 agreed to participate in the study, ranging in age from 52 to 84 years (median 72), the follow-up period ranged from 2.8–14.4 years (mean 5.9 years). In subjects with confirmed drusen regression, detailed phenotyping was performed according to the system defined by the International Classification for AMD, Fundus Autofluorescence (FAF) was recorded and high-definition sensitivity testing of the central 9° of the retina was performed using Fine Matrix Mapping (FMM). Phenotype and functional data were analyzed for correlations. FMM showed a generalised threshold elevation relative to normal controls both under photopic and scotopic conditions. Scotopic sensitivity loss exceeded photopic loss in all cases. Sensitivity loss over areas with drusen or regressed drusen did not differ significantly from that over non-drusen areas. We concluded that in AMD there is an early generalized preferential loss of central rod function. Macular soft drusen may fade or disappear without detectable ophthalmoscopic, FAF or psychophysical signs of local dysfunction. This phenomenon is a potential source of misclassification. The prognosis for cases with true regression of drusen compared with those without needs to be considered in future studies on AMD. In our morphological study we aimed to examine the ultrastructure of BrM in transgenic mice. AMD is characterized by the accumulation of lipid- and protein-rich deposits in Bruch's Membrane (BrM). A consequent decrease in hydraulic conductivity and impairment of transport through BrM may play a central role in the pathogenesis of AMD. The mechanism of deposit formation in AMD had been suggested to show similarities to atherogenesis in which the interactions of extracellular matrix proteoglycans with apoB-100 play an important role. A prime candidate for this interaction is the small leucin-rich proteoglycan biglycan. We aimed to test the effect of the simultaneous overexpression of human apoB-100 and biglycan genes in combination with a high-cholesterol diet on BrM morphology in transgenic mice. 6-weeks-old homo-

zygous apoB-100 or biglycan, hemizygous apoB-100/biglycan transgenic and wild type C57BL/6 mice were fed either a standard chow or a diet supplemented with 2% cholesterol for 17 weeks. Animals were sacrificed, serum lipid levels were measured and eyes were examined using transmission electron microscopy (TEM). Morphometric analysis of BrM showed that in apoB-100 and double transgenic animals fed a high-cholesterol diet, BrM thickness was significantly increased compared to wild-type animals. Both groups had electron-lucent profiles in clusters, scattered throughout BrM, and focal nodules of an amorphous material of intermediate-electron density between the plasma and basement membranes of the RPE. BrM thickness in these two groups correlated well with elevated cholesterol levels. Unexpectedly, animals overexpressing biglycan alone showed a marked, diet-independent increase in BrM thickness associated with a layer of a basement membrane-like material in outer BrM. The effects of biglycan overexpression are intriguing and further investigations are needed to elucidate the underlying mechanisms.

- Sallo FB, Rechtman E, Peto T, Stanescu-Segall D, Vogt G, Bird AC, Fitzke FW (2009) *Functional aspects of drusen regression in age-related macular degeneration. Br J Ophthalmol* 93: 1345–1350.
- Sallo FB, Bereczki E, Csont T, Luthert PJ, Munro P, Ferdinandy P, Sántha M, Lengyel I (2009) *Bruch's membrane changes in transgenic mice overexpressing the human biglycan and apolipoprotein b-100 genes. Exp Eye Res* 89: 178–186.
- Sallo FB, Peto T, Leung I, Xing W, Bunce C, Bird AC (2009) *The international classification system and the progression of age-related macular degeneration. Curr Eye Res* 34: 238–240.

---

## **PROGRAM 2/12.**

### **CLINICAL AND EXPERIMENTAL RESEARCH IN ANGIOLOGY**

#### ***Coordinator:***

**György ACSÁDY M.D., Ph.D., D.Sc.**

Department of Cardiovascular Surgery

68 Városmajor st, Budapest H-1122

Tel./fax: +36 1 458 6734

E-mail: [gyacsady@erseb.sote.hu](mailto:gyacsady@erseb.sote.hu)

#### **Program overview**

The education of clinicians and researchers having profound scientific background is the basic aim of the postgraduate Program, offering a possibility of research work on organ failures due to vascular disorders and of application of the results in the therapeutic processes. The study of the pathogenesis of the diseases of ischemic origin using the latest techniques is also part of the Program.



***Titles of research projects***

Relations of arteriosclerosis and chronic uraemia  
 Overview and planning of clinical studies of therapeutic angiogenesis  
 Physiological and pathological adaptation of venous system in cases hemodinamical stress  
 Assessment, monitoring of cardiopulmonal perioperative risks and treatment options in cardiovascular surgical diseases  
 The role and problems of endovascular graft implantation in treatment of aneurysms  
 The role of genetic factors in restenosis  
 Complex examination of left ventricle aneurysm  
 Clinical use of homologous vessel transplantation and experimental research on vessel preservation  
 Examination of vessel structures infected by *Chlamydia pneumoniae*  
 Molecular biological aspects of dilatative cardiomyopathy and myocarditis of viral origin  
 The role of regulatory function of Nociceptin in cardiovascular disease and its control  
 Radiological investigation and geometric analysis of aortic aneurysms  
 Experimental vascular surgery—clinical application  
 The cardiovascular effects of apelin

***Supervisors***

György Acsády  
 György Acsády  
 György Acsády  
 János Gál  
 Kálmán Hüttl  
 István Karádi  
 Béla Merkely  
 Attila Nemes  
 Zsuzsa Schaff  
 Péter Sótonyi  
 Péter Sótonyi  
 Péter Sótonyi  
 Attila Szijártó  
 Miklós Tóth

***Ph.D. students***

Zsuzsanna Cserép ft  
 Gergely Gósi pt  
 Miklós Krepuska ft  
 Gábor Ferenc Molnár ft  
 Tamás Mirkó Paukovits ft (a)  
 Csanád Várallyay ft (a)

***Supervisors***

János Gál  
 György Acsády  
 Péter Sótonyi  
 Attila Nemes  
 Kálmán Hüttl  
 Kálmán Hüttl

***Ph.D. candidates***

Gábor Bíró na  
 Zsófia Panna Patkó (Joóné) ft  
 Zoltán Szeberin pt  
 Gábor Szabó pt

***Supervisors***

Attila Nemes  
 György Acsády  
 György Acsády  
 György Acsády

***Ph.D. graduate***

Endre Gyurkovics pt

***Supervisors***

Attila Szijártó

a, absoltorium; ft, full-time; pt, part-time; na, not affiliated

## *Abstract of Ph.D. theses successfully defended in 2010*

**ENDRE GYURKOVICS (2010)**

### **Studies on the ischemic-reperfusion injury of lower limbs, systemic complication and prevention using postconditioning**

*Supervisor: Attila Szijártó*

**Introduction:** External aortic compression due to acute gastric dilation could be a rare etiology of the lower limb ischaemia. This phenomenon prompted the author to design experimental study for reperfusion syndrome. Vascular surgery on the lower limb causes ischemia-reperfusion (IR) injury of the tissues distal to the site of the procedure. The IR injury consists of local and systemic components. The most dangerous complication is the so-called reperfusion syndrome, which is critical to postoperative survival of the patients. A large number of technics were developed, which tried to reduce IR injury. The last and the most promising one is postconditioning. **Aim:** To examine in an experimental model, whether postconditioning is a practicable technique for infrarenal aortic surgeries.

**Materials and Methods:** Male Wistar rats underwent 180 minutes of infrarenal aortic occlusion. In one group of the animals we used postconditioning (10 sec. reocclusion/10 sec. perfusion in 6 cycles). Haemodynamics were monitored with intraarterial pressure gauge, the microcirculation events were recorded with Laser Doppler Flowmeter. Blood, urine, plasma and histologic samples were collected at the postischemic 4th, 24th, and 72th hour. **Results:** Postconditioning changed the characteristics of the flow curves. The flow stabilized with hyperaemia. Early inflammatory response (TNF $\alpha$ , free radicals) were reduced by postconditioning significantly. The method failed to affect the local muscle injury concerning the morphological and laboratory results, but it was able to reduce the late inflammatory response of the limb. Postconditioning was able to reduce the remote organ injury of lungs and kidneys. Morphological and laboratory results showed significant difference between the postconditioning and the control groups in these two organs. **Conclusion:** Postconditioning seems to be an applicable process to reduce both local and systemic complications of IR injury following vascular surgeries. However, more experiments are needed to determine the exact mechanism before routine clinical trials.

- Gyurkovics E, Tihanyi B, Szijártó A, Kaliszky P, Temesi V, Sas H, Kupcsulik P (2006) Fatal outcome from extreme acute gastric dilation after an eating binge. *Int J Eating Disord* 39(7): 602–605.
- Gyurkovics E, Arányi P, Stangl R, Ónody P, Ferreira G, Lotz G, Kupcsulik P, Szijártó A (2011) Postconditioning of the lower limb—protection against the reperfusion syndrome. *J Surg Res* 169(1): 139–147.
- Szijártó A, Gyurkovics E, Arányi P, Ónody P, Stangl R, Tátrai M, Lotz G, Mihály Z, Hegedűs V, Blázovics A, Kupcsulik P (2009) Posztakondicionálás kísérletes alkalmazása aortakirekesztés kapcsán. *Magy Sebészet* 62(4): 180–187.

**PROGRAM 2/13.**

**HORMONAL REGULATORY SYSTEMS**

**Coordinator:**  
**Károly RÁCZ M.D., Ph.D., D.Sc.**  
2nd Department of Internal Medicine  
46 Szentkirályi st, Budapest H-1088  
Tel.: +36 1 266 0926, fax: +36 1 266 0816  
E-mail: [racz.karoly@med.semmelweis-univ.hu](mailto:racz.karoly@med.semmelweis-univ.hu)

**Program overview**

There are numerous important interactions among the hormonal system and neural, immune and other regulatory mechanisms, by which hormones may influence the physiology or pathophysiology of organs or organ systems. The program includes research projects dealing with interactions between hormones and other regulatory mechanisms, such as neuroendocrine regulation of thyroid and gonadal function, and regulation of pituitary and adrenal hormone secretion. Other research projects include studies on the molecular mechanisms of hormone sensitivity, pathomechanism of metabolic bone disorders associated with endocrine disorders, hormonal disturbances associated with inborn metabolic errors, and the pathomechanisms of sporadic and hereditary adrenal and pituitary tumors.

<b><i>Titles of research projects</i></b>	<b><i>Supervisors</i></b>
Effect of endocrine alterations on the epidemiology of certain diseases	Nándor Ács
Neurohormonal regulation of thyroid function	Csaba Balázs
Steroids as antioxidants	Gábor Békési
Micro-RNA studies in endocrine tumors	Péter Igaz
Functional genomic studies in the pathogenesis of adrenal tumors especially focusing on the expression of cytokines and their receptors	Péter Igaz
Disorders of genes encoding mitochondrial electron transport enzymes in tumors of the endocrine system	Attila Patócs
Evaluation of the role of the glucocorticoid receptor beta in gene transcription	Attila Patócs
Molecular mechanisms determining glucocorticoid sensitivity	Károly Rác
Interactions of regulatory mechanisms in diseases of the pituitary and adrenal glands	Károly Rác
The metabolism of reactive aldehydes in type 2 diabetes mellitus, and the effect of antidiabetic treatment on aldehyde metabolism	Péter Reismann
Clinical and pathophysiological studies in gynecological and reproductive endocrine disorders and in experimental models	János Rigó
Mechanisms of endocrine disease associated bone metabolism disorders	Miklós Tóth

<b><i>Ph.D. students</i></b>		<b><i>Supervisors</i></b>
Tamás Bence Ács	ft	Attila Patócs
Ildikó Adler	pt	Gábor Békési

Karolina Feldman	ft	Károly Rácz, Attila Patócs
Zoltán Kender	ft	Péter Reismann
Nikoletta Lendvai	ft	Attila Patócs
Júlia Stark	ft	Gábor Békési
Balázs Stenczer	ft	János Rigó
Diána Rita Szabó	ft	Péter Igaz
Adrienn Zsippai	pt	Péter Igaz

***Ph.D. candidates***

Belema Boyle	ft
Henriett Butz	ft
Zoltán Magyar	na
Márta Sereg	ft
Péter Márton Sereg	ft
Ágnes Szappanos	ft
Zsófia Tömböl	ft

***Supervisors***

Csaba Balázs
Károly Rácz,
Attila Patócs
Gábor Békési
Miklós Tóth
Péter Igaz
Miklós Tóth
Péter Igaz

***Ph.D. graduates***

Rita Bertalan	ft
Péter Gergics	ft
Gábor Csongor Mezei	na
Ágnes Mondok	ft
Péter Reismann	na
Ágnes Éva Sallai	na
Judit Tőke	ft

***Supervisors***

Károly Rácz
Károly Rácz
János Rigó
Károly Rácz
Károly Rácz
Károly Rácz
Miklós Tóth

ft, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph.D. theses successfully defended in 2009 and 2010*****RITA BERTALAN (2010)****Molecular mechanisms influencing glucocorticoid and estrogen activity during the pregnancy**

*Supervisor: Károly Rácz*

In this work the potential clinical significance of the BclI, N363S and ER22/23EK polymorphisms of the glucocorticoid receptor gene was investigated in preterm neonates and in pregnant women with preeclampsia, HELLP syndrome and in women with healthy pregnancies. It was found that the BclI polymorphism of the GR gene was significantly associated with higher gestational age-adjusted birth weight in preterm neonates irrespective of maternal dexamethasone treatment. These polymorphisms failed to influence

the effect of maternal dexamethasone treatment on perinatal morbidities, including necrotizing enterocolitis (NEC), intraventricular hemorrhagia (IVH), patent ductus arteriosus (PDA), respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD) and sepsis. No associations were detected between any of the three GR gene polymorphisms and the development of NEC, IVH, PDA, RDS, BPD and sepsis in the two groups of neonates with and without maternal dexamethasone treatment.

It was found that the complete HELLP syndrome was significantly associated with the BclI polymorphism and that among women with pathologic pregnancies including both severe preeclampsia and HELLP syndrome, the BclI carriers had significantly higher AST, LDH and ALP levels and a tendency toward low PLT levels than non-carriers. Additionally, alignment analysis revealed that the BclI site of this polymorphic allele was present only in human, but not in the other 6 vertebral species examined.

Studies on a large group of healthy pregnant women indicated that heterozygous carriers and non-carriers of the ER22/23EK polymorphism had no differences in body weight and BMI recorded either at the beginning of pregnancy or before delivery, but the increase of body weight and BMI during pregnancy was significantly lower in heterozygous carriers than in non-carriers.

Finally, it was shown that markedly elevated serum testosterone level detected as early as the 7th week of pregnancy and increased gradually until delivery in a woman failed to cause virilisation of a female fetus. In addition, a progressive increase of maternal estradiol until delivery and substantially lower testosterone and androstendione in umbilical cord than in maternal blood samples at birth were observed, suggesting that placental aromatase activity likely played a role in preventing androgen exposure of the fetus. This possibility was confirmed with measurement of *in vitro* aromatase activity in microsomal fraction of placental tissues obtained after delivery.

- Bertalan R, Patócs A, Nagy B, Derzsy Z, Gullai N, Szappanos Á, Rigó J Jr, Rácz K (2009) Overrepresentation of BclI polymorphism of the glucocorticoid receptor gene in pregnant women with HELLP syndrome. *Clin Chim Acta* 405: 148–152.
- Bertalan R, Patócs A, Boyle B, Rigó J Jr, Rácz K (2009) The protective effect of the ER22/23EK polymorphism against an excessive weight gain during pregnancy. *Gynecol Endocrinol* 23: 1–4.
- Bertalan R, Patocs A, Vasarhelyi B, Treszl A, Varga I, Szabo E, Tamas J, Toke J, Boyle B, Nobilis A, Rigo J Jr, Rac K (2008) Association between birth weight in preterm neonates and the BclI polymorphism of the glucocorticoid receptor gene. *J Steroid Biochem Mol Biol*, 111: 91–94.

## PÉTER GERGICS (2010)

### Application of molecular biology methods for studying hormonal regulatory mechanisms

Supervisor: Károly Rácz

The appropriate selection of methods from numerous molecular biology techniques for studying the function and for understanding alterations underlying disorders of the hormonal regulatory system seems mandatory for accurate and rapid diagnosis of clinically relevant genetic abnormalities. In the first part of my work I developed a novel allele-

specific PCR method for the detection of the Bcl I polymorphism of the glucocorticoid receptor (*GR*) gene which has known influence on sensitivity to cortisol that mediates the metabolic and other effects of the hypothalamic-pituitary-adrenal axis. While preserving the high sensitivity of earlier techniques, this novel method is time- and labour-saving with a relatively low cost and it does not need expensive laboratory equipments. With the use of the novel method in a large cohort of healthy Hungarian subjects I showed that the frequency of the polymorphic Bcl I allele is 34.4%, which is similar to published data in other Caucasian populations.

In the second part of my work I analysed the disease-causing genetic alterations of the von Hippel-Lindau (*VHL*) gene in 7 families with 35 members with *VHL*-syndrome and in 37 unrelated patients with apparently sporadic pheochromocytomas (ASP) who were evaluated at the 2nd Department of Medicine, Semmelweis University between 1998 and 2008. My work represents the first comprehensive study of the *VHL* gene in Hungarian population. In addition to the analysis of point-mutations and small deletions with direct DNA-sequencing, I applied real-time PCR and multiplex ligation probe amplification (MLPA) for the detection of large gene deletions of the *VHL* gene. Combined application of these methods proved to be highly effective, as I detected different disease-causing *VHL* gene mutations in each of the 7 *VHL* families as well as in 3 of the 37 LSP patients. The 10 genetic abnormalities included one nonsense (R161X) and 6 missense mutations (L158V, R167Q, S80I, L63P, Y156C and R167G), one small (354\_355delCT) and 2 large deletions. The 354\_355delCT proved to be a novel gene defect, however, the other mutations have been already known in the literature. With the analysis of genotype-phenotype correlations I showed that nonsense mutation, 2 basepair small deletion and the 2 large deletions leading to truncated *VHL* protein were only found in *VHL* type 1 while missense mutations were predominantly found in *VHL* types 2B and 2C. Based on the results, *VHL* gene testing is recommended not only in patients/family members with *VHL*-syndrome but also in patients with LSP.

- Gergics P, Patocs A, Majnik J, Balogh K, Szappanos A, Toth M, Racz K (2006) Detection of the Bcl I polymorphism of the glucocorticoid receptor gene by single-tube allele-specific polymerase chain reaction. *J Steroid Biochem Mol Biol* 4/5: 161–166.
- Gergics P, Patocs A, Toth M, Igaz P, Szucs N, Liko I, Fazakas F, Szabo I, Kovacs B, Glaz E, Racz K (2009) Germline *VHL* gene mutations in Hungarian families with von Hippel-Lindau disease and patients with apparently sporadic unilateral pheochromocytomas. *Eur J Endocrinol* 3: 495–502.
- Gergics P, Tőke J, Szilágyi Á, Szappanos Á, Kender Z, Barta G, Tóth M, Igaz P, Rácz K, Patócs A (2009) A nagy géndelégiók kimutatásának módszerei és alkalmazásuk egyes örökletes betegségekben. *Orv Hetil* 150: 2258–2264.



**GÁBOR CSONGOR MEZEI (2010)****The mechanism of estrogen's effect in the hypothalamic regulation of energy homeostasis and circadian rhythm***Supervisor: János Rigó*

In addition to its well-described key role in the regulation of reproductive function, estrogen significantly modulates a variety of centrally regulated physiological functions. Cessation of ovarian function (natural or iatrogenic menopause) leads to imbalanced energy homeostasis favoring obesity and altered circadian rhythms. Estrogen is a potent antiobesity hormone that reduces food intake and increases energy expenditure. Its effect is comparable to leptin, which is believed to be the most potent anorectic. Estrogen also maintains the synchrony of endocrine and autonomic mechanisms by modulating the function of the biological clock in the suprachiasmatic nucleus (SCN) of the hypothalamus. The mechanisms, however, underlying these effects are not well understood. The aim of my thesis was to identify these mechanisms. Here we report that estrogen triggers a prompt and robust increase in the number of excitatory synapses on the pro-opiomelanocortin neurons in the hypothalamic arcuate nucleus, causing a decrease in food intake and body weight similar to leptin. This synaptic rearrangement is leptin independent since it was also observed in leptin-mutant obese animals. This effect seems to be mediated by the estrogen receptor  $\alpha$  (ER $\alpha$ ) and by the signal transducer and activator of transcription 3 molecule (STAT3). These above findings support the notion that the synaptic plasticity of feeding circuits is an inherent element in energy homeostasis regulation. Here we have also shown that locally formed estrogen has a developmental effect on the biological clock, and it may contribute to the emergence of gender-specific circadian rhythms. This effect takes place mostly late prenatally at the peak of aromatase activity in the brain. In the adult SCN estrogen enhances the light-induced activity of neurons. Estrogen might act directly on the SCN, and/or indirectly through the raphe serotonin system. The impressive developments of knowledge about these pathways suggest that targeting the estrogen cascade could be a real addition to the treatment of metabolic disorders and biological rhythm abnormalities.

- Abizaid A, Mezei G, Horvath TL (2004) *Estradiol enhances light-induced expression of transcription factors in the SCN. Brain Res 1010: 35–44.*
- Abizaid A\*, Mezei G\*, Thanarajasingam G, Horvath TL (2005) *Estrogen enhances light-induced activation of dorsal raphe serotonergic neurons. Eur J Neurosci 21: 1536–1546 (\*equally contributed to the work).*
- Gao Q, Mezei G, Nie Y, Rao Y, Choi C.S, Bechmann I, Leranth C, Toran-Allerand D, Priest CA, Roberts JL, Gao XB, Mobbs C, Shulman GI, Diano S, Horvath TL (2007) *Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. Nat Med 13: 89–94.*

**ÁGNES MONDOK (2010)****Clinical and pathophysiological studies in acromegaly***Supervisor: Károly Rácz*

In the first part of my work I evaluated the outcome of somatostatin analogue (SSA) treatment, and compared the results of conventional radiotherapy and radiosurgery by means of retrospective analysis of hormonal values and pituitary MRI scans of acromegalic patients. I found that at the end of the SSA treatment applied for  $3.1 \pm 0.3$  years, serum GH returned to the safe value ( $< 2.5$  ng/ml) in 36.7% of the patients, whereas serum IGF-1 normalised in 41.4% of the patients. There was no difference in the ratio of patients who reached the safe hormonal values as a result of SSA treatment between patients treated only with SSA, and those who underwent surgery and/or pituitary irradiation prior to SSA therapy. Adenoma regression occurred in 46% of the cases, and none of the patients had progression of the adenoma. Long term follow-up of acromegalic patients treated with conventional radiotherapy and gamma knife radiosurgery showed that the two methods lead to total or partial tumor regression in approximately the same percentage of patients (77.5% and 78%, respectively). I observed that the long term effects of the two radiotherapeutic techniques on GH overproduction are similar, but complications occur more rarely in patients treated with radiosurgery. In the second part of my work I stated that plasma cortisone (E) level is significantly higher and plasma cortisol/cortisone ratio (F/E) is significantly lower in patients with active acromegaly compared to the values measured in those cured by treatment. My further remarkable observation is that plasma E is significantly lower and plasma F/E is significantly higher in active acromegalic patients with type 2 diabetes mellitus or impaired glucose tolerance than in those with normal carbohydrate metabolism. I consider it possible that change of 11 $\beta$ -HSD1 activity, by alteration of the actual set-point of interconversion of cortisone and cortisol, could play a role in the development of carbohydrate metabolic disorders in acromegalic patients. In the third part of my work I observed for the first time in the literature that cessation of the high GH and IGF-1 levels by pituitary surgery in an acromegalic patient leads to rapid progression of the co-existing amyotrophic lateral sclerosis (ALS). I attribute the relationship between the cure of acromegaly and the rapid progression of ALS to the neuroprotective effect of IGF-1.

- Mondok Á, Szeifert GT, Mayer Á, Czirják S, Gláz E, Nyáry I, Rácz K (2005) *Treatment of pituitary tumors: radiation. Endocrine* 28: 77–85.
- Mondok Á, Szeifert GT, Mayer Á, Czirják S, Gláz E, Nyáry I, Rácz K (2006) *A hypophysis daganatok radioterápiája. Orv Hetil* 147: 1497–1503.
- Mondok Á, Varga I, Gláz E, Szücs N, Tóth M, Patócs A, Bekő G, Rácz K (2009) *11 $\beta$ -hydroxysteroid dehydrogenase activity in acromegalic patients with normal or impaired carbohydrate metabolism. Steroids* 74: 725–729.

**PÉTER REISMANN (2009)****Study of the Toll-like receptor 4 gene polymorphisms in diseases presenting with subclinical and chronic inflammation (diabetes mellitus, ischemic stroke, periodontitis)***Supervisor: Károly Rácz*

Toll-like receptor 4 (TLR4), a central mediator of the innate immune response has been shown to play an important role not only in the defense mechanism against microorganism, but also other non-infectious inflammatory diseases such as atherosclerosis. The common co-segregation polymorphisms of the TLR4 gene Asp299Gly and Thr399Ile have been shown to be associated with increased susceptibility for Gram-negative infections, but with a lower risk of carotid atherosclerosis and a reduced level of certain proinflammatory cytokines. Our aim was to study the influence of the TLR4 gene polymorphisms in different diseases.

Accumulating evidence suggests that acquired immunity as well as inflammation may take part in the pathogenesis of diabetes and its late complications. In the first study we investigated the association between the polymorphisms of the TLR4 gene and diabetes mellitus with its late microvascular complications.

Furthermore, based on the data presenting a reduced risk of carotid atherosclerosis in carriers of the TLR4 gene polymorphisms, we studied the association between these polymorphisms and the risk of cerebral ischemia.

Finally, we studied the association of the TLR4 gene polymorphisms and the risk of chronic periodontitis mainly caused by Gram-negative bacteria. In all studies the alleles of both polymorphisms were detected by PCR and subsequent cleavage by restriction endonucleases followed by gelelectrophoresis.

No difference was found in the prevalence of Asp299Gly and Thr399Ile polymorphisms in patients with type 1 and type 2 diabetes. No association with diabetic neuropathy or diabetic nephropathy was found in patient with type 1 diabetes. In patients with type 2 diabetes, heterozygote carriers of the Asp299Gly and Thr399Ile TLR4 genotypes had a significantly reduced prevalence of peripheral neuropathy, while there was no association with diabetic nephropathy.

Although TLR4 gene polymorphisms might have a protective role in carotid atherosclerosis risk, we failed to show any association between the TLR4 gene polymorphisms and the risk of cerebral ischemia in three independent populations.

Furthermore, TLR4 gene polymorphisms were not associated significantly with the risk of chronic periodontitis, however, in patients carrying the Asp299Gly and Thr399Ile genotypes significantly more clinical attachment loss and radiographic bone loss were detected. Taken together, the TLR4 gene polymorphisms are not enough sensitive genetic markers for risk stratification either in diabetes mellitus or in cerebral ischaemia or in chronic periodontitis. The data presented here do not lend themselves to use for clinical routine. However, the association found with peripheral neuropathy in type 2 diabetes should initiate further animal and cell culture studies.

- Rudofsky GJr\*, Reismann P\*, Witte S, Humpert PM, Iseman B, Chavakis T, Tafel J, Nosikov VV, Hamann A, Nawroth P, Bierhaus A (2004) Asp299Gly and Thr399Ile genotypes of the TLR4 gene are associated with a reduced prevalence of diabetic neuropathy in patients with type 2 diabetes. *Diabetes Care* 27: 179–183.

- Reismann P\*, Lichy C\*, Rudofsky G, Humpert PM, Genius J, Si TD, Dörfer C, Grau AJ, Hamann A, Hacke W, Nawroth P, Bierhaus A (2004) Lack of association between polymorphisms of the toll-like receptor 4 gene and cerebral ischemia. *J Neurol* 251: 853–858.
- Reismann P, Lichy C, Rudofsky G, Meiser H, Nawroth P, Staehle HJ, Bierhaus A, Dörfer C (2007) TLR4-polymorphism and the risk of chronic periodontitis. *Perio* 4: 41–45.

## ÁGNES ÉVA SALLAI (2010)

### The benefits of molecular genetic analysis in the prevention of certain endocrine tumors

Supervisor: Károly Rác

In my work I studied the benefits of molecular genetic analysis in the prevention of certain endocrine tumors associated with hereditary diseases. I showed that the novel semi-quantitative real-time PCR (RT-PCR) method developed by our group proved to be a fast, accurate and highly cost efficient tool for the detection of Ychromosome material in patients with Turner syndrome (TS). Of the 127 Hungarian Ychromosome negative TS patients diagnosed by conventional cytogenetic analysis, Ychromosome material was detected by the novel RT-PCR method in six patients. In one case prophylactic gonadectomy revealed bilateral gonadoblastomas. Based on our findings and previous literature we recommend routine molecular biological screening for hidden Ychromosome sequences in all TS patients, who are negative for Y-chromosome by conventional cytogenetic analysis. The molecular analysis should be performed as early as possible because the appearance of gonadoblastoma seems to occur at an early age of life. The autosomal dominantly inherited tumors in multiple endocrine neoplasia type 2 (MEN2) are caused by germline mutations of the *RET* proto-oncogene. By detailed exploration of medical history and clinical data of a family with MEN 2A syndrome I drew attention on the importance of genetic screening in family members of affected patients. Prophylactic thyroidectomy in patients having disease-causing *RET* mutations prevents the development of medullary thyroid cancer (MTC) improving their life expectancy significantly. I emphasized the importance of analysis of detailed clinical phenotype in two unrelated children with MEN 2B, a rare form of MEN2 syndrome and I showed that delayed diagnosis influences unfavorably the results of surgical treatment. In patients with non-autoimmune primary hyperthyroidism due to germline activating *TSHR* gene mutations multiple adenomas may develop within the thyroid gland. In my work I describe the first Hungarian patient with a de novo heterozygous germline I630L mutation. This mutation has been previously described in the literature as activating somatic mutation in toxic thyroid nodules. Considering the rarity of this disease, I collected and analyzed the clinical features of previously described cases reported in the international literature.

- Sallai Á, Hosszú É, Gergics P, Rác K, Fekete Gy (2008) Orolabial signs are important clues for diagnosis of the rare endocrine syndrome MEN 2B. Presentation of two unrelated cases. *Eur J Pediatr* 167: 441–446.
- Sallai Á, Sólyom J, Dobos M, Szabó J, Halász Z, Ságodi L, Niederland T, Kozári A, Bertalan R, Ugocsai P, Fekete Gy (2010) Y-chromosome markers in Turner Syndrome—screening of 130 patients. *J Endocrinol Invest* 33: 222–227.

- Bertalan R, Sallai Á, Sólyom J, Lotz G, Szabó I, Kovács B, Szabó É, Patócs A, Rácz K (2010) Hyperthyroidism caused by a germline activating mutation of the thyrotropin receptor gene: difficulties in diagnosis and treatment. *Thyroid* 20: 327–332.

## JUDIT TÓKE (2010)

### Extracellular calcium sensing under normal and pathological conditions Investigation of CaSR gene mutations in Hungarian patients

Supervisor: Miklós Tóth

In this work I summarized the results of clinical and genetic investigations of hypercalcemic disorders caused by mutations of calcium-sensing receptor. The diagnosis based on family screening and some simple laboratory methods. Molecular biological investigations were carried out using DNA samples of eight patients diagnosed with familial hypocalciuric hypercalcemia and one patient with neonatal severe hyperparathyroidism. CaSR gene mutations were identified in 25% of patients with FHH. Analysing the unique disease course of a patient with neonatal hyperparathyroidism, I demonstrated that NSHPT revert spontaneously to a symptomless hypercalcemic state. A novel heterozygous inactivating point mutation (R551K) and a known heterozygous polymorphism were demonstrated on the paternal allele of the CaSR gene of this patient. Using HEK-293 cells, I carried out functional molecular biological experiments in order to analyse the effects of these two point mutations on the signal transduction of the CaSR. My results studies demonstrated that the R551K mutation reduces the calcium sensitivity of mutant CaSR and this reduction is not alleviated by the simultaneous presence of A986S polymorphism located on the same allele. A retrospective analysis was performed studying the clinical characteristics and laboratory results of 141 consecutive patients diagnosed with PTH-dependent hypercalcemia between 1997 and 2007 at the 2nd Department of Medicine of Semmelweis University. Of the 141 patients with PTH-dependent hypercalcemia, 18 patients (12.7%) had a hereditary hypercalcemic condition. Each patient with PTH-dependent hypercalcemic disorder younger than 30 years was classified as having one of the hypercalcemic syndromes.

- Toke J, Czirjak G, Patocs A, Enyedi B, Gergics P, Csakvary V, Enyedi P, Toth M (2007) Neonatal severe hyperparathyroidism associated with a novel de novo heterozygous R551K inactivating mutation and a heterozygous A986S polymorphism of the calcium-sensing receptor gene. *Clin Endocrinol* 67: 385–392.
- Toke J, Patocs A, Balogh K, Gergics P, Stenczer B, Racz K, Toth M (2009) Parathyroid hormone-dependent hypercalcemia. *Wiener Klinische Wochenschrift* 121: 236–245.
- Tóth M, Tóke J, Horányi J, Stenczer B, Patócs A, Balogh K, Jakab Zs, Szücs N, Gergics P, Varga I, Rácz K, Tulassay Zs (2007) A sporadikus és familiáris primer hyperparathyreosis klinikai és laboratóriumi jellemzői. *Magy Belorv Arch* 60: 154–159.

**PROGRAM 2/14.****CLINICAL AND EXPERIMENTAL RESEARCH ON UROLOGICAL DISEASES*****Coordinator:*****Imre ROMICS M. D., Ph. D., D. Sc.**

Department of Urology

78/B Üllői st, Budapest H-1082

Tel.: +36 1 210 0280

E-mail: [romics.imre@med.semmelweis-univ.hu](mailto:romics.imre@med.semmelweis-univ.hu)**Program overview**

The program offers research on several fields of clinical and experimental urology and andrology.

***Titles of research projects***

Hereditary and acquired disorders of the external genital and urological tracts

Diagnostic and therapeutic challenges in urine retention and evacuation diseases

Up to date clinical experimental research of andrological diseases

Reconstructive urology

Novel diagnostic and therapeutic options in urological tumors

Etiology and etiopathogenesis of urinary tract stones, challenges in drug therapy, surgical treatment and prevention

Inflammatory disorders of the urinary organs

Congenital anomalies of the kidney, urinary tract and external genital tract in newborn infants

***Supervisors***

Zsolt Kelemen

Péter Nyirády

Péter Nyirády

Imre Romics

Imre Romics

Imre Romics

Imre Romics

Éva Görbe

***Ph. D. candidates***

Gergely Bánfi

na

Katalin Bedi

ft

András Horváth

na

István Laczkó

na

***Supervisors***

Imre Romics

Imre Romics

Péter Nyirády

Péter Nyirády

***Ph. D. graduates***

Fares Mohammed Osman

it

Attila Keszthelyi

na

Péter Riesz

na

Attila Szendrői

na

***Supervisors***

Péter Nyirády

Péter Nyirády

Imre Romics

Imre Romics

ft, full-time; na, not affiliated; it, international



## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**FARES MOHAMMED OSMAN (2010)**

### **Control of ureteral motility, synchronization of the circular and longitudinal muscle layers, a novel videomicroscopic technique**

*Supervisor: Péter Nyirády*

Ureteral motion propels urine from the renal calyces to the bladder. Despite its physiological, pathological and clinical importance our knowledge on the organization and control processes of ureteral movements is very limited. In the present Ph.D. thesis we analyzed practically all published literature on ureteral motility, its histological, physiological, cellular and pharmacological background, and attempted to give a coherent review of our present day knowledge on the Titles of research projects. For a better understanding of the mechanical events of the ureteral peristaltic cycle, the cooperation of the circular and longitudinal smooth muscle layers an *in vivo* technic was developed to follow the ureteral movements. With the aid of the newly developed videomicroscopic technic the synchronization of longitudinal and circular contractions could be analyzed under control conditions and under drug effects. Analysis of the literature revealed that several histologic, cytologic and physiologic characteristics of smooth muscle in general have not yet been tested on ureteral smooth muscle. Existing data reveals that substantial differences should exist between smooth muscle of the upper and lower urinary tracts. There are many differences between ureteral smooth muscle and other smooth muscle with peristalsis, e. g. intestinal muscle. Data obtained for other types of smooth muscle should not be automatically applied for ureteral muscle. For this reason we did everything to limit the scope of our overview to observations specifically on the ureter.

A surgical technique has been developed to isolate the middle portion of the left ureter of the rat. A tissue chamber was developed that could be positioned around the ureter ensuring its continuous superfusion with saline and added drugs while through the plastic window of the chamber its movements could be videomicroscopied. Urine propagation was analyzed by recording the movement of the urine level in a microcannula inserted into the left orifice. Characteristic points on the ureteral surface were identified using the pattern of the vasa vasorum on digitized, frozen videomicroscopic pictures. Their coordinates were determined during peristaltic cycles. Steric movements were analyzed as time functions of displacements of these points. In addition, autocorrelation functions were constructed to identify periodic components. Three types of movements were separated: (i) a longitudinal displacement as a result of axial contraction outside the observation territory (ii) a longitudinal contraction/relaxation at the observation territory (iii) contraction/relaxation of the diameter. Our observations proved the ordered sequence of these movements. As a new observation, we have found that the longitudinal smooth muscle layer plays a more important role in propelling the urine bolus as it was thought earlier. Contraction of the longitudinal muscle in the midportion passively, axially distends the upper parts of the ureter while the circular muscle is still in the relaxed state, the result is an axial “diastolic” phase that helps filling from the calyces. The longitudinal contraction of the lower part of the ureter, followed by the circular contraction ring helps inject urine into the bladder.

The longitudinal smooth muscle layer and its coordination with the circular one plays an essential role in ureteral function. Any attempt to analyze drug effects or upper urinary tract pathology should consider that fact.

- Osman F, Nádasz GL, Monos E, Nyírády P, Romics I (2009) A novel videomicroscopic technique for studying rat ureteral peristalsis. *World J Urol* 27: 265–270.
- Osman F, Romics I, Nyírády P, Monos E, Nádasz GL (2009) Ureteral motility. *Acta Physiol Hung* 96: 407–426.

## ATTILA KESZTHELYI (2009)

### Changes in the function of lower urinary tract following radical urological surgeries

Supervisor: Péter Nyírády

Radical urological procedures are known to cause a significant change in the quality of life. Incontinence is seen as most important factor affecting quality of life following radical prostatectomy and cystectomy. In this study we analyse the outcomes of these two common urological procedures. Radical prostatectomy involves the removal of both the prostatic urethra and the interruption of the internal sphincter, and is therefore responsible for the dysfunction of urine evacuation and flow. The removal of the urinary bladder on the other hand brings about a completely new function to the urinary system. Following cystectomy there are common two forms of urinary diversion, we examined urinary complaints and changes in quality of life of patients following both the Mainz pouch II type ureterosigmoidostomy and the urethral orthotopic Reddy ileal neobladder formation. We conducted a prospective and retrospective analysis using urodynamic analysis and rectal manometry to identify causes of incontinence. According to our findings the most common cause of incontinence following radical prostatectomy is a weakness of the urethral sphincter. The procedure itself does not influence bladder function nor does it affect the lower pelvic musculature. To identify causes of incontinence following cystectomy and neobladder formation we examined the function of the new bladder and sphincter. The surgical technique adopted in these procedures is important in preventing incontinence, moreover patients may be rendered incontinent if the length of bowel used for construction is not large enough. Urodynamic studies demonstrate that peristalsis returns in the neobladder bowel wall despite detubularisation. Incontinence following Reddy type ileal neobladder formation is due in part to uncontrolled neobladder contraction and weakening of sphincter function. Rectal manometry is therefore considered important in estimating the function of the lower pelvic musculature and likelihood of postoperative incontinence. Due to its ease of use and minimally invasive nature we recommend the routine use of this investigation before all radical urological surgeries. Analysis of Mainz II pouch results reveal that surgery lowers the rectal sphincter closing pressure. As a result of the risk of faecal and urinary incontinence ureterosigmoidostomy is recommended for urinary diversion only in selected cases where good sphincter function has been demonstrated preoperatively with anal manometry.

- Keszthelyi A, Majoros A, Nyírády P, Mayer P, Bach D, Romics I (2009) Voiding symptoms and urodynamic findings in patients with modified ileal neobladder. *Pathol Oncol Res* 15: 307–313.

- Majoros A, Bach D, Keszthelyi A, Hamvas A, Romics I (2006) Urinary incontinence and voiding dysfunction after radical retropubic prostatectomy (prospective urodynamic study). *Neurourol Urodyn* 25: 2–7.
- Majoros A, Bach D, Keszthelyi A, Hamvas A, Mayer P, Riesz P, Seidl E, Romics I (2007) Analysis of risk factors for urinary incontinence after radical prostatectomy. *Urol Int* 78: 202–207.

## PÉTER RIESZ (2009)

### New diagnostic and prognostic examinations of malignant tumors of urinary bladder

Supervisor: Imre Romics

Bladder carcinoma is the second leading malignant tumor in urology and it is getting more and more common in all over the world. The central query of the successful therapy are the early diagnostics, up-to-date valuation of the prognostical factors and with the help of these further indications of therapeutic steps. The goal of this thesis was to introduce the application of different methods for early clinical diagnostics such as genetic investigations, tumor markers and connections between the tissue structure of bladder cancer and the survival rate of the patients which are heading for the prognostication of bladder cancer. In the case of our tumor marker investigations, we analyzed the serum polypeptide antigene concentration in tumors with different tissue structure and in dissimilar stage and we made them compared with data from controlling groups. Summing up our experiments, we came to the conclusion that, however, the tumor marker concentration rises in muscular invasive processes, it is not sufficient as a classical marker for recognizing and screening of tumors. As for the molecular diagnostic tools, we developed two methods capable of recognizing chromosomal differences, moreover, we also aimed at increasing their efficiency. Both the fluorescent *in situ* hybridization method and the detection of microsatellites from urine samples considered as an excellent investigation methodology.

In the course of FISH experiments the followings were realized: if the UroVysion diagnostic are done both with the first urine per day and with the second which is a consequence of the forced fluid intake, we could get more accurate data, however, in the case of little histological bladder tumors in stage pTaG1 the results still remain uncertain. Concerning the investigations with microsatellites, we realized that the sensitivity of the check could further be increased with analyzing the DNS without cells only from the supernatant of the urine probes. As for the final conclusion, despite the above mentioned methods possessing excellent results, they are unable to take cytосcopy out from everyday practice. Patients diagnosed with inverted papilloma were followed up prospectively at prognostic medical checks and no renewal was detected but unfortunately bladder cancer occurred in a male patient among the twelve patients we treated. In spite of having no primary evidence (IA/IB) on taking stand on the follow-up of patients, we suggest that follow-up based on literature data according to pTaG1 bladder cancer. For our further investigations dealing with prognostics and carcinogenesis, we examined the strength of the E-cadherine expression, which is one of the most important cell adhesive molecule, in tissue intersections from patients with bladder tumor. Opposite to the

results of other work groups, we found no significant correlation among the stage and grade of differentiation of tumor, number of recidives and survival of patients. In one hand, the claudines, which are fall under the tight junction transmembrane proteins, are suspected to have a share in developing many tumors. During our experiments, we discovered the claudine expression pattern of normal vesical urothellum, of inflamed vesical tissue and of different pathological stages of bladder cancer. With these data we aimed at getting further to the role of cell-linking structures especially the role of claudines in the development and progression of bladder carcinoma. In the other hand, both the mRNS and protein expression of claudine-1, -4, -7 decrease in parallel with the progression in muscular invasive cases. Anyway, it is well known that tumor type pT1G3 creates an own entity among bladder tumors. Should the anticipating and invasive ability of pT1G3 carcinoma be estimated ahead, we could achieve curative intervention in more patients on time. As for stage pT1G3, it seems to be an internal stage between pT1G2 and pT2G3 tumors and in recidival cases the protein expression of claudine-1, 4, 7 is higher. That expression result could be the help with making correct therapeutic decisions in case of this inestimable group of carcinoma.

The goal of recent medical research is to develop medicines with less side effects and with more specificity for different kinds of tumors which would really be successful if molecular complexes could be synthesized with target points appropriate also for tumor cells. After having the pattern of claudine in bladder cancer been discovered, the claudine-4, which were detected both in mRNS and protein layer, seems very promising therapeutic target for the basic of further laboratory works.

- Riesz P, Lotz G, Páska Cs, Szendrői A, Majoros A, Németh Zs, Törzsök P, Szarvas T, Kovalszky I, Schaff Zs, Romics I, Kiss A (2007) Detection of bladder cancer from the urine using fluorescence in situ hybridization technique. *Pathol Oncol Res* 13: 187–194.
- Szarvas T, Kovalszky I, Bedi K, Szendrői A, Majoros A, Riesz P, Füle T, László V, Kiss A, Romics I (2007) Deletion analysis of urinary DNA to detect bladder cancer: urine supernatant versus urine sediment. *Oncol Rep* 18: 405–409.
- Riesz P, Székely E, Török V, Székely T, Romics I (2007) E-cadherin expression in transitional cell carcinomas. *Magy Urol* 3: 159–163.

## ATTILA SZENDRŐI (2010)

### The prognostic factors of renal cell carcinoma

Supervisor: Imre Romics

The renal clear cell carcinoma is the most malignant urological tumor and its mortality has increased 50% during the last 30 years in Hungary. Treatment options are highly based on the expected outcome of the disease, which has a significant importance due to the expansion of diagnostic and therapeutic possibilities. Furthermore, the behavior of the renal clear cell carcinoma is unforeseeable; therefore during the determination of patients' life expectancies many factors—closely interlinked with each other and have not totally been clarified yet—should be taken into account. This present dissertation is aiming at investigating the prognostic factors of renal clear cell carcinoma with special regards to the prognostic bearings of the symptoms which were induced by renal clear

cell carcinoma and to the investigations of the factors which can influence the outcomes of the disease in the case of bone metastases.

The investigations of the symptoms caused by primary renal clear cell carcinoma showed that patients with symptoms related to renal tumors (lumbar and abdominal pains) rarely consulted a physician. We proved the correlation between the symptoms caused by the carcinoma (independent from any other factors) and the intraoperative complications. Hereby, we have the opportunity to plan the surgery more precisely in order to avoid the possible complications in the case of patients with symptoms. It was also observed that the postoperative survival is shorter in the case of patients with symptoms. In addition, mortality was remarkably influenced by the characteristic of symptoms (local, systematic or symptoms caused by metastases). Accordingly, taking medical history in data which influence the outcome of the disease and the complications as well; moreover, it helps us with planning the treatment and with judging the prognoses.

According to our investigations concerning renal clear cell carcinoma with bone metastases, the following factors had no influences on the survival of the patients: age, gender, the stage as well as the Fuhrman grade of the primary renal tumor, the symptoms caused by the metastases, the presence of pathological fracture, the spreading of the metastasis to the soft tissues, the size of metastasis, localization of the metastasis in the skeletal system (spinal localization excluded), in the case of multiplex metastases, the multiorganic occurrence or sole skeletal involvement. The survival was otherwise significantly improved by the followings: the Fuhrman grade of metastasis, the multiplicity (solitary or multiplex occurrence) of metastases and the surgical radicality. If the radical removal of solitary metastasis was carried out, 35.5% of the patients lived the fifth postoperative year. In the case of multiple metastases or non-radical surgery, none of the patients lived the fifth postoperative year. Based on our results, it can be concluded that in the case of surgically resectable, solitary metastases with low Fuhrman grade, we should perform radical surgery. Here not only the life quality of the patient could be preserved, but in the case of solitary metastasis more than 10 years of survival might also be expected in certain cases.

- Szendrői A, Dinya E, Kardos M, Szász AM, Németh Z, Ats K, Kiss J, Antal I, Romics I, Szendrői M (2010) *Prognostic factors and survival of renal clear cell carcinoma patients with bone metastases. Pathol Oncol Res* 16: 29–38.
- Szendrői A, Tabák A, Riesz P, Szűcs M, Nyirády P, Majoros A, Haas G, Romics I (2009) *Clinical symptoms related to renal cell carcinoma are independent prognostic factors for intraoperative complications and overall survival. Int Urol Nephrol* 41: 835–842.
- Szendrői A, Antal I, Riesz P, Szendrői M, Romics I (2004) *Vesedaganat csontáttétjének műtéti kezelése. Magy Urol* 16: 9–17.

**PROGRAM 2/15.****MOLECULAR GENETICS, PATHOMECHANISMS,  
AND CLINICAL ASPECTS OF METABOLIC DISORDERS*****Coordinator:*****Péter LAKATOS M.D., Ph.D., D.Sc.**

1st Department of Medicine

2/A Korányi S. st, Budapest H-1083

Tel.: +36 1 210 0278, Tel./fax: +36 1 210 4874

E-mail: [lakatos.peter@med.semmelweis-univ.hu](mailto:lakatos.peter@med.semmelweis-univ.hu)**Program overview**

The program consists of 13 research sub-programs completed with appropriate theoretical courses for postgraduate students. Molecular as well as pathological and clinical aspects of different metabolic diseases are studied including metabolic bone diseases and disturbances of calcium metabolism and lipid metabolism, disorders in onco-hematology processes, endocrine glands, diabetes mellitus and vascular diseases. Ph.D. students are working under the supervision of a qualified scientist but also participate in the work of the laboratory. Publication in peer-reviewed international journals is a requirement for a successful Ph.D. thesis.

***Titles of research projects******Supervisors***

Molecular genetics, pathomechanism, early diagnosis, prevention and therapy of chronic liver diseases	Margit Abonyi
Regulation of endocrine functions of fat tissue and its relation to insulin resistance	Károly Cseh
Metabolic aspects of malignant hematological disorders	Judit Demeter
Investigation of disorders associated with macro- and microvascular complications and risk factors of atherothrombotic vascular diseases	Csaba Farsang
Pituitary gland dysfunction: clinical and experimental studies.	Miklós Góth
Ghrelin and cell proliferation	
Identification of genes participating in the stimulatory effect of ghrelin on cell proliferation	Miklós Góth
Effect of calcium and bone metabolism disorders and the drugs influencing it on mineral content, quality and mechanical properties of bone tissue	Csaba Horváth
Investigation of disorders associated with macro- and microvascular complications and risk factors of atherothrombotic vascular diseases	Zoltán Járαι
Gestational diabetes as preexisting condition for type 2 diabetes and metabolic syndrome	Zsuzsa Kerényi
Investigation the pathophysiology of insulin resistance. Development of early diagnostic tools and therapeutical interventions	László Korányi
Thyroid disorders and their effects on bone metabolism	Péter Lakatos
Molecular genetics, pathomechanism, early diagnosis, prevention and therapy of chronic liver diseases	Péter László Lakatos



Molecular mechanisms in bone metabolism	Attila Mócsai
Extraskeletal effects of key genes and proteins of bone metabolism	Gábor Speer
Epidemiology, pathogenesis and diagnosis of thyroid cancers	István Takács
Pathophysiological aspects of normoglycemic control of diabetes mellitus	Gyula Tamás
Prevalence and incidence of diabetes and pathophysiological points of normoglycemic treatment	Gyula Tamás
Endocrinological aspects, pathomechanism and therapy of polycystic ovary syndrome	Szabolcs Várbíró

***Ph. D. students***

Örs Levente Babos	ft
Judit Dénes	ft
Csaba Halászlaki	pt
Lajos Kiss	pt
Rita Magenheimer	ft (a)

***Supervisors***

Zoltán Járai
Miklós Góth
István Takács
László Péter Lakatos
Gyula Tamás

***Ph. D. graduates***

Péter Fuszek	na
Ádám Tabák	na
Krisztián Bácsi	ft
Bernadett Balla	ft
Henrik Csaba Horváth	pt
János Kósa	na
Áron Lazáry	ft
Eszter Madarász	ft
Judit Nádas	na
Zsolt György Nagy	na
Éva Palik	na
El Hajd Othmane Taha	it
András Khoór	na
Magdolna Krasznai	na

***Supervisors***

Gábor Speer
Gyula Tamás
Péter Lakatos
Péter Lakatos
Gábor Speer
Gábor Speer
István Takács
Zsuzsa Kerényi
Károly Cseh
Gábor Speer
Károly Cseh
Zoltán Járai
Károly Cseh
Károly Cseh

a, absolutorium; ft, full-time; pt, part-time; it, international; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**BERNADETT BALLA (2010)**

### **Investigation of bone metabolism in different molecular biological levels**

*Supervisor: István Takács*

Sex hormone deficiency after menopause has multifunctional role by influencing growth, differentiation, metabolism of the skeletal system and results in marked increases in bone resorption and formation, leading to rapid bone loss. However the complex genetic effects leads to osteoporosis are not exactly established.

Our aims were to determine genes characterized by significantly changed mRNA expression rates in postmenopausal osteoporotic and non-osteoporotic human bone tissue and to describe the relationships between these genes using multivariate data analysis. Messenger RNA was prepared from each sample and reverse transcribed to cDNA. The expression differences of 147 selected genes were analyzed in a TaqMan probe-based quantitative real-time reverse transcriptase-polymerase chain reaction system.

Mann-Whitney U-test indicated significant ( $p \leq 0.05$ ) differences in the expression of numerous genes involved in extracellular matrix formation and degradation, lipid metabolism, osteoblast/adipocyte differentiation. Some genes belonging to the transforming growth factor- $\beta$ /bone morphogenic protein pathway, genes controlled via estrogen receptor- $\alpha$ . Principal components analysis was used to evaluate data structure and the relationship between postmenopausal osteoporotic and non-osteoporotic phenotypes based on the multiple mRNA expression profiles of genes. According to the canonical variate analysis results, the groups of the examined women are separable by genes coding for cytokines, costimulator molecules, and cell surface receptors involved in antigen presentation and T cell stimulation processes which have high discriminatory power.

Based on a complex gene expression pattern analysis of human bone tissue, we could distinguish menopausal states from an immunological aspect. Significant differences observed in gene expression profiles of osteoporotic and non-osteoporotic human bone tissues. Our data might provide further insight into the changes of the intersystem cross-talk between immune and skeletal homeostasis, as well as local immune response in the altered microenvironment of postmenopausal bone. Characterization of the differences between osteoporotic and non-osteoporotic bones by expression profiling will contribute to the development of diagnostic tools in the future.

- Balla B, Kósa JP, Kiss J, Borsy A, Podani J, Takács I, Lazáry Á, Nagy Zs, Bácsi K, Speer G, Orosz L, Lakatos P (2008) Different gene expression patterns in bone tissue of aging postmenopausal osteoporotic and non-osteoporotic women. *Calcif Tissue Int* 82: 12–26.
- Balla B, Kósa JP, Takács I, Kiss J, Podani J, Borsy A, Lazáry Á, Bácsi K, Nagy Zs, Speer G, Orosz L, Lakatos P (2008) Menopauza hatása a csontszöveti génkifejeződésre posztmenopauzás és premenopauzás korú nem oszteoprotikus nőkben. *Magy Belorv Arch* 61: 208–219.
- Lazáry Á, Kósa JP, Tóbiás B, Lazáry J, Balla B, Bácsi K, Takács I, Nagy Zs, Mező T, Speer G, Lakatos P (2008) Single nucleotide polymorphisms in new candidate genes are associated with bone mineral density and fracture risk. *Eur J Endocrinol* 159: 187–196.

**HENRIK CSABA HORVÁTH (2010)****The expression of CYP24A1, the 1,25(OH)2D3 catabolizing enzyme during colorectal tumourigenesis***Supervisor: Gábor Speer*

Despite tremendous progress in the knowledge of disease pathomechanism during the past decades, colorectal carcinoma is still one of the most common malignant disease and cause of death from malignancy in Hungary and in the industrialised world. Epidemiological data suggest a protective role for vitamin D in colorectal tumourigenesis and tumour progression due to its antiproliferative, prodifferentiation and proapoptotic potential. Recent data showed evidence of extrarenal vitamin D metabolism and strong antitumour effect of vitamin D in a paracrine/autocrine manner.

We examined the expression of the catabolizing enzyme of the active vitamin D metabolite 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), 25-hydroxyvitamin D3 24-hydroxylase (CYP24A1) mRNA (by real-time PCR) and protein (by immunohistochemistry) in normal colon mucosa, colorectal adenoma and carcinoma samples. We also analyzed the mRNA expression levels of further major determinants of vitamin D actions, the synthesizing 25-hydroxyvitamin D3 1-alpha-hydroxylase enzyme (CYP27B1) and vitamin D receptor (VDR). We correlated the CYP24A1 expression data with the clinical features of the patients and pathomorphological parameters of the samples as well as with the expression of the Ki 67 proliferation marker. Since existence of CYP24A1 isoforms has been reported recently, we also investigated the presence of CYP24A1 splicing variants in our sample set. Significantly higher expression of both CYP24A1 mRNA and protein was found in adenocarcinomas and adenomas compared with normal mucosa. The expression of VDR was significantly decreased in benign and even more in malignant tumours, while CYP27B1 expression has not changed significantly, however a trend towards lower expression in adenocarcinomas was seen. We found no significant association between CYP24A1 expression and clinico-pathological characteristics of the samples. However, a strong positive correlation was demonstrated between CYP24A1 and Ki 67 confirming our hypothesis that CYP24A1 overexpression reduces local 1,25(OH)2D3 and thus its antiproliferative effect. Three splicing variants of CYP24A1 was found in colon tissue. These isoforms have dysfunctional catabolizing activity which may cause alterations in the microenvironmental regulation of vitamin D3 levels in colorectal tissue.

Our findings will lead to a better understanding of the role of vitamin D3 metabolism and action during colorectal tumourigenesis and thus, it can offer new preventive and/or therapeutic strategies against colorectal cancer.

- Horvath HC, Khabir Z, Nittke T, Gruber S, Speer G, Bonner S, Manhardt T, Kállay E (2010) CYP24A1 splice variants—implications for the antitumorigenic actions of 1,25-(OH)2D3 in colorectal cancer. *J Histochem Cytochem* 58: 277–285.
- Horváth HC, Lakatos P, Kósa JP, Bácsi K, Borka K, Bises G, Nittke T, Hershberger PA, Speer G, Kállay E (2010) The candidate oncogene CYP24A1: a potential biomarker for colorectal tumorigenesis. *J Histochem Cytochem* 58: 277–285.

## JÁNOS KÓSA (2009)

### Investigation of the genomic background of postmenopausal and glucocorticoid-induced bone loss

Supervisor: Gábor Speer

Primary (postmenopausal) and secondary (such as the glucocorticoid-induced) osteoporosis is a multifactorial disease with high heritability but its exact genetic background is still poorly understood. Oestrogen deficiency at the time of menopause results in marked increases in bone resorption and formation, leading to rapid bone loss. Also, glucocorticoids partly detain bone formation via the inhibition of osteoblastic function, however, the exact mechanism is not fully understood (of this inhibition remains elusive).

The aim of my study was to determine the influence of the menopausal changes on metabolism of bone tissue. So we investigated a genes characterized by significantly changed mRNA expression rates in postmenopausal versus premenopausal bone tissue and to describe the interrelationships among these genes using multivariate data analysis. Also, the functional interaction between the immune system and postmenoausal bone metabolism has been established at both molecular and cellular levels. Secondary, in our study we examined the effect of dexamethasone, (an active glucocorticoid analogue,) on cell viability and expression of bone formation.

The Mann-Whitney U test indicated significant differences in the expression of any genes of postmenopausal and premenopausal women. These genes, including extracellular matrix molecules and digesting enzymes, genes belonging to the transforming growth factor-beta/bone morphogenic protein pathway, transcription factors, growth factors, and other candidate genes, were significantly up-regulated in postmenopausal women compared with premenopausal women. Canonical variates analysis demonstrated that postmenopausal and premenopausal bone tissues can be distinguished by expression analysis of genes controlled via estrogen receptor-alpha and genes coding for extracellular matrix molecules. As a result of dexamethasone treatment we have detected significant apoptotic cell death, and others, including Smad3, type-2 and -9 collagen, matrix metalloproteinase-2, bone morphogenetic protein-4 and bone morphogenetic protein-8 showed significant changes in their expression on a time and concentration dependent manner. Bone morphogenetic protein-8, (a novel player in bone-metabolism,) exhibited a two orders of magnitude elevation in its mRNA level and highly elevated protein concentration by Western-blot in response to dexamethasone treatment. The knockdown of Bmp-8 by RNA interference significantly increases dexamethasone induced cell death, confirming a messenger role in the glucocorticoid-induced bone loss. Also, our results support the important role of BMP-8 in bone metabolism, especially induced by glucocorticoids.

- Kósa JP\*, Balla B\*, Kiss J, Borsy A, Podani J, Takács I, Lazáry Á, Nagy Z, Bácsi K, Speer G, Orosz L, Lakatos P (2009) Effect of menopause on gene expression pattern in bone tissue of nonosteoporotic women. *Menopause* 16: 367–377 (\*equally contributed to the work).
- Kósa JP\*, Balla B\*, Kiss J, Podani J, Takács I, Lazáry Á, Nagy Z, Bácsi K, Karsai A, Speer G, Lakatos P (2009) Postmenopausal expression changes of immune system-related genes in human bone tissue. *J Clin Immunol* 29: 761–768 (\*equally contributed to the work).
- Balla B, Kósa JP, Kiss J, Podani J, Takács I, Speer G, Bácsi K, Lazáry Á, Nagy Z, Lakatos P (2009) Transcriptional profiling of immune system-related genes in postmenopausal osteoporotic versus non-osteoporotic human bone tissue. *Clin Immunol* 131: 354–359.

**ÁRON LAZÁRY (2010)****Molecular biological approach of osteoporosis and surgical bone substitution***Supervisor: István Takács*

Osteoporosis is a common disease where heritability plays also important role in the pathogenesis beyond the environmental factors. Previous studies showed that the heritability of the bone quality and quantity parameters is around 60–80% but the exact role of the participant genes in the pathomechanism is still unclear. In our research work we investigated the effect of the allelic variants of five new candidate genes—*alkaline phosphatase* (ALPL), *matrix metalloproteinase 2* (MMP2), *tissue inhibitor of metalloproteinases 2* (TIMP2), *fibroblast growth factor receptor 1* (FGFR1), and *fatty acid-binding protein 3* (FABP3)—previously identified in an orthologous (red deer) model on bone mineral density (BMD) and fracture risk. We also studied the influence of intradiscal cement leakage—most common complication of minimal invasive surgical treatment for vertebral compression fracture—on the biology of the intervertebral disc. In our work we also analyzed the molecular biological background of the use of gypsum as synthetic bone graft. Three hundred sixty postmenopausal women were included into the genomic study and association of twenty-four single nucleotide polymorphisms (SNPs) in the five new genes with BMD and fracture risk were investigated. Applying robust statistical methods haplotype and gene-gene interaction analyses were also performed. For in vitro studies isolated human nucleus pulposus cells and mice preosteoblasts were cultured and treated with different type of synthetic bone grafts. Gene expression studies as well as cell viability and biochemical measurements were performed from the cell cultures.

We found previously not reported associations among the SNPs in the new candidate genes and the bone quality phenotypes. *rs6996321* in FGFR1 was significantly associated with lumbar BMD while *rs10914367* in the promoter of FABP3 was related to femoral BMD and *rs9900912* in TIMP2 significantly influenced the non-vertebral fracture risk. We identified a haplotype in FGFR1 associated with lumbar BMD and some highly suggestive gene-gene interactions having significant effect on bone phenotypes. We described that the commonly used PMMA had toxic effect on nucleus pulposus cells while hydroxy-apatite and gypsum based vertebroplasty filler materials proved to be more biologically compatible. Gypsum had also advantageous influence on the proliferation and differentiation of the bone cells.

In our research work, we identified new candidate genes and polymorphisms playing significant role in the process of osteoporosis. We also described that the most common complication of vertebral augmentation, the intradiscal cement leakage, could increase the subsequent new compression fractures and it could strongly depend on the type of the bone cement. We concluded that avoid of cement leakage and development of biologically and biomechanically optimal filler materials should be desired. We demonstrated the molecular biological background of the use of gypsum as synthetic bone graft as we first published that bone cells differentiated into the direction of new bone formation and this process was mediated by the high calcium content and special mineral structure of gypsum.

- Lazáry Á, Kósa JP, Tóbiás B, Balla B, Bácsi B, Nagy Zs, Takács I, Mező T, Speer G, Lakatos P (2008) Single nucleotide polymorphisms in new candidate genes are associated with bone mineral density and fracture risk. *Eur J Endocrinol* 159: 187–196.
- Lazáry Á, Speer G, Varga PP, Balla B, Bácsi B, Kósa JP, Nagy Zs, Takács I, Lakatos P (2008) The effect of vertebroplasty filler materials on viability and gene expression of human nucleus pulposus cells. *J Orthop Res* 26: 601–607.
- Lazáry Á, Balla B, Kósa JP, Bácsi K, Nagy Zs, Takács I, Varga PP, Speer G, Lakatos P (2007) Effect of gypsum on proliferation and differentiation of MC3T3-E1 mouse osteoblastic cells. *Biomaterials* 28: 393–399.

## ESZTER MADARÁSZ (2010)

### Gestational diabetes as preexisting condition for type 2 diabetes and metabolic syndrome

Supervisor: Zsuzsa Kerényi

Gestational diabetes (GDM) is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. GDM is a well-known risk factor of incident diabetes later in life, and is probably a predictor or an early manifestation of metabolic syndrome. In the present work we analyzed data of previous GDM women under our care during pregnancy in an average 4 years after delivery, and compared their results to that of a group women, who had been metabolically healthy during pregnancy. While glucose intolerance (type 2 diabetes, impaired fasting glucose and impaired glucose tolerance) was almost 3 times more frequent in the previous GDM group compared to controls (OR 4.10, 95%CI 1.51–11.05), type 2 diabetes was found exclusively among previous GDM women. The only independent predictor of follow-up glucose intolerance was the fasting blood glucose measured during the diagnostic oGTT during pregnancy. Previous GDM women had a worse cardiovascular risk profile compared to healthy subjects. They had more pronounced obesity (measured as body mass index, waist circumference, waist to hip ratio), higher fasting plasma insulin, triglycerides, LDL-cholesterol, and blood pressure levels. Using any criteria to diagnose the metabolic syndrome (a cluster of cardiovascular risk factors), its prevalence was increased among previous GDM women compared to controls.

Novel observations suggest that adipocytokines may be important determinants of later diabetes and cardiovascular disease. We found higher circulating fasting leptin and resistin levels, and lower adiponectin values in women with previous gestational diabetes. We described first, that leptin levels significantly decreased 90 minutes after an OGTT in women without glucose intolerance, while this decrease was significantly lower (and not different from 0) in women with present glucose intolerance, independently of prior GDM status. According to our novel observation, OPG levels (a marker of both bone metabolism and atherosclerosis) were independently associated with current glucose intolerance, higher gamma-glutamyl transferase and fasting serum C-peptide levels. Our results highlight that the investigations of gestational diabetes may help us to better understand the etiology of type 2 diabetes and cardiovascular disease and may also advance our knowledge on their prevention.



- Madarász E, Tabák GyÁ, Speer G, Lakatos P, Kerényi Zs, Tamás Gy (2009) Abnormal glucose tolerance is associated with diminished postload change in leptin levels in women. *Diabetes Metab Res Rev* 25: 632–638.
- Madarász E, Tamás Gy, Tabák GyÁ, Kerényi Zs (2009) Carbohydrate metabolism and cardiovascular risk factors 4 years after a pregnancy complicated by gestational diabetes. *Diab Res Clin Pract* 85: 197–202.
- Madarász E, Tamás Gy, Tabák GyÁ, Speer G, Lakatos P, Kerényi Zs (2009) Osteoprotegerin levels in women with prior gestational diabetes mellitus. *Diabetes Care* 32: e5.

## JUDIT NÁDAS (2010)

### Clinical aspects of the metabolic syndrome according to investigations in several cohorts of the Hungarian population

Supervisor: Károly Cseh

The clinical significance of the metabolic syndrome as a distinct entity has been debated in the past years, although the clustering of cardiovascular risk factors is unquestionable. For the first time in Hungary we have analysed the characteristics of cardiovascular risk factors typical to the metabolic syndrome in adult subjects with type 1 diabetes. We have found that the occurrence of these risk factors was frequent among patients with type 1 diabetes, showing a higher prevalence compared to the general population. According to our results the occurrence of metabolic syndrome was linked primarily to higher waist circumference. There was an inverse relationship between education level and the presence of metabolic syndrome. The rate of patients reaching the recommended target levels of cardiovascular risk factors was very low.

Recently, central obesity has become the central criterion of the metabolic syndrome, thus waist circumference has been recognised as one of the most important anthropometric parameters. According to our analysis comparing the reliability of earlier and more recent anthropometric measures characterising obesity, waist circumference is a useful and relevant parameter for the clinical practice, in spite of the larger intra- and interobserver variability compared to body mass index (BMI).

In our cross-sectional study within general practices we have confirmed that obesity characterised by BMI and abnormal waist circumference was common, affecting approximately 2/3 of the adult population screened. BMI and increasing waist circumference were both closely associated with the presence of cardiovascular risk factors.

According to our survey conducted within the general population, the term “metabolic syndrome” was not well known and the main source of knowledge was not medical information.

Our results emphasize that the concerted efforts of prevention of abdominal obesity, early identification of subjects at risk, more strict target-oriented treatment of cardiovascular risk factors and more effective patient education could improve the unfavourable cardiovascular morbidity and mortality rates in Hungary.

- Nádas J, Putz Z, Fövényi J, Gaál Z, Gyimesi A, Hídvégi T, Hosszúfalusi N, Neuwirth G, Oroszlán T, Pánczél P, Széles G, Vándorfi G, Winkler G, Wittmann I, Jermendy G (2009) Cardiovascular risk factors characteristic for the metabolic syndrome in adult patients with type 1 diabetes. *Exp Clin Endocrinol Diabetes* 117: 107–112.
- Nádas J, Putz Z, Fövényi J, Gaál Z, Gyimesi A, Hídvégi T, Hosszúfalusi N, Neuwirth G, Oroszlán T, Pánczél P, Vándorfi G, Winkler G, Wittmann I, Jermendy G (2009) Cardio-metabolic risk and educational level in adult patients with type 1 diabetes. *Acta Diabetol* 46: 159–162.
- Nádas J, Putz Zs, Kolev G, Nagy S, Jermendy G (2008) Intraobserver and interobserver variability of measuring waist circumference. *Med Sci Monit* 14: CR15–18.

## ZSOLT GYÖRGY NAGY (2010)

### New mechanism in the regulation of bone metabolism

Supervisor: Gábor Speer

The maintenance of bone involves a delicate balance between the actions of systemic hormones, local cytokines and growth factors, and physical forces. Disorders that interfere with, amplify, or mimic the effects of these factors can result in abnormal bone remodeling and elicit pathological conditions that result in increased susceptibility to fracture. My research to define the pathways that lead to the responses to these factors, in order to identify new potential targets for therapy.

Our studies on osteoblasts have focused on parathyroid hormone signaling. PTH can promote both the formation and resorption of bone. Intermittent stimulation by PTH promotes bone formation, likely through the local production of growth factors. Continuous stimulation by PTH promotes resorption through the local production of the osteoclast-stimulatory cytokines RANKL and interleukin-6 (IL-6). PTH acts through specific receptors to activate signaling pathways. Adenylyl cyclase and phospholipase C were previously recognized as signaling molecules activated by PTH-receptor interactions. We have found that the activation of phospholipase D by PTH occurs through the heterotrimeric proteins of the G $\alpha$ 12 and G $\alpha$ 13 family and the small G protein RhoA. We have shown that stimulation of the pathway by PTH in osteoblastic cells leads to activation of protein kinase C- $\alpha$  and increased IL-6.

We have found significant differences in the gene expression profiles of the bone tissue of postmenopausal and premenopausal non-osteoporotic women including genes that have not yet been associated with menopausal changes. The separation of the two groups by the multiparametric statistical methods applied suggests the involvement of new candidate gene subsets as well as genetic pathways (canonical TGF $\beta$  cascade, MAPK and ER signaling) that might be useful for the development of future diagnostic tools. Our findings may provide further insight into the process of postmenopausal changes of bone metabolism as well as it can contribute to the development of new statistical methods for the evaluation of batched genetic data.

Nuclear triiodothyronine (T<sub>3</sub>) receptors can be found in osteoblastic cell lines as well as in osteoclastomaderived osteoclasts. Thyroid hormones appear to affect bone either by a direct action on osteoclasts or by acting via osteoblasts. T<sub>3</sub> directly stimulates bone re-

sorption in organ cultures that can be inhibited by immunosuppressive compounds, indicating the involvement of immunologically active cytokines. Increased interleukin-6 (IL-6) production, a potent stimulator of osteoclast progenitors, has been reported in these cultures. Elevated serum IL-6 and IL-1/TNF concentrations were seen in hyperthyroid patients as well. A biphasic response in insulin-like growth factor I (IGF-I) production was observed in UMR-106 osteoblastic cells exposed to increasing doses of thyroid hormones: lower concentrations stimulated IGF-I secretion into the medium while higher concentrations inhibited it. Higher than normal serum IGF-I levels have been reported in hyperthyroid patients who exhibited a positive correlation with radius BMD. The anabolic effect of thyroid hormones during growth may be mediated, at least partly, via IGF-I.

- Radeff JM, Nagy Z, Stern PH (2001) *Involvement of pkc-beta in pth, tnf-alpha, and il-1beta effects on IL6 promoter in osteoblastic cells and on pth-stimulated bone resorption. Exp Cell Res 268: 179–188.*
- Nagy Z, Radeff J, Stern PH (2001) *Stimulation of interleukin-6 promoter by parathyroid hormone, tumor necrosis factor alpha, and interleukin-1beta in UMR-106 osteoblastic cells is inhibited by protein kinase C antagonists. J Bone Miner Res 16: 1220–1227.*
- Radeff JM, Nagy Z, Stern PH (2004) *Rho and Rho kinase are involved in parathyroid hormone-stimulated protein kinase C alpha translocation and IL-6 promoter activity in osteoblastic cells. J Bone Miner Res 19: 1882–1891.*

## ÉVA PALIK (2009)

### **The role of appetite regulation, adipose tissue cytokines and gene polymorphisms in obesity, insulin resistance and diabetes**

*Supervisor: Károly Cseh*

In my investigations I analyzed special conditions leading to diabetes. We found, that in the patient group taking atypical antipsychotics for at least one year the orexigenic effect if the fasting ghrelin levels significantly higher than in the normal weight and in the obese control groups may contribute to the obesitogenic potential of the drugs, together with the impaired suppression of the adiponectin level. The resulting fat tissue has similar cytokine profile and causes similar insulin resistance as fat tissue in normal obesity. The pathomechanism behind the ghrelin and adiponectin level alterations is yet unknown. The failing suppression of the ghrelin levels may be caused by the drug induced modifications of monoaminergic transmissions. The prevalence of TNF- $\alpha$  -308 G/A, TLR-4 Asp299Gly, Thre399Ile and PPAR $\gamma$  Pro12Ala polymorphisms were the same in our patient population as would be expected in the normal population. In the case of the TNF- $\alpha$  and TLR polymorphisms the less frequent alleles were associated with lower BMI and better insulin sensitivity. In the case of the PPAR $\gamma$  Pro12Ala polymorphism the less frequent allele was associated with higher BMI and insulin resistance.

In gestational diabetes the suppression of ghrelin levels in the 3. trimester was more marked than in normal pregnancy, and the adiponectin levels were also significantly lower. The adiponectin levels were the strongest independent determinants of the degree of insulin resistance (negative correlation). The adiponectin levels showed a significant positive correlation with the size of the offspring. The resistin levels did not correlate significantly with the BMI in GDM patients, but showed a strong significant positive cor-

relation with insulin resistance. As resistin is mainly secreted by adipose tissue macrophages in the visceral adipose tissue, it is possible, that hyperresistinaemia is associated not with the absolute amount of adipose tissue, but the ratio of visceral and subcutaneous fat.

In the case of MIDD we showed that the insulin secretion abnormality can be detected in normoglycaemic mutation carriers with IVGTT even before the manifestation of diabetes. The pathomechanism of insulin secretion abnormality is different from Type 1 Diabetes. In our patients we found no HLA haplotypes associated with susceptibility for T1D, and we found GADA or IA2A positivity in none of the patients. The carriers of the mutation had measurable C-peptide levels even with long diabetes duration.

- Palik E, Birkas KD, Faludi G, Karadi I, Cseh K (2005) *Correlation of serum ghrelin levels with body mass index and carbohydrate metabolism in patients treated with atypical antipsychotics. Diabetes Res Clin Pract* 68(Suppl1): S60–64.
- Palik E, Baranyi E, Melczer Z, Audikovszky M, Szocs A, Winkler G, Cseh K (2007) *Elevated serum acylated (biologically active) ghrelin and resistin levels associate with pregnancy-induced weight gain and insulin resistance. Diabetes Res Clin Pract* 76: 351–357.
- Cseh K, Baranyi E, Melczer Z, Palik E, Winkler G (2004) *Plasma adiponectin and pregnancy-induced insulin resistance. Diabetes Care* 27: 274–275.

---

## PROGRAM 2/16.

---

## DERMATOLOGY AND VENEROLOGY

### **Coordinator:**

**Sarolta KÁRPÁTI M.D., Ph.D., D.Sc.**

Department of Dermatology-Venerology  
and Skin Oncology

26 Üllői st, Budapest H-1085

Tel.: +36 1 266 0465

E-mail: [karpati.sarolta@med.semmelweis-univ.hu](mailto:karpati.sarolta@med.semmelweis-univ.hu)

### **Program overview**

The Ph.D. program of the Department Dermatology-Venerology and Skin Oncology at Semmelweis University Ph.D. School aims to fill a gap in development of skin and venereal diseases that will provide support for scientific research, education and postdoctoral training of the specialty. The foundation of this new doctoral program stems from a sub-program that belonged to the Molecular Medicine Ph.D. School since 1998. Graduates from this sub-program have already started an independent research work. As a result of the scientific activity of the group, it gained accreditation as a joined research group of the Hungarian Academy of Sciences. The previous Ph.D. program “Basics of Human Molecular Genetics and genetic Diagnostics” under which our sub-program was listed could no longer harbor all that diverse clinical and research activities that include venerology, STD-related microbiology, genetics, dermatologic immunology and dermatologic oncology. Our department has accumulated a very extensive dermato-venereologic clinical data that requires further research that eventually will benefit to our patients in understanding their diseases and will result in providing better healthcare. Our program

is being transferred from the Molecular Medicine Ph.D. School and will continue the research of monogenic inherited skin diseases. Our department is also the home of the National Epidermolysis Bullosa Center that carries out studies in the genetics of this severe inheritable group of diseases and has established successful prenatal diagnostics in the lethal forms. Diagnostics is now provided for more than 20 genes at the moment that are responsible for epidermolysis bullosa, Darier's disease and Hailey-Hailey disease and ichthyosis. The analysis of the genotype-phenotype relation as well as macro- and microalterations will give further insight into the pathophysiologic events in keratinocytes. Our facilities and expertise enable us to carry out clinical as well as basic science. A close scientific partnership is reflected by the introduction of two co-program leaders on the field of stem cell research. The skin is the largest organ in our body and also serves as the largest organ of our immune system. The skin is easily accessible and has great regenerating potential. The therapy of inherited, immunologic and all erosive skin diseases could benefit from a better understanding of the nature of *epidermal stem cells*. We wish to join the hot research area of stem cells with the tracking of stem cells of bone marrow transplanted recipient patients and with the use of an animal model. Our future aim, along with investigating skin differentiation and the dynamics of keratinocytes, is to explore the potential in gene therapy. In the current situation with the closing of the National Institute for Dermato-Venerology the university clinic got the obligation to further care of STD patients in form of a state center for *STD diseases* with national coverage that is based on the previous expertise from the above mentioned institution. To this area of dermatology is given special attention in our Ph.D. program. Beside the classical STD's, the altered immune reactions of HIV positive patients and opportunistic infections that frequently occur among HIV positive individuals are being investigated. This program is strongly supported by a complete microbiological laboratory, including the National Mycology Reference Laboratory, which also belongs to the department. This activity provides a diagnostic background for rare infectious diseases and also is in the process of introducing novel molecular biology diagnostic tools that yield new research data and scientific achievements. A long-lasting successful research activity on *autoimmune blistering skin diseases and gluten sensitive diseases*, like dermatitis herpetiformis and celiac disease, is well indicated by the fact, that in this field one Ph.D. work had been completed, and two further Ph.D. works are in progress. The large number of patients, the regular and careful study of circulating and tissue bound autoantibodies render good possibilities for the project. *Pharmacogenomics*, a new research area of the institute, is also based on the large number of patients with drug induced side effects on the skin. Within the planned biobanking, genetic and bioinformatic studies we started to elaborate material and data from patients with drug side effects. Our further goal is to focus to the predictivity of drug induced damages. Collecting data we plan to get important information about the pathomechanism of toxicodermas as well. With our *Pharmacogenomics* Ph.D. course we join the molecular toxicology, bioinformatics and pharmacology as well. Two years ago the Dermato-Venerology Clinic changed its name incorporating the *Skin Oncology* words as well, to underline the extended activity of the institute on the field. Scientific goal: the rapidly growing number of melanoma malignum forces us to organize extended preventive programs with organizing auto-investigation of the skin, and dermatological screening of the Hungarian population. The UV induced carcinogenesis, the development of skin tumors and its molecular biological background is also one of our ongoing studies. The Center of the Lymphoma Group of the Hungarian Dermatological Society is also in our clinic. Clinical, immunohistochemical, therapeutical and pathological features of cutaneous

lymphomas will be worked up. Epidemiology would be part of different themas: incidence of STD diseases, skin tumors, melanomas, cutaneous lymphomas is planned to be evaluated.

### ***Titles of research projects***

“New public health”: epidemiology, disease burden and disease progression, evidence based medicine, impact and cost assessment as well as analysis of policy implications	László Gulácsy
Pharmaco-economics	László Gulácsy
The examination of psoriasis immune pathomechanism	Péter Holló
Clinical and immunological studies in autoimmune bullous dermatological diseases	Sarolta Kárpáti
Pharmacogenomics: pharmacogenomic investigation of molecular mechanisms in toxicoderma	Sarolta Kárpáti
Stem cell research in dermatology	Sarolta Kárpáti
Occurrence, prognostic and etiologial factors and investigation of therapeutic modalities in cutaneous lymphoma	Márta Marschalkó
Molecular genetic investigations in genodermatosis	Márta Medvecz
Stem cell research in dermatology	Éva Mezey
Microbial organisms as pathogens, cofactors and opportunistic infections in retroviral infections	József Ongrádi
Antibacterial target proteins and peptides	László Ötvös
Health related quality of life and disease burden assessment in chronic conditions, with special focus on dermatologic diseases	Márta Péntek
Do MRSA strains form a distinct subspecies in the <i>Staphylococcus</i> genus?	Ferenc Rozgonyi
Prevalence, resistance to antibiotics, <i>in vitro</i> and <i>in vivo</i> pathogenic characteristics of coagulase negative staphylococci in nosocomial infections	Ferenc Rozgonyi
Application of molecular microbiological methods in the rapid microbiological diagnostics	Ferenc Rozgonyi
Molecular genetic diagnosis of <i>Neisseria gonorrhoeae</i> infections and resistance to antibiotics	Ferenc Rozgonyi
Molecular pathogenic and taxonomic examination of Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	Ferenc Rozgonyi
Characterisation of bacterial species, molecular methods	Ferenc Rozgonyi
Antifungal susceptibility of <i>Candida</i> clinical strains and pathogenicity properties	Ferenc Rozgonyi
Elaboration of a prevention program to improve the early recognition of melanoma	Beáta Somlai
Allergic skin diseases	Erzsébet Temesvári
The role of enviromental and meteorological factors in mortality	Klára Törő

### ***Ph.D. students***

István Almási	pt (a)	Ferenc Rozgonyi, Ian M. Gould
Andrea Horváth	ft	Ferenc Rozgonyi
Hajnalka Jókai	ft	Péter Holló

### ***Supervisors***

László Gulácsy
László Gulácsy
Péter Holló
Sarolta Kárpáti
Sarolta Kárpáti
Sarolta Kárpáti
Márta Marschalkó
Márta Medvecz
Éva Mezey
József Ongrádi
László Ötvös
Márta Péntek
Ferenc Rozgonyi
Ferenc Rozgonyi
Ferenc Rozgonyi
Ferenc Rozgonyi
Ferenc Rozgonyi
Ferenc Rozgonyi
Beáta Somlai
Erzsébet Temesvári
Klára Törő
Ferenc Rozgonyi, Ian M. Gould
Ferenc Rozgonyi
Péter Holló



Orsolya Kontár	ft	Márta Marschalkó
Gábor Malmos	ft	Ferenc Rozgonyi
Veronika Tóth	pt (a)	Beáta Somlai

***Ph. D. candidate***

Zsuzsa Szabó	ft
--------------	----

***Supervisor***

Ferenc Rozgonyi
-----------------

***Ph. D. graduates***

Nóra Erős	na
Györgyi Pónyai	na
Tibor Vág	na

***Supervisors***

Márta Marschalkó
Sarolta Kárpáti
József Ongrádi

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****NÓRA ERŐS (2009)****Analysis of clinicopathological characteristics of cutaneous lymphomas**

*Supervisor: Márta Marschalkó*

Uncommon cutaneous lymphoma entities were analyzed in this study with diagnostic and differential diagnostic difficulties: rare CBCL and CTCL forms, CD4+/CD56+ hematodermic neoplasm, multiple malignant lymphoproliferative disorders. Marginal zone B-cell lymphoma was found to be the most common form of CBCL followed by follicle center lymphoma, diffuse large B-cell lymphoma leg type, and intravascular large B-cell lymphoma. Two patients were analyzed with anaplastic large T-cell lymphoma, and 5 patients with CD4+ small/medium-sized pleomorphic T-cell lymphoma belonging to the peripheral T-cell lymphoma group. Two cases with multiple T-cell malignancies (LyP + MF, LyP + systemic ALCL), and 2 patients with coexistent B- and T-cell lymphomas (B-CLL + MF, B-CLL + cutaneous ALCL + MF) were evaluated. The value of the complex diagnostic procedure of cutaneous lymphomas was analyzed investigating the diagnostic usefulness of the concomitant histopathological, immunohistochemical and molecular biologic analysis. Skin biopsy specimens and peripheral blood samples of 60 patients were analyzed with skin symptoms suspicious for cutaneous lymphoma, especially for MF and Sézary syndrome. The complex analysis proved to be useful in the differentiation between benign, reactive dermatoses and cutaneous lymphomas in 80% of cases. Subsequent skin biopsies and complex histopathological analysis were performed in the indeterminate cases and proved to be diagnostic in further 10% of cases. Comparing the results of the TCR  $\gamma$  gene rearrangement of the skin and blood samples T-cell clonality proved to be more common in the peripheral blood than in the skin, and it was typically observed in elderly patients. Monoclonal gene rearrangement of the TCR  $\gamma$  gene in the skin was detected only in patients with cutaneous lymphoma, however peripheral blood monoclonality

was observed furthermore in one patient with benign dermatosis. This peripheral T-cell clone should not be considered as a tumour clone, it was not associated with an identical cutaneous clone, and the clinical picture and follow-up results did not support a malignant process.

- Marschalkó M, Csomor J, Erős N, Szigeti Á, Hársing J, Szakonyi J, Désaknai M, Matolcsy A, Demeter J, Kárpáti S (2007) Coexistence of primary cutaneous anaplastic large cell lymphoma and mycosis fungoides in a patient with B-cell chronic lymphocytic leukemia. *Br J Dermatol* 157: 1291–1293.
- Erős N, Károlyi Zs, Marschalkó M, Kárpáti S, Matolcsy A (2008) Clinical, histopathological, immunophenotypic and molecular analysis of 60 patients with cutaneous T-cell infiltrates with follow up of indeterminate cases to identify T-cell lymphoma. *Pathol Oncol Res* 14: 63–67.
- Erős N, Marschalkó M, Lőrincz Á, Hársing J, Csomor J, Szepesi Á, Matolcsy A, Kárpáti S (2009) CD30 positive anaplastic large T-cell lymphoma of the tongue. *J Eur Acad Dermatol Venereol* 23: 231–232.

## GYÖRGYI PÓNYAI (2010)

### The role of contact provoking factors in adult atopic dermatitis

Supervisor: Sarolta Kárpáti

**Background:** The increasing number of adult atopic dermatitis (AD) cases observed in the last years has turned the attention to ascertaining factors eliciting skin symptoms. Of these, great importance is attached to environmental contact allergens (ECA) and aeroallergens (AA), but there are only a few investigations focusing on adult AD population. **Aim of the study** was to study the sensitivity rate, the common and relevant ECA and AA of adult AD patients divided into intrinsic and extrinsic groups. **Methods:** A total number of 65 adult AD cases (47 women and 18 men) over 18 years were included in the study. ECA and AA were examined with Atopy Patch Test (APT) and epicutaneous standard tests, which were supplemented by Prick tests and *in vitro* allergy tests. **Results:** 15 AD patients were classified into the intrinsic, 50 into the extrinsic group. The hypersensitivity to ECA was 49% (intrinsic: 33% extrinsic: 54%). The detected allergen was relevant in 44%. Relevant allergens were wood tar, thiomersal, lanalcolm, fragrance mix I., nickel, iodine chlorhydroxyquin, benzoic acid, propolis and paraben. We detected AA hypersensitivity in 37% of the patients (intrinsic: 7%, extrinsic: 46%). Most common AA are house dust mites. In extrinsic AD more than 70% of the APT positive patients are sensitized to house dust mite (relevant 94%), more than 40% to pollen (relevant 40%) and more than 25% to cat epithel (relevant 66.6%) Dog epithel positivity was detected in 2 cases (1 relevant). Multiplex positivity by APT, specific IgE tests and/or Prick tests was shown in 71% of the APT positivities in extrinsic group, mostly by cat epithel (100%). We detected in 37% of the APT-positive patients new positive test reactions in 96 hours or on day 7. **Conclusion:** Contact sensitization to ECA and to aeroallergens is remarkable in adult AD. The present study is the only one in the literature which examined the sensitivity rates, typical ECA and aeroallergens in adult AD. The number of the tested aeroallergens (10) is high compared to previous reports. The present study is unique in com-

parison of the environmental contact and aeroallergen sensitivity rate of intrinsic and extrinsic AD patients.

- Pónyai Gy, Hidvégi B, Németh I, Sas A, Temesvári E, Kárpáti S (2008) Contact and aeroallergens in adulthood atopic dermatitis. *J Eur Acad Dermatol Venereol* 22: 1346–1355.
- Temesvári E, Pónyai Gy, Németh I, Hidvégi B, Sas A, Kárpáti S (2009) Periocular dermatitis: a report of 401 patients. *J Eur Acad Dermatol Venereol* 23: 124–128.
- Pónyai Gy, Temesvári E, Kárpáti S (2007) Atópiás dermatitis felnőttekben: epidemiológia, klinikai jellemzők, provokáló és prognosztikai faktorok. *Orvosi Hetil* 148: 21–26.

## TIBOR VÁG (2009)

### The role of human herpesviruses 6B, 7 and human parvovirus B19 in the pathogenesis of pityriasis rosea and papular-purpuric “Gloves and Socks” syndrome

Supervisor: József Ongrádi

Reports on the causative agents of pityriasis rosea (PR) and papular-purpuric “gloves and socks” syndrome are varying. HHV-6B and 7, two viruses from the genus *Roseolovirus* have been discussed to play a role in PR. Parvovirus-B19 (PV-B19) from the genus *Erythrovirus* on the other hand as well as HHV-6B have been implicated as etiological agents for PPGSS. In the present study, we therefore addressed following questions: (1) Do HHV-6B and HHV-7 play a role in the etiology of PR, and if yes, what kind of infection (reinfection/reactivation or primary infection) is the underlying cause? (2) Do HHV6B, HHV7 or PV-B19 play a role in the etiology of PPGSS? To answer these questions we performed serological studies using indirect immunofluorescence assay (IFA) for the aforementioned viruses on a cohort of 33 patients suffering from PR, on 4 patients suffering from PPGSS, on 25 healthy adults and on 21 healthy children (6–18 months). Further investigations included viral nucleic acid detection by PCR, virus isolation in cell cultures and electron microscopy. Serology on healthy children demonstrated significantly elevated antibody titres (dilutions up to 1:320) to HHV-6B and HHV-7 in 85.7% and 66.7% respectively, whereas antibody titres to HHV-6B and HHV-7 in healthy adults were not detectable in 80% and only slightly elevated (dilutions up to 1:40) in 20%. Serology on patients suffering from PR demonstrated significantly elevated antibody titres (IgM, IgG and HA-IgG) with dilutions reaching from 1:20 to 1:320. Antibody constellation suggests a primary infection in 17 patients (whereas three of them probably exhibited a delayed IgM production) and a reinfection in 8 patients. In the remaining 8 patients no determination between primary and recurrent infection could be made due to a lack of HA-IgG determination. Antibody titres to HHV-6B were generally low in the tested PR-patients. In 6 patients IgG was slightly elevated up to 1:20, in one patient all three antibody subtypes were slightly elevated. PCR was performed in 23 patients suffering from PR. In 12 cases (51.6%) HHV-7 DNA could be demonstrated in their lymphocytes compared to 30% in the control group. Co-cultivation of patients' lymphocytes (3 patients) with a HHV-7 susceptible cell line revealed high HHV-7 virus load proven by detecting virus antigens by immunofluorescence, visualisation of virus particles by electron microscopy and detecting viral nucleic acid by PCR. Based on these results, we conclude that primary HHV-6B and HHV-7 infection usually occurs in early childhood (<18

months). A primary HHV-7 infection in seronegative adults and in some rare cases a re-infection with the virus or reactivation from latent state however can cause the onset of PR. Patients suffering from PPGSS demonstrated elevated antibodies to HHV-7 in all 4 cases (100%), antibody pattern suggesting a primary infection in one case, an infection in the near past in two cases (whereas it was not possible to distinguish between primary and reinfection) and a reinfection, reactivation or persistent infection in one case. Elevated antibody levels to PV-B19 were demonstrated in the blood samples of two cases, whereas antibody pattern suggests a primary infection in one case and a re-infection in the other case. Antibodies to HHV-6 could not be demonstrated in any patient. HHV-7 DNA was detected in the lymphocytes of three patients. In one patient, PCR for HHV-7 DNA was not performed. HPV-B19 DNA was found in the serum of two patients, two patients on the other hand were free of viral DNA. Reviewing our results we suggest, that PV-B19 and HHV-7 both play an etiological role in PPGSS.

- Vág T, Sonkoly E, Kemény B, Kárpáti S, Horváth A, Ongrádi J (2003) *Az emberi 7-es herpesvírus újabb szerepének vizsgálata bőrbetegségekben (Studies on the novel association of human herpesvirus-7 with skin diseases)*. *Orv Hetil* 144: 1623–1629.
- Vág T, Sonkoly E, Ongrádi J, Kárpáti S, Horváth A (2004) *Avidity of antibodies to human herpesvirus-7 suggests primary infection in young adults with pityriasis rosea*. *J Eur Acad Dermatol Venereol* 18: 738–740.
- Vág T, Sonkoly E, Kemény B, Kárpáti S, Horváth A, Ongrádi J (2004) *Familial occurrence of papular purpuric “gloves and socks” syndrome with human herpesvirus 7 and human parvovirus B19 infection*. *J Eur Acad Dermatol Venereol* 18: 639–641.

---

# SCHOOL OF PH.D. STUDIES

---

## 3. PHARMACEUTICAL AND PHARMACOLOGICAL SCIENCES

---

**Chairwoman:**

**Éva SZŐKE M.Sc., Ph.D., D.Sc.**

Department of Pharmacognosy

26 Üllői st, Budapest H-1085

Tel./fax: +36 1 317 2979

E-mail: [szokee@drog.sote.hu](mailto:szokee@drog.sote.hu)

### General overview

The Doctoral School of Pharmaceutical and Pharmacological sciences focuses on two scientific disciplines:

- *Pharmacological research* is needed to select new active substances, to develop and use medicinal products. In addition, new scientific results and discovered relationships may help to understand functions of the living human organism.
- *Pharmaceutical research* is related to drug research, development of drug delivery systems as well as it is a prerequisite to produce and apply pharmaceutical preparations. Although pharmaceutical science involves the knowledge of other disciplines (e.g. chemistry and medical science), but the evaluation of medicinal products requires specialised knowledge from the viewpoint of this interdisciplinary science.

The objective of the Doctoral School is to train qualified experts with an internationally recognized scientific degree (Ph.D.) for pharmacological and pharmaceutical research. Scientific results of the above mentioned research topics will be summarized in doctoral thesis and research papers which will be published in international journals of high impact. Research topics provide students with theoretical and practical experience in different fields of pharmaceutical and pharmacological sciences. Special problems are covered by the research projects of the educational Program:

- Study of bioactive substances of plant origin in connection with phytochemical and biological evaluation as well as biotechnological production; pharmaceutical chemistry and analysis; design, manufacturing and biopharmaceutical evaluation of novel dosage forms; clinical pharmacy and pharmacoeconomics; study of organic compounds with potential bioactivity; investigation of medical and pharmaceutical aspects of biology and environmental protection;
- Pharmacodynamic investigations; pharmacokinetic and drug metabolism; influence on neurochemical transmission; study of neurodegenerative and neuroprotective mechanisms; cardiovascular pharmacological investigations; separation methods and their applications; study of drugs affecting on calcium and bone metabolism; human study of cytostatic drugs; role of ion transport mechanisms controlling neurochemical transmission.

**PROGRAM 3/1.****MODERN TRENDS IN PHARMACEUTICAL SCIENCES****Coordinator:****István ANTAL M.Sc., Ph.D.**

Department of Pharmaceutics

7 Hőgyes E. st, Budapest H-1092

Tel./fax: +36 1 217 0914

E-mail: [antist@gyok.sote.hu](mailto:antist@gyok.sote.hu)**Program overview**

The scientific-education scope of the participating 15 institutes/departments provides the eligible professionals (pharmacists, medical doctors, chemists, biologists, physicists, chemical engineers) with a wide selection of topics in the fields of fundamental and specific drug-oriented research, including current problems of inorganic, organic, physical, analytical, bioinorganic, bioorganic, and coordination chemistry, biology, biophysics, biotechnology, botany, microbiology and virology.

The specific topics of drug- and pharmaceutical research are drug design and synthesis, structure-activity relationships, mechanism of action of drugs, drug-receptor binding, isolation of active compounds from natural sources, drug metabolism, biochemical toxicology, relationships between physicochemical properties and biological function, pharmacognosy, elucidation of biosynthesis of natural compounds, pharmacokinetics, drug-drug interactions, transport mechanisms, biopharmaceutics, pharmaceutical technology, physical pharmacy, chemical pharmacy and social pharmacy.

***Titles of subprograms and research projects******Coordinators, supervisors*****Subprogram 1.****Production of bioactive compounds by biotechnological methods**

Optimization of active substance formation by biotechnological methods (fermentation, bioregulation, gene transformation) in tissue and cell cultures of medicinal plants

Study on role of endogenous formaldehyde in C1 metabolism and biosynthesis related to C1 fragments

Research and production of bioactive lignanes of plant origin by *in vitro* cell cultures for therapeutical use

Study on protective response of plants induced by elicitors in case of *in vivo* and *in vitro* systems

Screening of antioxidant activity of plant compounds in HTS-conditions

**Subprogram 2.****Phytochemical and biological evaluation of bioactive substances of plant origin**

Active ingredient content assays of medicinal plants and their preparations

**Éva Szőke**

Éva Szőke,

László Kursinszki

Lehel Hullán

Miklós László,

István Gyurján,

Zoltán Krisztóf

Károly Bóka

György Tibor

Balogh

**Éva Lemberkovics**

Éva Lemberkovics,

Éva Szőke



Formation of bioactive compounds in medicinal plants	Éva Szőke, Andrea Balázs
Research of plant-derived active substances for phytotherapeutical purpose	Ágnes Kéry
Symptoms of heavy metal contaminations in medicinal plants	Béla Böddi
Metals and metal ions in medicinal plants and their extracts in consideration of dosage forms	Klára Szentmihályi

**Subprogram 3.****Pharmaceutical chemistry and drug analysis**

Microspeciation of bio- and drug molecules	<b>Béla Noszál</b> Béla Noszál
Study on cyclodextrines regarding the ability to form inclusion complexes	Ágnes Barcza-Buvári
Appplication of chiroptical, CD/UV and NMR spectroscopy in analysis of chiral and natural compounds	András Gergely
Development and application of high resolution separation methods for analysis of bioactive molecules and drug candidates	Miklós Idei
Rational drug desing in signal transduction therapy	György Kéri
Preparation of kinase inhibitor molecules by rational drug design	György Kéri
Study on novel molecules with selective tyrosine kinase effect: modelling relationships between chemical stucture and biological effect	György Mészáros
Study on pathobiochemical processes of cancerous and inflammatory diseases regarding to role of kinases and to development of drug candidates	Tibor Vántus
Investigations of nonlinear chemical phenomena	Krisztina Kurin Csörgei
Role of combinatorical chemistry and informatics in the design and preparation of new drug candidates	László Órfi
Study on relationships between molecular properties and chemical structure, role of lipophilicity	Krisztina Novák Takács
Chromatographic analysis of amino acids and amines	Ibolya Molnár Perl
Development of microanalytical methods and speciation of elements for studying biological systems	Gyula Záray

**Subprogram 4.****Design and preparation of modern dosage forms**

Investigation of excipient systems used as drug carriers	<b>István Antal</b> Judit Dredán
Investigation of drug carrier systems with controlled and programmed drug release	István Antal
Study and optimization of pharmaceutical technological procedures	István Antal
Investigation of competitive interfacial processes in colloidal drug carriers	Ferenc Csempezs

**Subprogram 5.****Biopharmaceutical considerations of design and evaluation of pharmaceutical preparations**

Investigation of drug carrier systems with improved bioavailability	<b>Sylvia Marton</b> Sylvia Marton
---	---------------------------------------

Investigation of drug carrier systems with controlled and programmed drug release	Sylvia Marton
Analytical assay of drug carrier systems	Krisztina Ludányi
Study of intelligent, thermoresponsive drug carrier systems	Gabriella Csóka
<b>Subprogram 6.</b>	
<b>Pharmacoeconomics and clinical pharmacy</b>	
Pharmacoeconomical investigations	Zoltán Vincze
Studies in clinical pharmacy	Zoltán Vincze
Application of novel dosage forms in the clinical pharmacy	Romána Zelkó
Health care-economics, technological analysis	Romána Zelkó
	Ágnes A. Mészáros
<b>Subprogram 7.</b>	
<b>Study of potentially bioactive organic compounds</b>	
Study of potentially bioactive organic, heterocyclic compounds	Péter Mátyus
Design and synthesis of potentially bioactive compounds	Péter Mátyus
Synthesis of potentially bioactive “O” and “N”-heterocyclic compounds and investigating their effect	György Keserű
	Sándor Antus
<b>Subprogram 8.</b>	
<b>Pharmaceutical considerations of biology and environmental protection</b>	
Pharmacological and molecular biological investigations of histamine, its receptors responsible for action, histamine agonists and antagonists regarding the role in cell proliferation	Éva Ádám
Biological and clinical principles of chemotaxis	Sára Tóth
Study on action mechanism of pharmaceutical substances influencing membranes	László Kőhidai
Study of molecular dynamic interactions on model membranes by spectroscopical methods	Gabriella Csík
Study of glycoproteins and biomarkers by mass spectrometry	Pál Gróf
Structural bases and medical aspects of protein aggregation	Károly Vekey
	Judit Fidy,
	László Smeller
Computer study of protein dynamics: role of dynamics in ligand binding	Erika Balog
Significance of glutamaterg neuron phenotype in parvicellular and magnocellular neurosecretoric systems	Ferenc Tölgyesi
Study on structural bases of functional interactions in proteins	Judit Fidy,
	László Smeller,
	Levente Herényi,
	Szabolcs Osváth
Study on structural bases of functional interactions in proteins	András Kaposi
Molecular imprinting polymers	György Horvai

**Ph.D. students**

Tamás Ágh	pt (a)
Ágnes Alberti-Dér	ft
Nóra Edina András	ft
Márton Argay	pt (a)
Borbála Bizderi	ft
Réka Bodnár	ft
Emese Ficsor	ft
Krisztina Futosi	crc (a)
Petra Füredi	ft
Rita Garamvölgyi	ft
Gabriella Godina	pt
Pál Gyulavári	ft
Seyed Farzag Hashemi	it
Imola Isó	ft
Nóra Valéria Kaszás	ft
Dóra Kertesy	ft
Diana Kostyalik	ft
Kristóf Mária Kovács	ft
Kinga Kövér	ft
Ibolya Kurkó	crc
Árpád Könczöl	pt
Erzsébet Laczkó	ft
Eszter Lajkó	ft
Júlia Láng	ft
Azizian Nastaran	it
Gábor Neumajer	ft
Noémi Anna Niczinger	ft
Zoltán Pálmai	ft
Mátyás Imre Pápai	ft
Liza Pelyhe	ft
Kinga Péntes	crc
Péter Petscher	ft
Nazila Ram	it
Eszter Riethmüller	ft
Deme Ruth	ft
Márta Schalkhammer	pt
Tamás Sohajda	ft
Barnabás Szabó	pt
Laura Simon-Szabó	ft
Zsuzsa Szabó	ft
Gergely Szakonyi	pt

**Supervisors**

Ágnes Andrea Mészáros
Ágnes Kéry
Ibolya Molnár
Perlné
Romána Zelkó
Kornélia Tekes
Ágnes Andrea Mészáros
Andrea Balázs
Tibor Vántus
Imre Klebovits
László Őrfy
Zoltán Járai,
Judit Kapocsi
Tibor Vántus
Kornélia Tekes
Éva Lemberkovics
Pál Gróf
Éva Szőke
László Tóthfalusi
Krisztina Ludányi
Ágnes Andrea Mészáros
Tibor Vántus
György Balogh
Zsuzsa Fürst
László Kőhidai
László Kőhidai
István Antal
Béla Noszál
István Antal
Erika Balog
Péter Mátyus
György Mészáros
György Kéri
László Tóthfalusi
Huba Kalász
Ágnes Kéry
Péter Mátyus
Romána Zelkó
Béla Noszál
Romána Zelkó
György Kéri
Gyula Záray
Romána Zelkó

Péter Szegi	ft	Kornélia Tekes
Csilla Anikó Székely	ft	András Telekes
Apolka Kinga Szentirmay	ft	Pál Riba
Nóra Szókontor	pt	Romána Zelkó
Mária Takács	ft	András Gergely
Bálint Péter Tóbiás	ft	Péter Lakatos
Gergő Tóth	ft	Béla Noszál
László Tóth	ft	Péter Mátyus
Lilla Turiák	pt	Károly Vékey
László Urbán	pt	Ágnes Andrea
		Mészáros
András Váradi	ft	András Gergely
Attila Varga	crc	Tibor Vántus
Zsolt Wágner	ft	Éva Szökő
Lilian Sára Zsákai	ft	György Kéri

***Ph.D. candidates***

Gergely Agócs	ft
Ferenc Baska	crc
István Bokkon	ft
Balázs Balogh	i
Gyula Bencze	crc
Gábor Csaba Borbély	crc
Andrea Böszörményi	ft
Máté Dániel Bubenyák	ft
István Dicházi	pt
Petra Zsófia Dunkel	ft
Péter Erdélyi	pt
Ágota Anna Földi	ft
Anikó Gáva	pt
Balázs Herczeg	pt
József Horgos	pt
Viktor Horváth	ft

Mónika Huszár	ft
András Inotai	ft

László Kékesi	crc
Csilla Major	pt
Erika Tünde Mészáros-Alapi	pt
Gábor Németh	crc
Gábor Orgován	ft
József Papp	pt
Zsófia Márta Polgári	ft
Zsófia Éva Sáska	pt
Zita Sebestyén	ft

***Supervisors***

Szabolcs Osváth
László Örfy
Péter Kovács
Péter Mátyus
László Örfy
György Kéri
Éva Lemberkovics
Béla Noszál
László Kursinszki
Péter Mátyus
Péter Mátyus
Péter Mátyus
Ágnes Kéry
Zoltán Vincze
Romána Zelkó
Krisztina Kurin
Csörgei
Miklós Idei
Ágnes Andrea
Mészáros
György Kéri
Zoltán Vincze
Péter Mátyus
László Örfy
Béla Noszál
Sylvia Marton
Gyula Záray
István Antal
Ágnes Buvári
Barcza

Éva Sedlák	ft	István Gyurján
Bálint Sinkó	ft	Krisztina Novák
		Takács
György Szabó	pt	Péter Mátyus
Szabolcs Szarka	ft	Éva Szőke
Edina Rita Székely	crc	György Kéri
Virág Szenté	pt	Romána Zelkó
Blanka Tóth	ft	György Horvai

***Ph. D. graduates***

Gábor Bánhegyi	crc
Angéla Benedek	pt
Balázs Blazsics	ft
Livia Budai	ft
Katalin Deák	pt

Zsófia Dobos	crc
Zsófia Fenyvesi	pt
Barbara Hevesi Tóth	ft
Andrea Jámbo	ft
Nikollett Kállai	ft
Róbert Kiss	ft
Ágnes Kőrös	ft
István Kövesi	pt
Ádám Makó	pt
Tamás Németh	na
Norbert Orbán	ft
Ágnes Bálint Polonka	ft
András Süle	ft
Krisztián Szigeti	ft
Viktória Vukics	ft

***Supervisors***

László Örfy
Péter Mátyus
Ágnes Kéry
Károly Vékey
Krisztina Novák
Takács
Miklós Idei
Sylvia Marton
Ágnes Kéry
Ibolya Molnár Perl
István Antal
Miklós Józan
Ibolya Molnár Perl
Judit Fidy
Gabriella Csóka
András Gergely
Károly Bóka
Péter Mátyus
Ferenc Csempez
Judit Fidy
Ágnes Kéry

a, absolutorium; ft, full-time; pt, part-time; it, international; i, individual; crc, Cooperation Research Centre

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**PÉTER GÁBOR BÁNHEGYI (2009)**

### **Design and synthesis of benzothiophene and benzothienopyrimidine derivatives with kinase inhibitory activity**

*Supervisor: László Órfi*

During my Ph.D. research I participated in the development of novel potential pharmaceutical agents against human and bacterial kinase enzymes. In this process I synthesized pyrrolo-pyrimidine leads with highly active Epidermal Growth Factor Receptor Protein Tyrosine Kinase (EGFR-PTK) inhibitory activity, as well as the 1-benzothiophene lead with mycobacterial Protein Kinase G (PknG) inhibitory activity, that are acknowledged in the literature. I have synthesized focused molecule libraries with new patentable structures around these core structures and our research group examined the biological activity profile of these derivatives. By changing the substituents, introducing new molecule fragments, or by changing their positions, more efficient, patentable structures can be created. During the biological test of the focused benzothiophene and the benzothienopyrimidine molecule libraries we found that several analogues exhibited increased inhibitory activity compared to the parent lead molecules. Moreover we discovered an unusual chemical method for the amidation of the slightly reactive benzothiophene carboxylate esters by reaction with lithium amide in THF. The detailed exploration of the structure-activity relationships of these derivatives and the optimization of their biological activity requires further research.

- Bánhegyi P, Wáczek F, Székelyhidi Z, Hegymegi-Barakonyi B, Kéri G, Órfi L (2008) New method for the synthesis of 2-acylamino-1-benzothiophene-3-carboxamide derivatives from the corresponding esters. *Synthetic Communications* 38: 3270–3276.
- Bánhegyi P, Wáczek F, Székelyhidi Z, Hegymegi-Barakonyi B, Kéri G, Órfi L (2008) Novel method for the synthesis of carboxamides from low reactivity esters: synthesis of 2-acylamino-1-benzothiophene-3-carboxamides. *Acta Pharmaceut Hung* 78: 75–78.
- Bánhegyi P, Wáczek F, Horváth Z, Pató J, Greff Z, Markó P, Hegymegi-B. B, Kéri G, Órfi L (2008) The development of novel kinase inhibitors against mycobacterial infections. *Acta Pharmaceut Hung* 4: 145–155.

**ANGÉLA BENEDEK (2009)**

### **Analysis of the early phase of ischemic-reperfusion brain damage using histological, neurochemical and functional methods**

*Supervisor: Péter Mátyus*

The failure of efforts to develop a drug for the treatment of stroke can be explained, in part, by the difficulty of delineating sufficient and firm animal data required for establishing the initiation of a clinical stroke trial. Our aim was to study how precisely the ischaemic-reperfusion damage (IR) is reflected by the methods routinely applied in the



preclinical practice. At Division of Preclinical Research of EGIS Pharmaceuticals Plc, we evaluated the IR damage of brain tissue using triphenyl-tetrazolium chloride (TTC) staining and histological analysis in rats after a 1-hour middle cerebral artery occlusion followed by different reperfusion periods. Neuronal function of the territory judged infarcted by TTC staining was investigated by the help of corticostriatal brain slice with radioactively labelled dopamine, *in vitro*. The animals underwent *in vivo* assessment applying 4 different neurological scales, which were completed with foot fault, beam balance, locomotor activity tests.

The results of TTC staining performed by transcerebral perfusion or in brain slices were different in the cortex and striatum, and considering the size of the core and penumbra. After transcerebral TTC staining the infarct volume temporarily decreased during the early reperfusion period. At 24 hours of reperfusion astrocytes died within the area judged infarcted by TTC staining, and despite extensive tissue damage, several morphologically viable neurons and intact mitochondria were observed. Dopamine content decreased and dopamine turnover increased in the infarcted striatum, but dopaminergic nerve terminals retained functional activity. All neurological scales scored sensorimotor deficit similarly in rats after stroke. Beam balance, foot fault and locomotor activity tests can not substitute for neurological scales.

Taken together, the pathological process in consequence of an ischaemia-reperfusion challenge can be accurately assessed using a number of complementary methods.

- Benedek A, Móricz K, Jurányi Z, Gigler G, Lévy G, Hársing LG Jr, Mátyus P, Szénási G, Albert M (2006) Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. *Brain Res* 1116: 159–165.
- Matucz E, Móricz K, Gigler G, Benedek A, Barkóczy J, Lévy G, Hársing LG Jr, Szénási G (2006) Therapeutic time window of neuroprotection by non-competitive AMPA antagonists in transient and permanent focal cerebral ischemia in rats. *Brain Res* 1123: 60–67.
- Gigler G, Móricz K, Ágoston M, Simó A, Albert M, Benedek A, Kapus G, Kertész S, Végh M, Barkóczy J, Markó B, Szabó G, Matucz E, Gacsályi I, Lévy G, Hársing LG Jr, Szénási G (2007) Neuroprotective and anticonvulsant effects of EGIS-8332, a non-competitive AMPA receptor antagonist, in a range of animal models. *Br J Pharmacol* 152: 151–160.

## BALÁZS BLAZICS (2010)

### Analysis of medicinal plant phenoloids by coupled tandem mass spectrometry

Supervisor: Ágnes Kéry

Medicinal plant extracts and herbal preparations are complex mixtures of active- and ballast substances which may contain numerous, not infrequently up to several hundreds of different constituents with not exactly defined structures. Quality, safety and efficacy of these herbs is thus a great issue and their analysis is challenging. Tandem mass spectrometry coupled to high performance liquid chromatography (LC-MS/MS), as a sensitive, powerful and robust technique, capable of analysing very diverse complex liquid samples, may offer a solution.

The aim of our work was to reevaluate traditionally well known, but less characterized herbs of various phenoloid content by the qualitative and quantitative analysis of their active substances, and to study the versatile capabilities of mass spectrometry in phyto-

chemistry, and draw general conclusions regarding phytoanalytical mass spectral applications.

For the analyses of the methanolic and/or aqueous methanolic extracts of *Euphrasia rostkoviana* Hayne, *Satureja hortensis* L., *Filipendula ulmaria* L. MAXIM, *Sempervivum tectorum* L., and *Epilobium parviflorum* Schreb. LC-DAD-MS/MS methods were adopted and developed. Samples were analysed with a triple quadrupole analyzer with electrospray ionsource (ESI) in negative ion mode. For revealing the potential active components of *Euphrasia rostkoviana* a bioassay (*in vitro* antioxidant decolorization assay) guided extraction and fractionation was accomplished.

All measurements were accomplished with great selectivity and sensitivity at high throughput. LC coupled mass spectrometry served indeed as a universal analytical tool for the qualitative and quantitative analysis of phenolics from simple phenolic acids and salicylates towards more complex structures, like flavonoid glycosides and macrocyclic phenolics.

The single reaction monitoring (SRM) mode enabled the quantitation of the leading active substance of the *Euphrasia* sample, acteoside, at ppb level by great precision and accuracy. A total of seventeen different phenolic acids and flavonoid glycosides were identified or characterized in the extract and in its fractions [1, 2]. Quantification of the antioxidant rosmarinic acid in *Satureja hortensis* was not amenable nor in selected ion monitoring (SIM) nor in SRM mode due to non-quantitative dimer formation, thus UV based quantitation was performed. Contents of six salicylates were determined with salicin and salicylic acid standards in SIM mode in the *Filipendula ulmaria* sample, and several flavonoids were characterized [3]. A comprehensive LC-MS characterization of the glycosilation profile of the *Sempervivum* flavonoids was accomplished based upon rel. intensities of the fragment ions and radical fragments [4]. The simultaneous formation of the single  $[M-H]^-$  and double  $[M-2H]^{2-}$  charged molecule ion of oenothien B helped the interpretation of its fragmentation in the *Epilobium* sample [5]. However some limitations regarding constitutional isomeria and stereochemistry (sugar moieties) of mass spectrometry in phytochemistry were pointed out which, still, are of almost no significance if compared to the possibilities of the technique.

- Blazics B, Ludányi K, Szarka Sz, Kéry Á (2008) Investigation of *Euphrasia rostkoviana* Hayne Using GC-MS and LC-MS. *Chromatographia* 68: S119–S124.
- Blazics B, Alberti Á, Béni Sz, Kursinszki L, Tölgyesi L, Kéry Á (2011) Identification and LC-MS/MS determination of acteoside, the main antioxidant compound of *Euphrasia rostkoviana*, using the isolated target analyte as external standard. *J Chrom Sci* 49: 203–208.
- Blazics B, Papp I, Kéry Á (2010) Qualitative analysis and simultaneous determination of six *Filipendula* salicylates with two standards by LC-MS. *Chromatographia* 71: 61–67.
- Alberti Á, Blazics B, Kéry Á (2008) Evaluation of *Sempervivum tectorum* L. flavonoids by LC and LC-MS. *Chromatographia* 68: S107–S111.

**LÍVIA BUDAI (2010)****The analysis of the isoforms of the alpha-1 acid glycoprotein as a biomarker**

*Supervisor: Károly Vékey*

The investigation of the isoforms of the alpha-1 acid glycoprotein (AGP) was carried out with liquid chromatography coupled to mass spectrometry technique (LCMS).

We have determined the ratio of the genetic variants in healthy individuals and in cancerous patients. The different ORM1/ORM2 ratio in healthy and in cancerous groups indicate that the level of the increase of the genetic variants is not equal.

Significant difference was found in the proportion of ORM2 variant comparing the healthy individuals to the cancerous groups. The results of our study confirm that the ORM1 variants are more responsible for the acute phase response of the AGP than ORM2 variant. In our research pathological conditions were characterized not by establishing the presence or the absence of a protein or glycoprotein, but it was based on the glycosylation isoforms of an important plasma glycoprotein, the AGP [1].

In order to characterize the glycosylation pattern of glycoproteins we worked out an LC-MS method. With the mass spectrometric detection we identified the oligosaccharides measuring the molecular masses. The separation of certain isoforms of oligosaccharides was possible applying graphitized carbon column. The fragmentation of the isoforms of oligosaccharides were measured with tandem MS.

With the use of our LC-MS method, detailed characterization of the glycosylation pattern in biological samples became possible, by characterizing the major and minor micro-heterogeneities of the corresponding oligosaccharides [2].

- Budai L, Hajdu M, Budai M, Grof P, Beni S, Noszal B, Klebovich I, Antal I (2007) Gels and liposomes in optimized ocular drug delivery: studies on ciprofloxacin formulations. *Int J Pharmaceut* 343: 34–40.
- Petrikovics M, Budai M, Baskin SI, Rockwood GA, Childress J, Budai L, Grof P, Klebovich I, Szilasi M (2009) Characterization of liposomal vesicles encapsulating rhodanese for cyanide antagonism. *Drug Delivery* 16: 312–319.
- Budai M, Hajdu M, Budai L, Antal I, Lenti L, Klebovich I (2009) Liposomal hydrogels and soft contact lenses for the delivery of antibiotics in ophthalmology. Pp 663–674 in: *Hydrogels: Properties, Preparation and Applications Book*. Nova Science Publishers, Inc., USA, ISBN: 978-1-61668-167-8.

**KATALIN DEÁK (2009)****Physico-chemical profiling of centrally acting molecules for prediction of pharmacokinetic properties**

*Supervisor: Krisztina Novák Takács*

Physico-chemical profiling is a fundamental tool at the early stage of drug discovery in screening drug-like candidates. Complex physico-chemical profiling, including molecular properties such as solubility, ionization, lipophilicity and permeability, has been found to be of predictive power in ADME (absorption, distribution, metabolism, elimination).

Within the framework of the research cooperation between Semmelweis University and Gedeon Richter Plc. the physico-chemical properties of centrally acting compounds were investigated. We determined the protonation (macro)constants ( $K$ ) (in case of one molecule also the microconstants ( $k$ )), the partition coefficient in octanol/water ( $P_{\text{oct}}$ ) and cyclohexane/water ( $P_{\text{ch}}$ ) systems of antidepressive sertraline and 15 antipsychotic piperidine and piperazine derivatives and calculated the  $\Delta\log P$  ( $\log P_{\text{oct}} - \log P_{\text{ch}}$ ) values of the molecules. Due to the poor water solubility of the compounds potentiometry using the “co-solvent” technique was applied for the determination of the protonation constants. The  $\log P$  values were measured by the dual-phase potentiometric titration in octanol/water system and the traditional shake-flask method was used in cyclohexane/water system. Highly precise physico-chemical data were obtained by these validated methods. The relationship between the structure of the molecules and the physico-chemical data was investigated. Simple parameters such as number of heteroatoms, surface and Abraham’s descriptors which influence the partition in different organic solvent/water systems were also studied. The pharmacokinetic properties of the compounds were predicted by the physicochemical parameters. Linear relationship has been found between the brain penetration characterized by the  $\log BB$  values and the  $\Delta\log P$  values. The validity of the equation was controlled by the  $\Delta\log P$  and the  $\log BB$  values of sertraline.

- Deák K, Takács-Novák K, Tihanyi K, Noszá B (2006) *Physico-chemical profiling of antidepressive sertraline: solubility, ionization, lipophilicity*. *MedChem* 2: 385–389.
- Deák K, Takács-Novák K, Kapás M, Vastag M, Tihanyi K, Noszá B (2008) *Physico-chemical characterization of a novel group of dopamine D3/D2 receptor ligands, potential atypical antipsychotic agents*. *J Pharm Biomed Anal* 48: 678–684.
- Völgyi G, Deák K, Vámos J, Valkó K, Takács-Novák K (2008) *RPTLC determination of  $\log P$  of structurally diverse neutral compounds*. *JPC-J. Planar Chromatogr Modern TLC* 21: 143–149.

## ZSÓFIA DOBOS (2010)

### Application of high performance separation techniques in the analysis of biologically active and drug candidate molecules

Supervisor: Miklós Idei

In this thesis I introduce a new technique using micellar electrokinetic chromatography (MEKC) for the determination of hydrophobicity. Comparison was made between the formerly introduced micellar phase residence time and the calculated hydrophobicity (CLOGP) values of the analytes, and linear correlation was found between them. I introduced a new parameter called micellar proportion ( $t_{\text{prop,mic}} = t_{\text{mic/tm}}$ ). A good linear correlation was obtained between  $t_{\text{prop,mic}}$  and the CLOGP of the analytes for the six different pseudostationary phases. There were both anionic and cationic among the studied pseudostationary phases is. Considering a given pseudostationary phase,  $t_{\text{prop,mic}}$  as a relative quantity is a suitable parameter to characterize and compare experimentally the behaviour of the various analytes in MEKC.

Applying a set of probe molecules with known hydrophobicity, the CLOGP<sub>50</sub> value (showing the value of hydrophobicity of a virtual molecule spending exactly 50% of its migration time in the pseudostationary phase) has been calculated for each pseudosta-

tionary phase applied. This experimentally determinable numerical value (characterizing the pseudostationary phase) can be utilized to compare the hydrophobicity and hence retention ability of the pseudostationary phases. The order of the pseudostationary phases: SDS>HTAB>TTAB>SC>LiPFOS>SDC.

The  $t_{\text{prop,mic}}$  value was found to be applicable to compare the methylene selectivity of the different pseudostationary phases as well:  $\log t_{\text{prop,mic}} = A \cdot Z + B$ , where Z is the number of carbon atoms of the alkyl chain in the alkyl benzene homologous series. Considering the methylene-selectivity the order of the pseudostacionary phases is: LiPFOS>SDC>TTAB>SDS>SC>HTAB.

- Dobos Z, Kiss É, Hallgas B, Kéri G, Idei M (2005) *Micellar proportion: a parameter to compare the hydrophobicity of the pseudostationary phases or that of the analytes in micellar electrokinetic chromatography*, *Electrophoresis* 26: 849–857.
- Varga M, Huszár M, Dobos Z, Kiss É, Horváth A, Idei M (2009) *Characterisation of mixed lithium dodecylsulphate/lithium perfluorooctanesulphonate pseudo-stationary phases in MEKC*. *Electrophoresis* 30: 1923–1928.

## ZSÓFIA FENYVESI (2010)

### Formulation and investigation of diclofenac sodium containing drug products with modified release

Supervisor: Sylvia Marton

The aim of my work was to develop pH sensitive multiparticulate system providing the drug liberation with increasing pH only in the intestine after leaving the stomach. Therefore a stomach protective dosage form was developed without the application of any type of coating. The model active compound was diclofenac sodium mainly used as anti-inflammatory agent, but it is widely used in arteriosclerosis too. The most frequently side effects of orally administered NSAID are gastric ulcer and hemorrhage in stomach. Two different methods (coacervation and *in situ* gelation) were used to prepare microcapsules as multiparticulate dosage form and their physical and mechanical properties were checked.

The *in situ* gelation method was studied in detail while the coacervation was not successful to prepare suitable particles.

Using three-component gel system the parameters influencing the production of microcapsules were optimized on the base of swelling and erosion determinations and by DSC method. The dissolution properties of different samples were evaluated by mathematical models describing the mechanism of the process. The absorption was simulated *in vitro* and using survivor intestinal ring, while the ulcerogenity was tested *in vivo* in rats. The changing of crystal form during preparation has deep impact on the structure of microcapsules, which was studied in free films by NIRS, x-ray and DSC method.

From the prepared microcapsules tablet with similar liberation profile to marketed Voltaren® tablet and transdermal patches with prolonged drug liberation were prepared.

- Fenyvesi Zs, Auner A, Schmalz D, Pásztor E, Csóka G, Gyires K, Marton S, Klebovich I, Antal I (2009) *Preparation of pH-sensitive beads for NSAID using three-component gel systems*. *J Pharm Sci* 98: 4285–4295.

- Fenyvesi Zs, Ashour KOA, Zelkó R, Müller U, Antal I, Klebovich I, Marton S (2010) Impact of crystalline form changing on behaviour of microcapsules prepared from three-component gel system. *Pharm Dev Technol* 15: 553–561.
- Fenyvesi Zs, Marton S (2005) Mikro kapszulák, mikrorészecskék, mikrokapszulázás. „Képzés egy életen át”—Továbbképző Szakfolyóirat Gyógyszerészek Számára 9: 7–12.

## BARBARA HEVESI TÓTH (2009)

### Phytochemical and *in vitro* biological evaluation of potentially active compounds in *Epilobium* species

Supervisor: Ágnes Kéry

*Epilobium* species (willow-herbs) are common members of the Hungarian flora. Their blooming, perennial shoots are used as traditional medicine in the therapy of prostatic diseases, especially in benign prostatic hyperplasia (BPH). In spite of good therapeutic experiences, none of the species are registered neither in the Hungarian (Ph.Hg. VIII.) nor in the European (Ph. Eur. 6.) Pharmacopoeia. Due to the lack of adequate amount of *in vitro* and *in vivo* studies regarding the mechanism of action, *Epilobium* species may be less popular among other medicinal herbs used in the same indication. On the other hand, macromorphology of *Epilobium* species is very similar and mistaken identity is recurrent, what increases the uncertainty of their use even more.

Aim of our study was to improve the knowledge of *Epilobium* species, through comparative phytochemical analysis and *in vitro* studies, modeling the pathological process, on mechanism of action.

We have investigated five *Epilobium* species, commonly occurring in Hungary: *E. parviflorum* Schreb., *E. roseum* Schreb., *E. tetragonum* L., *E. montanum* L., *E. angustifolium* L. Both the collected and cultivated plant material was examined and identified by cautious macro- and micromorphological methods. During phytochemical analysis, we have mainly focused on revelation of the possibly potent substances: phenoloids and sterols. After thin-layer chromatographic (TLC) examination, we have elaborated a new, high performance liquid chromatographic (HPLC-UV, HPLC-MS/MS) method for the analysis of willow-herb phenoloids. Due to “HPLC fingerprints”—which revealed similarities and differences in phenoloid composition of species—16 components, among them 11 flavonol-glycosides, have been identified. A macrocyclic tannin, oenothetin B, considered as active compound, has been observed in all samples. Explored fragmentation patterns of oenothetin B resulted in completely new data on its mass-spectrometric analysis. Quantitative data have been published on total polyphenol (22.3–34.8 g/100 g herb), tannin (18.7–25.8 g/100 g herb) and flavonoid (0.69–0.83 g/100 g herb) content of *Epilobium* species [1, 2].

Sterol composition of species has been studied by TLC and gas chromatographic (GC-MS) methods.  $\beta$ -sitosterol, a pharmacologically important compound, was the sole identifiable sterol component in n-hexane extract of samples, whose quantitative content in *E. parviflorum* ( $0.13 \pm 0.02$  g/100 g herb) has been published first.

We have compared the *in vitro* antioxidant capacity of the polar extract of species by two different spectrophotometric methods (ABTS, DPPH), and a significant activity of *E.*



*parviflorum* has been established. Elements of BPH pathomechanism have been *in vitro* simulated in order to reveal the mechanism of action of *E. parviflorum*.

Our team was the first to prove the lipid-peroxidation inhibitory (IC<sub>50</sub>: 2.37 mg/ml) effect of *E. parviflorum* in TBA assay and also its antioxidant cell-protective effect, comparable to that of catalase enzyme on fibroblast cells. The anti-inflammatory effect of *E. parviflorum* has been confirmed by verifying its COX enzyme inhibitory action (IC<sub>50</sub>: 1.4 µg/ml) [3]. We have investigated the aromatase enzyme inhibitory effect of the extract (0.32–3.2 µg/ml) on choriocarcinomic placenta cells, however the study applied has not provided significant results to verify this activity.

We have investigated the *E. parviflorum* extracts agonistic and antagonistic action on steroid receptors, and we were the first to state that the willow-herb extracts has not been acted on steroid receptors in the concentration range examined (0.01–1000 µg/ml) [4]. Since there is no evidence on the components which are undoubtedly responsible for beneficial effect, it is not possible to draw conclusion from the revealed variances occurring in phytochemical composition of *Epilobium* species to the possible differences in biological effect or the replace ability. Although, it can be established that simultaneous application of botanical and phytochemical examination is essential. Investigation of “HPLC fingerprint” of flavonoids is particularly important, especially in case of *E. angustifolium*.

*E. parviflorum* has the highest antioxidant capacity among the species examined, however further comparative investigations are necessary to prove other selective activities of the species.

Based on our *in vitro* studies *E. parviflorum* possesses multifactorial antioxidant and anti-inflammatory (COX inhibitory) effects, but does not influence the steroid homeostasis on receptorial level.

- Hevesi Tóth B, Balázs A, Vukics V, Szőke É, Kéry Á (2006) Identification of *Epilobium* species and willow-herbs (Onagraceae) by HPLC analysis of flavonoids as chemotaxonomic markers. *Chromatographia* 63: 119–123.
- Hevesi Tóth B, Blazics B, Kéry Á (2009) Polyphenol composition and antioxidant capacity of *Epilobium* species. *J Pharmaceut Biomed Anal* 49: 26–31.
- Hevesi Tóth B, Houghton PJ, Habtemariam S, Kéry Á (2008) Antioxidant and antiinflammatory effect of *Epilobium parviflorum* Schreb. *Phytotherapy Res* 23: 719–724.

## ANDREA JÁMBOR (2010)

### Amino acid analysis as their 9-fluorenylmethyloxycarbonyl-derivatives by HPLC

Supervisor: Ibolya Molnár Perl

In the frame of my studies the controversial points of literature data about the reaction conditions of amino acids and 9-fluorenylmethyloxycarbonyl-chloride (FMOC-Cl) have been critically evaluated.

The quantitative interaction between the amino acids and the FMOC reagent(s) has been determined under various conditions: aspartic acid proved to be the rate limiting amino acid. The optimum derivatization conditions were defined (borate buffer pH 9, ACN containing 0.5 mM FMOC reagent, {[FMOC]/[amino acids]}<sub>T</sub>=5.5:1, solvent/water=1:1 (v/v)}, reaction time 20 min, reagent excess elimination by 1-aminoadamantane). It has

been proved that the reaction time of the quantitative derivatization is determined by the FMOc concentration (0.5–5 mM), while, on the basis of derivatizations, performed under various molar ratios of the reactants {[FMOc]/[amino acids]T=2.5:1–66:1}, it has been confirmed that the reaction rate could not be influenced by increasing these molar ratios. In addition, it turned out that in order to ensure the necessary FMOc excess the ratio of [FMOc]/[amino acids]T should be 5.5:1.

The formation and transformation of the single and two FMOc group containing derivatives of histidine (His-1, His-2) and tyrosine (Tyr-1, Tyr-2) have been quantitatively characterized. Impurities, coeluting with the FMOc-amino acids, have been quantified for the first time. Based both on FL and on UV detections it has been demonstrated that to take into consideration the amount of these impurities is inevitable necessary. The separation of 22 FMOc-amino acids was carried out by eluents of pH 7.2. The simultaneous deproteinization and derivatizations method was introduced and applied to the quantitative determination of the plasma free amino acids.

- Hanczók R, Jámbor A, Perl A, Molnár-Perl L (2007) *Advances in the o-phthalaldehyde derivatizations: comeback to the o-phthalaldehyde-ethanethiol reagent. J Chromatogr A* 1163: 25–42.
- Jámbor I, Molnár-Perl L (2009) *Amino acid analysis by high-performance liquid chromatography after derivatization with 9-fluorenylmethyl-oxycarbonyl chloride: literature overview and further study. J Chromatogr A* 1216: 3064–3077.
- Jámbor I, Molnár-Perl L (2009) *Quantitation of amino acids in plasma by high performance liquid chromatography: simultaneous deproteinization and derivatization with 9-fluorenylmethyloxycarbonyl chloride. J Chromatogr A* 1216: 6218–6223.

## NIKOLETTA KÁLLAI (2010)

### Application of inert spheres for formulation of multiparticulates dosage form

*Supervisor: István Antal*

The pharmacokinetic properties of an active ingredient can be improved with the choice of and adequate dosage form. One such choice seems to be the formulation of multiparticulate dosage form comprising pellets, which proved to be successful in the improvement of the efficacy and tolerability of numerous compounds (e.g.: antiarrhythmics). Consequently, there is a growing interest toward the application of inert pellets as starting excipients for pharmaceutical pellet manufacturing. They serve as alternatives to develop and adapt a relatively simple manufacturing technology compared to pellet agglomeration. The aim of the present work was to investigate the application possibility of isomalt as a new, starter pellet and to compare the most important characteristics of this core to the commonly used sugar and MCC-based inert spheres. My results show that the new pellet core, similarly to the previously used pellet cores, exhibits adequate shape, particle size distribution and mechanical properties in view of further processing. I was the first to use isomalt based starter core for the production of layered structured pellets. I examined the effect and importance of the type of inert pellet core, the solubility of the drug, the composition of the coat, and the osmolality of the dissolution medium on drug release. My results prove that, in case of a water-soluble drug the only factors effecting drug release are the composition of the coat and the osmolality of the dissolu-

tion medium. The type of starter inert core does not play a major role. On the other hand, in case of a poorly water soluble drug aside the previously listed factors, the type of inert starter core is also important. With the help of following the particle size changes occurring during the drug release from pellets produced with permeable coats, I concluded that the rate of swelling is independent of the neutral pellet core and that the cracking of the coat does not play an important role during drug release. In case of layered pellets made of isomalt and sugar inert cores an additional osmotic pressure is formed during dissolution, which enhances the drug release.

- Mátyus P, Varga I, Rettegi T, Simay A, Kállai N, Károlyházy L, Kocsis Á, Varró A, Péntes I, Papp JG (2004) Novel antiarrhythmic compounds with combined class IB and class III mode of action. *Curr Med Chem* 11: 61–69.
- Kállai N, Antal I (2006) Neutrális pelletmagok jelentősége és gyógyszer technológiai vonatkozásai I. *Acta Pharm Hung* 76: 208–212.
- Balogh E, Kállai N, Dredán J, Lengyel M, Klebovich I, Antal I (2007) Számítógépes képalizis alkalmazása gyógyszeres pelletek jellemzésére. *Acta Pharm Hung* 77: 123–131.
- Kállai N, Luhn O, Dredán J, Kovács K, Lengyel M, Antal I (2010) Evaluation of drug release from coated pellets based on isomalt, sugar, and microcrystalline cellulose inert cores. *AAPS PharmSciTech* 11: 383–391.

## RÓBERT KISS (2009)

### Structural analysis of histamine receptors and its application in drug design

Supervisor: Miklós Józán

We developed the structural model of the human histamine H1 receptor (hH1R) by means of homology modeling. We proposed a possible activation mechanism of hH1R induced by histamine. Briefly, the binding of histamine causes a movement of Lys191 (5.39) in the direction of histamine, consequently significant conformational changes occur in helix TM5 and in the subsequent G-protein binding part (IC3 loop) of the receptor. Four structurally different H1 antagonists were docked to the binding site with flexible receptor side chains. These ligand-receptor complexes can be used in the improvement of H1 antagonists (e. g. by exploiting newly identified lipophil sites of the binding cavity), as well as in the development of novel H1 ligands. Docking results pointed out the ligand-binding role of several residues that have not been analyzed in the literature so far: Tyr108 (3.33), Phe184 (5.32), Phe190 (5.38), Phe199 (5.47), Phe424 (6.44), Trp428 (6.48), Tyr431 (6.51).

We have also developed the structural model of the human histamine H4 receptor (hH4R). Docking results, surface analysis and the optimization of the ligand-receptor complexes as well as the experimental results of Shin *et al.* suggested a novel binding mode compared to the previously proposed one in literature. Optimization of the histamine-hH4R and OUP-16-hH4R complexes resulted in interactions with Asp94 (3.32) and Glu182 (5.46). Furthermore, these agonists formed an additional H-bond with Thr323 (6.55). The H4 antagonist JNJ-7777120, however, did not form any interaction with Thr323 (6.55). In summary, six hH4R models developed by different protocols were evaluated for virtual screening by enrichment tests. We found that the choice of the ligand, the pharmacophore constraints and the scoring functions had a great influence on the enrichment

factors. We compiled a database containing more than 8.7 millions of compounds. This database was screened virtually on one of the hH4R models. In summary, 255 virtual hits were tested by a radioligand binding assay. Sixteen of these showed significant H4 activity in a concentration of 5  $\mu$ M. These compounds can be used as starting points in the development of potent and selective H4 agonists and antagonists.

- Kiss R, Kovári Z, Keserű GM (2004) Homology modelling and binding site mapping of the human histamine H1 receptor. *Eur J Med Chem* 39: 959–967.
- Kiss R, Noszál B, Rácz Á, Falus A, Erős D, Keserű GM (2008) Binding mode analysis and enrichment studies on homology models of the human histamine H4 receptor. *Eur J Med Chem* 43: 1059–1070.
- Kiss R, Kiss B, Könczöl Á, Szalai F, Jelinek I, László V, Noszál B, Falus A, Keserű GM (2008) Discovery of novel human histamine H4 receptor ligands by large-scale structure-based virtual screening. *J Med Chem* 51: 3145–3153.

## ÁGNES KŐRÖS (2009)

### Analysis of amino acids and amines in biological tissues and food matrices by HPLC

*Supervisor: Ibolya Molnár Perl*

The stability and characteristics of the derivatives of amino acids and amines obtained with the *ortho*-phthalaldehyde (OPA)/ethanethiol (ET)/9-fluorenylmethyl chloroformate (Fmoc) reagent has been investigated. The stoichiometry of the introduced, two-step derivatization process (1. step: OPA/ET, 2. step: Fmoc) has been followed by photodiode array (DAD) and fluorescence (FL) detections, simultaneously, while the composition of derivatives was confirmed by HPLC/mass spectrometry (MS) measurements. Optimum elution condition (18 min, including equilibration) was developed for the simultaneous quantitation of four biogenic amines (putrescine, cadaverine, spermidine, spermine), their precursor amino acids (ornithine, lysine) and an internal standard (1,7-diaminoheptane), in the presence of the rest of protein amino acids. The optimum extraction/deproteinization procedure of these constituents from biological tissues was also demonstrated. Applying perchloric acid deproteinization two approaches have been followed: (i) deproteinization with subsequent neutralization by potassium hydroxide and lyophilization, as well as, (ii) deproteinization without neutralization and lyophilization. Results obtained from standard solutions and from biological tissues proved that in order to get quantitative recovery ornithine, lysine and the amine contents of biological tissues should be determined directly in the supernatant of their perchloric acid deproteinized samples.

As a continuation of this study simultaneous analysis of 21 amino acids and 11 amines as their OPA/ET/Fmoc derivatives was described. For this purpose a quaternary elution system was elaborated containing pH and ionic strength gradients. The practical utility of the method was demonstrated by the analysis of mouse tissues.

This method was extended to the determination of amino acids and amines in various cheese samples. The proposal is based on acidic deproteinization and gradient optimization studies, resulting in the identification and quantification of 21 amino acids and 9 amines from a single solution. The optimized, simple protocol consists of deproteinization (1 M

perchloric acid), centrifugation, filtration and the subsequent derivatization with the OPA/ET/FMOC reagent. The developed method was successfully applied in the determination of the amino acid and amine contents of Hungarian port salut cheese, blue cheese and smoked cheese samples.

- Kőrös A, Hanczók R, Jámboor A, Quian Y, Perl A, Molnár-Perl I (2007) Analysis of amino acids and biogenic amines in biological tissues as their o-phthalaldehyde/ethanethiol/fluorenylmethyl chloroformate derivatives by high-performance liquid chromatography: a deproteinization study. *J Chromatography A* 1149: 46–55.
- Kőrös Á, Varga Z, Molnár-Perl I (2008) Simultaneous analysis of amino acids and amines as their o-phthalaldehyde-ethanethiol-9-fluorenylmethyl chloroformate derivatives in cheese by high-performance liquid chromatography. *J Chromatography A* 1203: 146–152.
- Hanczók R, Kőrös Á, Tóth F, Molnár-Perl I (2005) Behavior and characteristics of biogenic amines, ornithine and lysine derivatized with the o-phthalaldehyde-ethanethiol-fluorenylmethyl chloroformate reagent. *J Chromatography A* 1087: 210–222.

## ISTVÁN KÖVESI (2010)

### Effects of functionally significant small molecules' (antagonists, allosteric effectors) binding to the structure of peptides. Computational simulation study

Supervisor: Judit Fidy

The problems studied in this work are related to the question how small molecules affect the conformation of proteins upon functional binding. The complexes of two proteins were examined by molecular dynamics simulation.

1. The conformational effects of binding allosteric effectors on human hemoglobin (HbA). Lacking crystallographic data, we examined whether oxy-HbA is also able to bind allosteric effectors. We successfully determined the structure of the complex, and unraveled significant tertiary changes due to effector binding in the case of three allosteric effectors. The stability of the structures shows that the models are reliable. The analysis of the binding site in oxy-HbA showed specific like in the deoxy (T) state, but different in structure from it. The analysis of the subunit interfaces showed that effector binding decreased the stability of the HbA tetramer in the deoxy(T) and increased in the oxy(R) state.
2. The effects of binding antagonists in complexes in two kinds of Ca-Calmodulin (CaM) complexes: CaM-2TFP and CaM-2DPD. The x-ray structures of the two complexes were similar in the literature, but the dissociation constants differed by two orders of magnitude showing DPD a better antagonist. This contradicted the x-ray results. Based on the analysis of the structures of 12ns molecular dynamics simulations we found out, that the residues interacting with antagonists differ from those in the x-ray structure: the structure of the CaM-2DPD complex became more compact during the dynamics. According to electrostatic Poisson-Boltzmann calculations the desolvation terms are more favourable in case of DPD. Analysing the interactions of the amino acids, we can tell that the reason of the TFP's less efficiency is that its positive charge cannot form charge-charge interaction with the surrounding polar amino acids because the charged group is on a too short carbon chain. Therefore TFP molecule blocks these polar parts to interact with the solvent.



- Laberge M, Kövesi I, Yonetani T, Fidy J (2005) R-state hemoglobin bound to heterotropic effectors: models of the DPG, IHP and RSR13 binding sites. *FEBS Letters* 579: 627–632.
- Kövesi I, Schay G, Laberge M, Yonetani T, Fidy J (2006) High pressure reveals that the stability of interdimeric contacts in the R- and T-state of HbA is influenced by allosteric effectors: insights from computational simulations. *Biochim Biophys Acta—Proteins and Proteomics* 1764: 516–521.
- Kövesi I, Menyhard DK, Laberge M, Fidy J (2008) Interaction of antagonists with calmodulin: insights from molecular dynamics simulations. *J Med Chem* 51: 3081–3093.

## ÁDÁM MAKÓ (2010)

### Formulation of thermoresponsive and bioadhesive gel for treatment of oesophageal pain and inflammation

Supervisor: Gabriella Csóka

Several illnesses related to the oesophagus result in an inflammation which may cause pain, dysphagia, weight loss of the patient. It is essential for us to focus on the effective analgetic and antiinflammatory therapy, as the availability of the conventional oral administered dosage forms is limited. Due to its short transit time and the relative impermeability of the stratified squamous epithelium, drug absorption from the oesophagus is not significant in comparison to the other parts of the gastrointestinal tract. However it would be desirable that locally acting agents should be used in the treatment of the pain and inflammation in several cases. In order to reach a considerable drug effect and absorption from the mucosal surface of the oesophagus, contact time should be prolonged by using different methods. The aim of this study was to formulate a novel thermoresponsive and bioadhesive *in situ* gelling drug delivery hydrogel system which can adhere to the mucosal surface in the oesophagus, improving the transit time and the bioavailability and decreasing the side effects. In my present work a water-soluble cellulose derivative hydroxypropyl methylcellulose (Metolose® 60SH 4000) was used as a thermoresponsive and bioadhesive material. The Metolose® 60SH 4000 aqueous system is in sol phase at room temperature, and at a certain temperature it turns into gel phase. The temperature where the gelation can be observed is referred to as thermal gelation temperature (T<sub>2</sub>). In normal conditions the gelation temperature of Metolose® 60SH 4000 is above body temperature (62 °C), while by using 5% NaHCO<sub>3</sub>, this temperature can be shifted to body temperature. The alteration of the pH had no influence on T<sub>2</sub>. Based on my experiments, piroxicam and acetylsalicylic acid proved to have the best permeation and release abilities from the thermal gel. Based on my investigations that were carried out to examine the dependence of these processes on pH, I can conclude that the basic medium is preferable for the release and permeation. My investigations proved that by using different additives (water-soluble salts, sugar esters) a special behaviour Metolose® based drug carrier system can be developed from which the incorporated drug can release up to 100%.

- Makó Á, Csóka G, Pásztor E, Marton S, Horvai G, Klebovich I (2009) Formulation of thermoresponsive and bioadhesive gel for treatment of oesophageal pain and inflammation. *Eur J Pharm Biopharm* 72: 260–265.



- Csóka G, Gelencsér A, Makó Á, Marton S, Zelkó R, Antal I, Klebovich I (2007) Potential application of Metolose® in a thermoresponsive transdermal therapeutic system. *Int J Pharm* 338: 15–20.

## TAMÁS NÉMETH (2010)

### Practical aspects of the application of separation techniques for the analysis of pharmaceutical substances and preparations

Supervisor: András Gergely

The subject of my Ph.D. work was to study some practical aspects of the application of modern high performance separation techniques (HPLC, capillary electrophoresis) in pharmaceutical analysis. We developed a capillary electrophoresis method for the quality control of paracetamol containing products and an HPLC method for the assay of a magistral suppository. I was participating in a project dealing with the comparison of HPLC column classification systems. A rapid capillary electrophoresis method was developed and validated for the assay of paracetamol containing products and for the determination of 4-aminophenol impurity (main degradation product of paracetamol) in these products. As CZE does not allow an appropriate separation of paracetamol and 4-aminophenol, sodium dodecyl sulphate was added to the running buffer, so the separation was based on micellar electrokinetic chromatography (MEKC). HPLC methods were developed and validated in the laboratory of National Institute of Pharmacy for the assay of benzocaine and mazipredon in a magistral preparation (suppository) to investigate a patients complaint. The different selectivity of HPLC columns made by different manufacturers might cause problems, especially in impurity tests. Several research groups have developed column classification systems, which help the analyst to find a column of similar selectivity compared to a defined one. The systems developed by the research groups of Hoogmartens, Euerby and Snyder use different parameters to characterize columns. Our aim was to compare these 3 systems, therefore the parameters of several HPLC columns were determined using the 3 methods; the correlation between the methods was investigated by calculating the correlation coefficients between the parameters, by principal component analysis and by the comparison of the selectivity of the columns shown in pharmaceutical separations with the parameters determined by the 3 methods. It was found that the methods of Hoogmartens and Euerby are comparable, the parameters used in the two methods show correlation with each other while the method of Snyder uses different parameters, showing no correlation to the method of Hoogmartens; however, all the 3 methods showed a good applicability to find columns of similar selectivity.

- Németh T, Jankovics P, Németh-Palotás J, Kőszegi-Szalai H (2008) Determination of paracetamol and its main impurity 4-aminophenol in analgesic preparations by micellar electrokinetic chromatography. *J Pharm Biomed Anal* 47: 746–749.
- Németh T, Jankovics P, Lohner Sz, Angi ER, Némethné Palotás J, Kőszeginé Szalai H (2008) Benzokain és mazipredonium-klorid meghatározása egyedi magisztrális kúpban fordított fázisú folyadékkromatográfiás módszerekkel. *Acta Pharm Hung* 78: 139–144.
- Németh T, Haghedooren E, Noszál B, Hoogmartens J, Adams E (2008) Three methods to characterize reversed phase liquid chromatographic columns applied to pharmaceutical separations. *J Chemometr* 22: 178–185.

## NORBERT ORBÁN (2009)

### Examination of elicitor-induced plant defence response

Supervisor: Károly Bóka

The number of profitable (pharmaceutical) molecules produced by plant cell cultures is growing because of the several advantages of the applied methods. In our work, we studied the callus formation and the features of reprogrammed cells, and we also evaluated particular steps of the elicitor induced plant defence response in cell cultures. The applied methods allowed us to examine the *in vitro* cultures of *Rubia tinctorum* and *Capsicum annuum* and their behaviour from several points of view after elicitor treatments. The structural investigations were carried out by microscopy and electron microscopy, while the appearance of particular steps of the plant defence response was followed by applying quantitative methods (spectrofluorimetry, spectrophotometry, HPLC-DAD-MS). In our work, we observed that the callus formation from non-wounded parts of leaves of *Rubia tinctorum* was initiated by the transfer cells of vascular bundles. In the transfer cells significant structural changes were observed before the callus formation. In the mesophyll cells similar changes were also started, but these cells were not able to divide. The accumulation of anthraquinone derivatives during the callus formation exhibited two-step kinetic process. The elicitor induced H<sub>2</sub>O<sub>2</sub> production (which is a known part of the plant defence response) exhibited several maximum values, and the appearance of these maxima (duration, time-course, amplitude) depended on the applied elicitor. The electron microscopic investigations affirmed the phenomenon of H<sub>2</sub>O<sub>2</sub> production, and we found that this H<sub>2</sub>O<sub>2</sub> production had local characteristics independent of the direction of the signal. By using inhibitors during the experiments, we pointed out that the presence of the calcium signal had important role in the elicitor induced H<sub>2</sub>O<sub>2</sub> production. Direct linkage with the IP<sub>3</sub> signalization is presumable. We confirmed by using liquid chromatographic method that application of different elicitors in *Rubia tinctorum* cell cultures resulted different anthraquinone compositions. This fact may give the opportunity to develop more selective anthraquinone-producing strategies in the *in vitro* systems. Our results contribute to better understanding of the elicitor induced plant defence response. In this way, the influence on signaling pathways gives an effective tool to change the quantity and quality of the selected products.

- Orbán N, Boldizsár I, Szűcs Z, Dános B (2008) Influence of different elicitors on the synthesis of anthraquinone derivatives in *Rubia tinctorum* L. cell suspension cultures. *Dyes and Pigments* 77: 249–257.
- Orbán N, Boldizsár I, Bóka K (2007) Structural and chemical study of the differentiation associated with callus formation initiated from leaves of *Rubia tinctorum* L. *Biologia Plantarum* 51: 421–429.
- Bóka K, Orbán N (2007) New aspect of H<sub>2</sub>O<sub>2</sub> signaling. *Plant Signaling & Behaviour* 2: 498–500.

**ÁGNES BÁLINT POLONKA (2009)****Studies on and extensions of the tert-amino effect:  
synthesis of tetrahydropyridine- and azocine-annelated ring systems***Supervisor: Péter Mátyus*

The Ph.D. work, led by Prof. Péter Mátyus, was carried out at the Department of Organic Chemistry, Semmelweis University. In the Ph.D. work, novel applications of the tert-amino effect, achieved since September 2004, are described. The name of tert-amino effect was originally coined by Meth-Cohn and Suschitzky in 1972, to describe the thermal isomerization with a ring closure reaction of ortho substituted tertiary anilines. Six types of tert-amino effect have been described so far. In my studies two main lines were followed: the first one was related to type 2 tert-amino effect of anilines with tertiary amino groups incorporating a series of aza-rings, and containing various cyclic and acyclic electron withdrawing groups in the vinyl group. The possible extension of the type 2 tert-amino effect to biaryl ring systems was also investigated. The most important results could be summarized as follows.

It was found that in the reactions of benzaldehydes having an azepane or azocane ring in ortho position with active methylene compounds at room temperature tetrahydroquinolines could be isolated instead of the expected vinyl derivatives, i. e. isomerization also occurred. In cases of other aza rings, the isolation of vinyl compounds could be smoothly achieved, and their heating was necessary for ring closure.

In two series of biaryl compounds, vinyl and tertiary amino groups were attached to the two aromatic rings in ortho,ortho' position for extension of type 2 tert-amino effect to biaryl ring systems. Vinyl compounds were synthesized from the corresponding biaryl aldehydes via Knoevenagel condensation reaction, which were in turn prepared by Suzuki reaction. In several cases, phenanthridinium derivatives, formed through ring closure, could be isolated instead of vinyl compounds in the condensation reactions. On the other hand, azocine annelated ring systems were obtained by the thermal isomerization of vinyl compounds. The mechanism of ring closure may involve a [1,7] hydride transfer. The ways of formation of phenanthridinium compounds and azocines, respectively, represent two new types of the tert-amino effect. Interestingly, phenylpyridazine analogues behaved differently and their thermal reactions led to the formations of unexpected ring systems, too. Structures of biaryl aldehydes, vinyl compounds, phenanthridinium derivatives and azocines were confirmed by single crystal X-ray diffraction method. Some interesting non-bonding interactions in aldehydes and vinyl compounds were also identified.

The antiplasmodial activity or effect on semicarbazide sensitive amino oxidase (SSAO) enzyme of some compounds synthesized as a part of the PhD work were investigated. Three compounds have been found to exhibit significant antiplasmodial activity, which form a rational basis for further studies.

- Mátyus P, Éliás O, Tapolcsányi P, Polonka-Bálint Á, Halász-Dajka B (2006) Ring closure reactions of ortho-vinyl-tert-anilines and (di) aza-heterocyclic analogues via the tert-amino effect: recent developments. *Synthesis* 16: 2625–2639.
- Franck P, Hostyn S, Dajka-Halász B, Polonka-Bálint Á, Monsieurs K, Mátyus P, Maes BUW (2008) Pd-catalyzed intramolecular direct arylations at high temperature. *Tetrahedron* 26: 6030–3037.

- Polonka-Bálint Á, Saraceno C, Ludányi K, Bényei A, Mátyus P (2008) Novel extensions of the tert-amino effect: formation of phenanthridines and diarene-fused azocines from ortho-ortho'-functionalized biaryls. *Synlett* 18: 2846–2850.

## ANDRÁS SÜLE (2009)

### Colloids for controlled drug delivery: molecular and colloidal interactions of cyclodextrins

*Supervisor: Ferenc Csempesz*

Statins, as efficient HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme-A) reductase inhibitors are widely used in the management of cardiovascular diseases. Interactions in aqueous solutions between highly lipophilic statins and cyclodextrins of different chemical structures ( $\alpha$ -,  $\beta$ -,  $\gamma$ -CD, and methylated  $\beta$ -CD) were studied in the absence and the presence of a dissolved small or macromolecular additive, respectively. It was shown that the formation of host-guest type inclusion complexes considerably improves the solubility of the studied statin derivatives. Complexation may lead to improvement of the aqueous solubilities of both drugs by 1 or 2 orders of magnitude in a wide temperature range. At physiologically relevant pHs considerable solubilization could be detected even at moderate CD-concentrations. In binary systems statin-CD complexes dominantly of 1:1 molar ratios and associates of 1:2 molar ratios with less probability may alike form. Stability constants for the associates of different molar ratios have been calculated by two independent calculation procedures. The solubility of the statins in statin-CD-additive ternary systems could be further improved. The enhanced drug solubilities in the solutions of suitable small molecular mass solutes are likely related to their ability to disintegrate the H-bonding system of  $\beta$ -CD. In polymer-containing systems, statin-CD-polymer associates of supramolecular structure may presumably form in a way that portions of surface-active statin- $\beta$ -CD complexes are anchored at the macromolecular chains. The non-colloidal analogue of the polymer competitively reduced the efficacy of CDs. Properties and probable structure of the statin-CD associates formed in binary and ternary solutions were characterized by spectroscopic (UV-vis, NMR, IR, DSC) and colloid-physical (surface activity, immersion wetting enthalpy) measurements. The possible utilization of solid statin-CD complexes in pharmaceutical preparations was proven by the detected favourable physical parameters and dissolution properties of model tablets.

- Süle A, Szente L, Csempesz F (2009) Enhancement of drug solubility in supramolecular and colloidal systems. *J Pharmaceut Sci* 98: 484–494.
- Süle A, Csempesz F (2008) Complexation of statins with  $\beta$ -cyclodextrin in solutions of small molecular additives and macromolecular colloids. *Progress in Colloid and Polymer Sciences* 135: 93–100.
- Süle A, Csempesz F (2008) Szupramolekuláris gyógyszerhordozó rendszerek kolloid-fizikai jellemzése. *Acta Pharmaceut Hung* 78: 3–10.

**KRISZTIÁN SZIGETI (2009)****The role of  $\text{Ca}^{2+}$  in the active structure of horseradish peroxidase***Supervisor: Judit Fidy*

The aim of my work was to study the effect of containing  $\text{Ca}^{2+}$  ions bound in the structure of enzymes on their biochemical activity. I selected horseradish peroxidase isoenzyme C (HRP), a globular heme protein for the studies as a model protein that contains two bound  $\text{Ca}^{2+}$  ions in its native form. Literature data show that the removal of these ions decreases the activity to 40% of its native level. I performed comparative studies by several spectroscopy methods on the native form of the enzyme and on its  $\text{Ca}^{2+}$  depleted form. In these measurements, I varied several environmental parameters like the temperature, pressure, viscosity and pH. Literature data show that in the procedure of  $\text{Ca}^{2+}$ -depletion, it is especially hard to remove the  $\text{Ca}^{2+}$  distal to the heme and bound in a rigid region of the conformation. In the literature the results are not convincing concerning the success of  $\text{Ca}^{2+}$ -depletion and thus concerning the role of their binding by the native enzyme. In my work I carefully investigated the possible reasons that may hamper the success of  $\text{Ca}^{2+}$ -depletion and adapted a method suggested in the literature based on these experiences. I completed the technique by an independent physical measurement—total reflexion X-ray luminescence—to control the molar amount of  $\text{Ca}^{2+}$  in the samples. In this way I was successful in elaborating a reliable and reproducible method for the  $\text{Ca}^{2+}$  depletion of HRP. Based on the analysis of literature data it could be expected that the deprotonation of the distal His42 may strengthen the effect of  $\text{Ca}^{2+}$  depletion on the conformation and structure of the heme pocket. Thus, I determined the  $\text{pK}_a$  of this His residue and studied the protein in both the His42-protonated and -deprotonated states of the structure. Optical spectroscopy based on the absorption spectrum of the heme group performed in a broad temperature range, and CD spectroscopy lead to the conclusion that indeed, His42 takes part in a H-bonding network involving a distal water molecule that connects the distal  $\text{Ca}^{2+}$ -binding site to the heme. The importance of His42 in this network was revealed by the significant structural rearrangements in the heme pocket when in the absence of  $\text{Ca}^{2+}$  the His42 residue is deprotonated. It was also shown that although the H-bonds are maintained in case the His42 is protonated even in the absence of the distal  $\text{Ca}^{2+}$ , the conformation of the heme and heme group is different from that in the native form. This difference was clearly demonstrated also by my optical spectroscopy study under increasing pressure. CD spectroscopy in the farUV range showed that the  $\text{Ca}^{2+}$ -binding does not alter the secondary structural—alpha helical—order of the protein. H/D exchange FTIR spectroscopy measurements, the compressibility data and studying the effect of viscosity showed that the structure is significantly influenced at the level of the tertiary structure by  $\text{Ca}^{2+}$  depletion. The conformational dynamics loses its features specific for the structural regions of native HRP and becomes uniformly characterized by significantly enhanced structural fluctuations. The structure can be characterized as a molten globule in the absence of  $\text{Ca}^{2+}$ . The results suggest that out of the two bound  $\text{Ca}^{2+}$  ions, the distal  $\text{Ca}^{2+}$  plays more important role in optimizing the structure for enzyme activity. The changes in the heme pocket, in the tertiary structure and conformational dynamics may act together to reduce the peroxidase activity upon the removal of the  $\text{Ca}^{2+}$  ions. The decrease of activity is within one order

of magnitude as the effect of the absence of  $\text{Ca}^{2+}$ , thus I interpret the role of binding  $\text{Ca}^{2+}$  in HRP as “structural fine tuning” of the enzyme activity.

- Szigeti K, Smeller L, Osváth S, Majer Z, Fidy J (2008) *The structure of Horseradish Peroxidase C characterized as a molten globule state after  $\text{Ca}^{2+}$  depletion BBA. Proteins and Proteomics 1784: 1965–1974.*
- Laberge M, Szigeti K, Fidy J (2004) *The charge transfer band in horseradish peroxidase correlates with heme in-plane distortions induced by calcium removal. Biopolymers 74: 41–45.*
- Huang Q, Laberge M, Szigeti K, Fidy J, Schweitzer-Stenner R (2003) *Resonance Raman spectroscopy study of change of iron spin state in horseradish peroxidase C induced by removal of calcium. Biopolymers 72: 241–248.*

## VIKTÓRIA VUKICS (2010)

### Antioxidant flavonoid glycosides in *Viola tricolor* L.

Supervisor: Ágnes Kéry

In this thesis the chemical composition of heartsease (*Viola tricolor* L.) was studied to support the evidence-based determination of its biological activities. As the traditional internal administration of heartsease herb is as a tea, we primarily aimed at the analyses of components, which are supposed to be present in this aqueous extract. Henceforth, a proper sample preparation method was developed, yielding a fraction rich on polar constituents, which was further separated by conventional Sephadex LH-20 column chromatography. HPLCUV analysis of the fractions suggested that the two main flavonoid components had been successfully isolated. They were definitely identified by LC-MSn and NMR studies as violanthin (6-C-glucosyl-8-C-rhamnosyl apigenin) and rutin (3-O-rhamnoglucosyl quercetin). Furthermore, sixteen of the minor flavonoid components (C-glycosides, O-glycosides and C,O-glycosides) were tentatively identified by nanoLC-MSn. Although violanthin could not be quantified in the lack of a commercially available reference molecule, rutin was quantitatively determined by HPLC with UV detection (( $0.42 \pm 0.01$ )%). The antioxidant capacities (electron-donor and hydrogen donor activities) of the fractions were determined by the TEAC and DPPH assays, respectively. In respect to their antioxidant properties, the highest electron-donor capacity was measured with rutin, and a fraction enriched on flavonoids exhibited the highest hydrogen donor activity. Garden pansies (*V. x wittrockiana* Gams.) are plants of complex hybrid origin. In this thesis, beside a comparative HPLC study, the anthocyanidin and flavonoid contents as well as the antioxidant capacities of garden pansies of different petal color and heartsease were compared. The anthocyanidin and flavonoid contents of the samples were quantified by spectroscopic methods registered in the European Pharmacopoeia 5.0. While the highest anthocyanidin content was measured in the violet flower sample, the white and yellow pansy samples showed the highest flavonoid content. The antioxidant capacity of the samples was determined by the TEAC assay. The heartsease and pansy samples were observed to be as good antioxidants as the well-known ginkgo leaf. In addition, significant correlation was found between the flavonoid content and the antioxidant capacity of the samples.



- Vukics V, Hevesi Toth B, Ringer T, Ludanyi K, Kery Á, Bonn GK, Guttman A (2008) Quantitative and qualitative investigation of the main flavonoids in heartsease (*Viola tricolor* L.). *J Chrom Sci* 46: 97–101.
- Vukics V, Kery Á, Bonn GK, Guttman A (2008) Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities. *Anal Bioanal Chem* 390: 1917–1925.
- Vukics V, Ringer T, Kery Á, Bonn GK, Guttman A (2008) Analysis of heartsease (*Viola tricolor* L.) flavonoid glycosides by micro-liquid chromatography coupled to multistage mass spectrometry. *J Chromatogr A* 1206: 11–20.

---

## **PROGRAM 3/2.**

---

### **EXPERIMENTAL AND CLINICAL PHARMACOLOGY**

**Coordinator:**

**Kálmán MAGYAR M.D.,**

**member of the Hungarian Academy of Sciences**

Department of Pharmacodynamics

4 Nagyváradi sq, Budapest H-1089

Tel.: +36 1 210 4411

E-mail: [magykal@net.sote.hu](mailto:magykal@net.sote.hu)

#### **Program overview**

The topic of pharmacokinetics becomes increasingly important for studying bioequivalence and planning modern drug preparations for optimal drug treatment. The study of the mechanism of drug action by pharmacodynamic tools aims at recognising new active substances and various opioid receptor types and subtypes. It is advised to get acquainted with their physiological and pathophysiological role in the development of opioid dependence or in the protection of gastric mucosa. The Program also covers the research into the metabolism of neurotransmitters in the CNS in order to elucidate the relationship between disturbances of neurotransmission and certain psychiatric disorders. Research in the field of presynaptic regulation of the neurochemical transmission in the peripheral and central nervous system is also included in the Program. Investigation of compounds affecting calcium and bone metabolism is also carried out. The mode of action of cardiovascular drugs and endogenous substances are planned to be tested, as well as antihypertensive agents in order to find optimal treatment of the diseases. Anticancer agents are involved in the studies to improve the effectiveness of the treatment. Structure activity relationship studies, including the chirality of drugs is also part of the Program.

***Titles of subprograms and research projects******Supervisors*****Subprogram 1.****Drug and mechanism-oriented pharmacodynamic studies**

Central and peripheral mechanisms as potential drug targets

The role of ORL-1 receptor-mediated neuromodulation in the central autonomic control

The role of local mediators in vascular reactions, functional integrity of mucosa and the adrenomedullary-functions

Receptor-mediated protection of the gastrointestinal mucosa

Analysis of the centrally-mediated protection of the gastrointestinal mucosa

The role of opioid receptors in the cellular immunomodulation

Mechanisms of spinal and supraspinal opioidergic control of pain perception

**Klára Gyires**

Zsuzsanna Fürst

András Rónai

Klára Gyires

Klára Gyires

László Köles

Julianna Kardos

Pál Riba

**Subprogram 2.****Pharmacokinetic and drug metabolism studies**

Drug pharmacokinetic studies in humans, and animal experiments

Regulation of extrahepatic cytochrome P450 enzymes; the role of inhibition of drug metabolism in drug interactions

Studies on induction of cytochrome P450 enzymes

Studies on drug interactions

Neuropsychopharmacology, drug discovery and development

Selective detection methods in studies of xenobiotic metabolism

Pharmacometric analysis of bioequivalence studies

Application of quantitative electroencephalography (qEEG) in drug research

Analysis of teratogenic effects and circumstances of drug use during pregnancy

**Imre Klebovich**

Imre Klebovich

Károly Tihanyi

László Vereczkey

László Vereczkey

György Lévy

István Hazai

László Tóthfalusi

László Tóthfalusi

Ferenc Bánhidly

**Subprogram 3.****Modulation of neurochemical transmission by drugs; neurodegenerative and neuroprotective mechanisms**

Analysis of correlation among nociceptinerg, nocistatinerg and biogenaminerg systems

Biochemical basis of affective and anxiolytic disturbances

Animal models for studies of drugs affecting neurochemical transmission

Neurochemistry of mental diseases

Investigation of neuroprotective and neuroregenerative effects

Studies on the role of nitric oxide synthase and transcription factors in neurodegenerative and neuroprotective processes

Biochemical basis of neuropsychiatric symptoms of chronic hepatic diseases

**Kornélia Tekes**

Kornélia Tekes

Kornélia Tekes

Júlia Tímár

Gábor Faludy

Kálmán Magyar

Éva Szökő

Ferenc Szalay

**Subprogram 4/A.****Preclinical and clinical cardiovascular pharmacological studies**

Effect of cardiovascular or other drugs on cardiac parameters  
(mechanical, electrophysiological) on isolated cardiac preparations  
under physiological and pathological conditions

**Valéria Kecskeméti**

Valéria Kecskeméti

**Subprogram 4/B.****Preclinical and clinical cardiovascular pharmacological studies**

Possibilities of drug treatment of macro- and microvascular diseases  
The role of imidazoline receptors in haemodynamic regulation in  
hypertension

**Csaba Farsang**

Zoltán Járai

Judit Kapocsi

Reduction of cardiovascular risks by antihypertensive drugs in  
chronic renal failure patients

István Kiss

Experimental and clinical cardiovascular pharmacological studies

Csaba Farsang

**Subprogram 5.****Separation methods and their applications in pharmacological studies****Huba Kalász**

Investigation of fate of drugs in the body and their effects by  
chromatographic methods

Huba Kalász

***Ph. D. students***

Borbála Bizderi

ft

Veronika Dóczy

ft

Petra Füredi

ft

Ádám Galamb

pt

Gabriella Godina

pt

Tamás Kubicsek

pt

Rudolf Laufer

ft

Péter Szegi

ft

Zoltán Tömösváry

pt

Csilla Anikó Székely

ft

Zsolt Wagner

ft

***Supervisors***

Kornélia Tekes

Kálmán Magyar

Imre Klebovich

Ferenc Bánhidly

Zoltán Járai

Zoltán Kazy

Huba Kalász

Kornélia Tekes

Ferenc Bánhidly

András Telekes

Éva Szökő

***Ph. D. candidates***

Mónika Laura Bakk

ft

Éva Hellinger

ft

Erzsébet Kató

ft

Kornél Király

ft

Katalin Pápai

ft

Nashwan Shujaa Aldeen

ft

Melinda Sobor

a

Syed Muhammad Nurulain

it

***Supervisors***

Károly Tihanyi

Károly Tihanyi

András Rónai

András Rónai

Imre Klebovits

Klára Gyires

Zsuzsanna Fürst

Huba Kalász

***Ph. D. graduates***

Bernadett Benkő

pt

Melinda Gyenge

pt

Zsuzsa Iffiú-Soltész

ft

***Supervisors***

Károly Tihanyi

Kornélia Tekes

Éva Szökő

Andrea Szebeni  
Zoltán Zádori

na  
ft

Valéria Kecskeméti  
Ágnes Kéry

a, absolutorium, ft, full-time; pt, part-time; it, international; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

### **BERNADETT BENKŐ (2009)**

#### **Influence of diabetes on cytochrome P450 enzyme mediated drug metabolism —case studies on diclofenac and K-48**

*Supervisor: Károly Tihanyi*

Insulin dependent diabetes mellitus (IDDM) is a complex metabolic disorder, which develops changes in the cytochrome P450 (CYP), mediated metabolism in the liver and in the small intestine and it may also produce altered bioavailability. The main goal of this study was to reveal these metabolic changes in experimental diabetic rats and to evaluate their significance in the drug metabolism. Decreased intestinal CYP3A mediated metabolism in spite of the statistically unaltered total CYP content resulted, which suggests either posttranslational regulation of the enzyme via covalent down-regulation (e. g. phosphorylation) or a change in the intestinal isoenzyme composition. Insulin may be involved in the intestinal CYP3A regulation since inverse correlation was found between the blood glucose concentration (as a marker for insulin level) and the CYP3A function. The hepatic total CYP content and the hepatic CYP2E1 and FMO3 gene expression and function were seen to change remarkably in untreated long-term diabetes and following insulin treatment. Our study concentrated on rat hepatic CYP2C11, CYP2C13, CYP2C22 and CYP2C23 isoforms and reduced gene expressions with the exception of CYP2C23 were found in diabetes, which is explained, by its different physiological role and regulation. The mRNA level of CYP2C11 and CYP2C13 isoforms were sensitive to insulin showing the role of insulin in their regulation. The study resulted in unaltered CL<sub>int</sub> of the CYP2C substrate; diclofenac in either insulin treated or untreated diabetic rats. Similarly, unchanged biotransformation of the cholinesterase reactivator oxime, K-48 was seen in diabetes. These results suggest no influence of diabetes and particularly compensated diabetes on the metabolism of the two drugs investigated. The *in vitro* and *in vivo* metabolism studies of K-48 resulted in a weak metabolism. None of the *in silico* predicted metabolites but the K-48 was found in serum, CSF and brain while an epoxide metabolite was detected in urine. The presence of K-48 in the brain shows a moderate penetration of K-48 to the CNS.

- Borbás T, Benkő B, Szabó I, Dalmadi B, Tihanyi K (2006) *Insulin in flavin-monooxygenase regulation. Flavin-containing monooxygenase and cytochrome P450 activities in experimental diabetes. Eur J Pharmaceut Sci* 28: 51–58.
- Benkő B, Kalász H, Ludányi K, Petroianu G, Kuca K, Darvas F, Tekes K (2007) *In vitro and in vivo metabolism of K-48. Anal Bioanal Chem* 389: 1243–1247.
- Benkő B, Laufer R, Ohmacht R (2007) *HPLC Analysis of microsomal metabolism of K-48. Acta Chromatogr* 19: 61–72.

**MELINDA GYENGE (2010)****Interaction between the nociceptinergic and aminergic systems in central nervous system***Supervisor: Kornélia Tekes*

The dissertation is dealing with the interrelation of nociceptinergic/orphanin FQ-erg and biogenic amine system in animal experiments and in clinical samples.

In animal studies both the effect of the *exogenously* (i. c. v.) administered nociceptin on histamine and serotonin release and changes in *endogenous* nociceptin, nocistatin and also on tissue biogenic amine levels in such animal models as chronic diabetes, stress and alcohol consumption were examined. In hormonal imprinting studies nociceptin, nocistatin and  $\beta$ -endorphin were applied as imprinters and nociceptin, nocistatin as well as dopamine, serotonin and their metabolites from different biological samples (brain areas, CSF, plasma) were measured. To get ready for analysing the nociceptinergic-acetylcholinergic interaction in the CNS, an HPLC-EC method was developed for the determination of a newly synthesized acetylcholine-esterase reactivator, K-27 (1-(4-hydroxyimino-methyl-pyridinium)-3-(4-carbamoylpyridinium)-propane-dibromide) from different biological samples. Effect of acute ischemic CNS damage (stroke, TIA) on plasma nociceptin level and serotonergic measures were studied in clinical samples. Experimental data demonstrated that nociceptin has significant histamine releasing activity in the CNS both from neuronal pool and also from mast cells, however decreases tissue serotonin levels. Evidence was given that chronic diabetes (model system for neuropathic pain) does not influence nociceptin level, however produces significant elevation in nocistatin levels both in the CSF and blood plasma. It was found that either long-term low-dose or short-term high-dose alcohol-load of pregnant mothers result in significant nocistatin-level increase in the offspring.

Hormonal imprinting studies showed that not only  $\beta$ -endorphin, but both nociceptin and nocistatin have imprinting activity when administered neonatally and imprinting by these compounds cause long-term changes in the dopaminergic and serotonergic measures of the adult rats.

Examining the tissue penetration of K-27, it was found that in spite of the hydrophilic nature of the compound it can penetrate the CNS in an effective acetylcholin-esterase reactivating concentration, however this amount of the compound in the CNS has no effect on dopaminergic and serotonergic measures.

Studying groups of acute ischemic CNS damage patients (stroke and TIA) we were the first in demonstrating that in these patients plasma nociceptin levels are significantly higher compared to healthy controls. Experiments gave also further evidence on the disturbances of the serotonergic system in these patients.

- Tekes K, Gyenge M, Sótónyi P, Csaba G (2009) *Effect of neonatal nociceptin or nocistatin imprinting on the brain concentration of biogenic amines and their metabolites. Brain & Development* 31: 282–287.
- Tekes K, Gyenge M, Hantos M, Csaba G (2007) *Effect of  $\beta$ -endorphin imprinting during late pregnancy on the brain serotonin and plasma nocistatin levels of adult male rats. Horm Metab Res* 39: 479–481.

- Gyenge M, Kalasz H, Petroianu GA, Laufer R, Kuca K, Tekes K (2007) Measurement of K27, an oxime-type cholinesterase reactivator by high-performance liquid chromatography with electrochemical detection from different biological samples. *J Chromatogr A* 1161: 146–151.

## ZSUZSA IFFIÚ-SOLTÉSZ (2010)

### Semicarbazide-sensitive amine oxidase substrates in obesity and diabetes

Supervisor: Éva Szökő

Obesity and diabetes are serious public health problems in developed countries and considerable efforts are put in the development of drugs improving impaired carbohydrate metabolism. Semicarbazide-sensitive amine oxidase (SSAO) may be a possible target. The enzyme catalyzes the oxidation of primary amines. SSAO is highly expressed in white adipose tissue (WAT) and in the vasculature. It has been found that its soluble form in plasma is an independent cardiovascular risk factor in diabetes. However, the administration of exogenous SSAO substrates has been shown to reduce hyperglycemia in diabetic animals. Hydrogen peroxide formed during the enzyme reaction has been proven responsible for such effect. However, this reactive oxygen species may contribute to vascular complications of obesity and diabetes, as well. Our aim was to explore the implication of SSAO substrates on certain parameters of diabetes and obesity. We investigated the influence of WAT extension (in obesity and fasting) on its SSAO content. We have analysed the insulin-like actions of benzylamine (BzA), an exogenous substrate of SSAO, *in vitro* and *in vivo*. Furthermore, being aware of the dual action of SSAO in diabetes, we also studied the effect of longterm BzA treatment on glucose handling and on putative vascular complications. Then, we tested SSAO substrate candidates in human adipocytes. SSAO activity was higher in subcutaneous WAT of obese than lean mice. BzA injection was effective alone to improve glucose homeostasis in type 1 and type 2 diabetic animals. Chronic BzA injections also improved endothelial function of diabetic rats when vanadate was simultaneously administered. Chronic oral administration of BzA improved glucose homeostasis in three mouse models of insulin-resistance. In such conditions, we did not observe any adverse effect of hydrogen peroxide. Moreover, increased aorta nitrite concentration, indicative of NO production, accompanied the reduction of fasting plasma glucose levels. In keeping with this, we have set up a pharmacological screening, based on human adipocytes, which allowed the detection of better SSAO substrates than BzA. The results obtained in obese and diabetic models thus confirmed the beneficial metabolic action of BzA and encouraged us to propose SSAO substrates as possible anti-hyperglycemic drug candidates.

- Iffiú-Soltész Z, Prévot D, Carpéné C (2009) Influence of prolonged fasting on monoamine oxidase and semicarbazide-sensitive amine oxidase activities in rat white adipose tissue. *J Physiol Biochem* 65: 11–23.
- Iffiú-Soltész Z, Prévot D, Grès S, Bour S, Szökő E, Knauf C, Burcelin R, Fernandez-Quintela A, Lomba A, Milagro FI, Carpéné C (2007) Influence of acute and chronic administration of benzylamine on glucose tolerance in diabetic and obese mice fed on very high-fat diet. *J Physiol Biochem* 63: 305–315.



- Soltész Z, Tábi T, Halász AS, Pálfi M, Kocsis E, Magyar K, Tóth M, Szökő E (2007) *Studies on the insulinomimetic effects of benzylamine, exogenous substrate of semicarbazide-sensitive amine oxidase enzyme in streptozotocin induced diabetic rats. J Neural Transm* 114: 851–855.

## ANDREA SZEKENI (2010)

### The immune background of type-1 diabetes mellitus pathomechanism and the cardiovascular complications

Supervisor: Valéria Kecskeméti

T1 diabetes mellitus is (T1DM) considered as an immune mediated disease where the  $\beta$ -cells are destroyed by T-cells. Among the possible targets in the autoimmune process are 60 kD heat shock proteins (hsp60) and hsp epitopes p277 and LAK peptide. The immune response to hsps is considered as a functional constituent of natural autoimmunity controlled by the regulatory T cells. The decreased activity of these regulatory T cells and a shift in the physiological Th1/Th2 immune balance-can lead T-cell mediated autoimmune destruction of  $\beta$ -cells. If we accept this theory, a possible way of the T1DM treatment may be the specific immunization with hsp, as one of the possible target antigens of the autoimmune process. Patients with T1DM exhibit a high incidence of diabetic cardiomyopathy. The most prominent electrical alteration is the prolongation of the repolarisation (QT interval). In STZ induced diabetic rat and mice models the APD has been found significantly prolonged and the Vmax significantly decreased. The aim of my human experiments was to analyze the nature of immune regulation disorder. Therefore I determined the shift in Th1/Th2 immune response characterized in terms of cytokine production upon stimulation with presumed target antigens (p277, LAK). The aim of *in vivo* experiment was to examine the effects of immunisation with heat shock proteins on STZ induced diabetic WT/HDC-KO mice and rats. The novelty of my electrophysiological experiments was the characterization of changes of cardiac action potential in HDC-KO mice as compared to the heart parameters of WT animals. Furthermore the STZ diabetes-induced alterations in cardiovascular electrophysiological functions were also compared in WT and HDC-KO mice, respectively. In type 1 diabetic patients the p277 induced Th1 response was significantly higher, meanwhile Th2 was lower as compared to healthy controls. Therefore, this peptide may be considered as a target-antigen of the autoimmune diabetic process. The significant shift toward Th1 immune response can be taken as a further support to the theory that T1DM is an autoimmune disease caused by the disorder of immune regulation. The results obtained in the experiments with WT/HDC-KO mice immunized with the purported target antigens hsp65 and p277 indicated that immunization can moderate the development of multiple low dose of STZ induced autoimmune diabetes. Therefore vaccination may be regarded as a potential novel treatment mode in T1DM. In rat experiments treatment with hsp65 failed to prevent the development of the diabetes but prolonged the survival of animals. Based on the electrophysiological measurements it can be concluded that the action potential alterations (prolonged APD, decreased Vmax) detected in control HDC-KO mice are very similar to the changes found in STZ induced diabetic WT animals. It may also have a well-defined, still not yet clarified reason why STZ treatment failed to cause any further significant

changes in the APD and Vmax in HDC-KO mice. The differences in the STZ treatment-related alterations of AP parameters (APD, Vmax) in HDC-KO and WT animals might be attributed to the increased susceptibility of HDC-KO mice to autoimmune diabetes.

- Szebeni A, Schloot N, Kecskemeti V, Hosszúfalusi N, Panczel P, Prohaszka Z, Füst G, Uray K, Hudecz F, Meierhoff G (2005) *Th1 and Th2 cell responses of type 1 diabetes patients and healthy controls to human heat-shock protein 60 peptides AA437-460 and AA394-408. Inflamm Res 54: 415–419.*
- Szebeni A, Prohászka Z, Buzás E, Falus A, Kecskeméti V (2008) *Effects of vaccination with heat shock proteins on streptozotocin induced diabetes in histidine decarboxylase knockout mice. Inflamm Res 57: 1–5.*
- Szebeni A, Falus A, Kecskeméti V (2009) *Electrophysiological characteristics of heart ventricular papillary muscles from control and diabetic histidine decarboxylase knockout and wild-type mice. J Interv Card Electrophysiol 26: 155–158.*

## ZOLTÁN ZÁDORI (2009)

### Analysis of the mechanisms involved in the regulation of gastric mucosal integrity and gastrointestinal motility

Supervisor: Klára Gyires

Treatment of peptic ulcers can raise difficulties even nowadays, therefore the investigation of mucosal protective factors is particularly important. In the first part of my doctoral work I analyzed the gastroprotective effect of endomorphins and that of nociceptin (N/OFQ) and nocistatin (NST). Gastric ulcers were induced by ethanol, which is an acid-independent ulcer model, thus suitable for the analysis of gastroprotection. It was found that both endomorphin-1 and endomorphin-2 induced mucosal protection after intracerebroventricular (i. c. v.) administration in the rat, similarly to other opioid peptides. Inhibition of the key enzyme responsible for degradation of endomorphins also induced protective effect, which indicates the role of endogenous endomorphin system in the maintenance of mucosal integrity. The central effect of endomorphins is mediated mainly by NO and CGRP, but partly also by prostaglandins in the periphery. Furthermore, it was first demonstrated that beside N/OFQ also centrally injected NST can induce gastroprotection, thus it is a biologically active peptide per se. NST exerts its effect irrespectively of N/OFQ, through activation of its own (and still unidentified) receptor. Two types of interaction were found between N/OFQ and NST: when higher doses were applied, NST reduced the protective effect of N/OFQ. In contrast, addition of their effects was observed, when lower doses were used. The effect of both peptides is mediated by the endogenous opioid system. The central effect is conveyed by the vagal nerve to the periphery, where prostaglandins and NO are likely to mediate the mucosal protection. In the second part of my work I investigated the role of  $\alpha 2$ -adrenoceptors and imidazoline receptors in the regulation of gastrointestinal motility. The results indicate that oxymetazoline, a selective  $\alpha 2A$ -receptor subtype agonist, which is extensively used for the analysis of gastrointestinal functions, inhibits gastric motility through two distinct mechanisms: beside activation of presynaptic  $\alpha 2A$ -receptors on myenteric neurons also postsynaptic mechanisms may be involved. It was also found that beside  $\alpha 2$ -adrenoceptors I1 receptors may regulate the gastrointestinal motility as well.

- Zádori ZS, Shujaa N, Köles L, Király KP, Tekes K, Gyires K (2008) Nocistatin and nociceptin given centrally induce opioid-mediated gastric mucosal protection. *Peptides* 29: 2257–2265.
- Zádori ZS, Shujaa N, Fulop K, Dunkel P, Gyires K (2007) Pre- and postsynaptic mechanisms in the clonidine- and oxymetazoline-induced inhibition of gastric motility in the rat. *Neurochem Int* 51: 297–305.
- Gyires K, Zádori ZS, Shujaa N, Minorics R, Falkay G, Matyus P (2007) Analysis of the role of central and peripheral alpha2-adrenoceptor subtypes in gastric mucosal defense in the rat. *Neurochem Int* 51: 289–296.

---

# SCHOOL OF PH.D. STUDIES

---

## 4. MENTAL HEALTH SCIENCES

---

**Chairman:****István BITTER M.D., Ph.D., D.Sc.**

Department of Psychiatry and Psychotherapy

6 Balassa st, Budapest H-1083

Tel./fax: +36 1 210 0330; fax: +36 1 210 0336

E-mail: [bitter.istvan@med.semmelweis-univ.hu](mailto:bitter.istvan@med.semmelweis-univ.hu)

### General overview

The Ph.D. training and research programs of the Mental Health Sciences Doctoral School IV of Semmelweis University aim to offer research areas and methodological training for those interested in conducting research in *psychiatry, behavioural and mental health sciences as well as in community and social mental health*. The school promotes initiatives relating to preventative medicine. One of its goals is to facilitate the Ph.D. candidates' skills for promoting their research topic in public and provides feedback on their academic competence during the Ph.D. training period.

Our research projects highlight the interactions between clinical neurosciences, social sciences, mental health and in various other helping professions. Our school is proud to have a history of promoting interdisciplinary research.

We offer insight into research areas and the methodology of social sciences for all the Ph.D. students of Semmelweis University who are interested in attending our courses. Furthermore, the inter-disciplinary feature of our doctoral school manifests itself in the basic principle of its training philosophy; i.e. Ph.D. students with state scholarships and PhD candidates entering into the qualification phase (without training) should become familiar with the theoretical terminology and methodology of both the natural and social sciences. However, our school is prepared to welcome only such titles of research projects which can be researched and evaluated by the principles of natural sciences.

Our school promotes methods which facilitate this process (e.g. methods of epidemiology, representative surveys, comparative studies, etc.).

**PROGRAM 4/1.****PSYCHIATRY*****Coordinator:*****László TRINGER M.D., C.Sc.**

Department of Psychiatry and Psychotherapy

6 Balassa St, Budapest H-1083

Tel./fax: +36 1 210 0330; fax: +36 1 210 0336

E-mail: [tringer.laszlo@med.semmelweis-univ.hu](mailto:tringer.laszlo@med.semmelweis-univ.hu)**Program overview**

This program offers education and research possibilities for, medical doctors, psychologists and other eligible persons in behavioural sciences, clinical psychology and in the neurobiological, psychosocial, diagnostic and treatment aspects of psychiatric disorders. The research profile of the program has considerably widened in the last few years. New research topics include psychiatric, genetics and psychophysiology, as well as cognitive neuropsychology and social cognition in several psychiatric diseases, including psychotic disorders and adult ADHD. Another important area covers methodological research in the field of clinical psychopharmacology in cooperation with genetics, biochemistry and bioinformatics.

***Titles of research projects***

Genetic factors and gene x environment interactions in psychiatric disorders: the NEWMOOD study

Psychiatric disorders in children and adolescents

Clinical psychopharmacology

Psychophysiological and neuropsychological mechanisms of psychiatric disorders

Common neuropsychological mechanisms of psychiatric disorders

Survey of electroconvulsive therapy's clinical use

Epidemiology, clinical and psychosocial characteristics of the addictive diseases

Mental deficits in the developmental psychopathology

The application of neuro-cognitive tests in psychiatric disorders

Family pathology and communication

Sleep disorders in patients with chronic kidney diseases

The research of factors relating to the quality of life of patients with organ transplant

The psycho-social aspects of chronic diseases

Sleep disorders among chronic kidney diseases patients

Experimental behavioural physiology and pharmacology

The role of the disturbed circadian rhythm in the development and procession of psychiatric disorders and conditions of internal medicine

***Supervisors***

György Bagdy

Judit Balázs

István Bitter

Pál Czobor

Ede Frecska

Gábor László Gazdag

József Gerevich

György Gergely

Szabolcs Kéri

Tamás Kurimay

Miklós Zsolt Molnár

István Mucsi

István Mucsi

István Mucsi

Csaba Nyakas

György Purebl

The research of energetic aspects of mental health by the use of actigraphy	Péter Rajna
Clinical and biological aspects of affective disorders	Zoltán Rihmer
Molecular psychiatry: genetic, epigenetic, genomic and proteomic studies of psychiatric disorders	János Réthelyi
Virtual reality therapy	Lajos Simon
The recognition and expression of emotions in psychiatric disorders	Lajos Simon
Biological aspects of some sleep disorders	Anna Szűcs
Psychotherapy in the medical practice	László Tringer
Epidemiology of eating disorders	Ferenc Túry

***Ph.D. students***

Lívia Balogh	ft
Boglárka Bánsági	pt
Judit Benkovits	ft
Eszter Bertha	ft
Gyöngyvér Dallos	pt (a)
Ajándék Eőry	pt
Ágnes Fábián	pt
Dóra Filipovits	pt
Nóra Garamvölgyi	pt
Krisztina Kocsis-Bogár	pt

Sarolta Komlósi	ft (a)
Daniella Kováts	pt
Krisztina Magyar	pt
Balázs Matuszka	pt
Mónika Barbara Miklósi	ft

Eszter Molnár	ft (a)
Dorottya Pap	ft
Szilvia Papp	ft
Ágnes Udvardy-Mészáros	ft (a)
Johanna Takács	pt
László Tombor	pt
Anabella Veress	ft (a)

***Ph.D. candidates***

Anita Benkő	ft
Nikoletta Bódi	ft
Árpád Dóró	pt
Judit Futó	ft
Csaba Petővári	ft
Pál Salacz	na
József Szabó	pt

***Supervisors***

Pál Czobor
Szabolcs Kéri
István Bitter
Judit Balázs
Judit Balázs
Zoltán Rihmer
János Réthelyi
István Bitter
Zoltán Rihmer
Dóra Perczel-Forintos
Pál Czobor
Tamás Kurimay
István Bitter
József Gerevich
Dóra Perczel-Forintos
György Bagdy
György Bagdy
Pál Czobor
István Bitter
László Tringer
Pál Czobor
Lajos Simon

***Supervisors***

György Bagdy
Szabolcs Kéri
Tamás Kurimay
György Gergely
József Gerevich
Zoltán Rihmer
József Gerevich



***Ph. D. graduates***

Brigitta Baran	na
Anna Bátki	ft
Gábor Csukly	pt
Judit Lazáry	ft
László Mayer	na
Orsolya Nagy	na
Patrícia Polgár	pt
Viktória Simon	na
Tamás Telek	ft

***Supervisors***

István Bitter
György Gergely
Lajos Simon
György Bagdy
Judit Balázs
Szabolcs Kéri
István Bitter
István Bitter
István Bitter

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010***

**BRIGITTA BARAN (2009)**

**The introduction of convulsive therapy: data contributing to the history of the treatment of schizophrenia**

*Supervisor: István Bitter*

This thesis has had three main objectives. First, it has sought to describe the personal and professional background against which László Meduna developed his idea, namely curing schizophrenia by chemically-induced epileptic seizures. Second, it has aimed to present the circumstances in which the first courses of chemically-induced convulsive therapy took place by making use of the original documents and by analysing the relevant literature. This has allowed a re-evaluation of the safety and effectiveness of Meduna’s method. Third, the thesis has defined the impact of Meduna’s method on the development of biological psychiatry and its role in changing the conceptualization of schizophrenia. Meduna compared the findings of his glia research in epileptic brains with pathological alterations found in schizophrenic patients by his colleagues. Based on his experimental data, he hypothesised that schizophrenia and epilepsy were antagonistic to each other. As this hypothesis was supported by clinical observations, Meduna came to the conclusion that induced epileptic seizures would have a curative effect on schizophrenia, and started considering the clinical implications. Following animal experiments with a variety of substances to induce seizures, he started camphor-induced convulsive therapy on six patients on 2 January 1934; within a month he had treated 11 patients. This thesis describes those 11 cases in detail, aiming to identify Meduna’s selection criteria and to re-evaluate the safety and effectiveness of the first courses of convulsive therapy. Finally, the thesis has underscored the broader significance of Meduna’s hypothesis, which eventually proved to be inaccurate yet led him to introduce a revolutionary new treatment method, convulsive therapy. Meduna had a pioneering role in the advancement of biological psychiatry because he initiated a paradigm shift towards the biological conceptualization of schizophrenia by establishing the experimental approach in clinical psychiatry.

- Baran B, Bitter I, Fink M, Gazdag G, Shorter E (2008) *Károly Schaffer and his school: the birth of biological psychiatry in Hungary, 1890–1940*. *Eur Psychiatry* 23: 449–456.
- Baran B, Bitter I, Ungvári GS, Nagy Z, Gazdag G (2008) *The beginnings of modern psychiatric treatment in Europe: lessons from an early account of convulsive therapy*. *Eur Arch Psychiatry Clinical Neurosci* 258: 434–440.

## ANNA BÁTKI (2010)

### The development of emotion-regulation and representational abilities in adopted children

Supervisor: György Gergely

Several international studies have demonstrated that years after being adopted, post-institutionalized children still experience social and emotional difficulties, regardless of their improvement in other areas. Yet we know very little of the processes through which early experience leads to these developmental problems. The study described in the dissertation aimed to explore emotion-regulation abilities and related social-cognitive (representational) skills of adopted children. Such abilities and skills are especially important because these are prerequisites of mental health and efficient social interactions. The general hypothesis of the study was that the emotion-regulation abilities of children who had spent at least the first 6 months of their lives in institutional care were less developed, and it was more difficult for them to employ cognitive representational skills in social interactions.

90 children, between the age of 4 and 6 participated in the study. Each child belonged to one of the 3 groups, with 30 children in each of the following groups: 1. Children placed in institutional care at birth and lived there for at least six months before being adopted; 2. Children adopted within the first 6 weeks of their lives; 3. (control) Children reared with their biological families.

The study examined emotion-regulation abilities by analyzing play-narratives (MacArthur Story Stem Battery), and explored representational abilities by false-belief tests and a test of coordination of symbolic representations. In addition, an IQ test (Snijders-Oomen) and a test of receptive vocabulary (Peabody Picture Vocabulary Test) were administered to the children. Finally, the parents completed the Child Behaviour Checklist.

The results of comparing the three groups confirm the hypothesis that one critical consequence of institutional care is the delay in both the development of emotion-regulation abilities and the efficient use of social cognitive representational skills in emotionally charged situations.

We found that even moderate institutional deprivation may lead to long-term, serious consequences in emotional development. This is a new finding in relevant international literature.

At the same time our results also draw attention to the fact, that even early adopted children differ from the control group (children in birth-families) in several measures of emotion-regulation development (although to a lesser extent than late-adopted children do).

This result can be explained by pre- and perinatal factors on the one hand and by various characteristics of the adoptive parents and vulnerabilities following directly from the fact of being adopted.

- Bárti A, Pléh Cs (2008) *Evolúciós pszichopatológia: jelszavak és kérdőjelek*. Pp 239–250 in: Pléh Cs. *A lélek és a lélektan örömei*. Gondolat Kiadó, Budapest.
- Batki A, Baron-Cohen S, Connellan J, Wheelwright S, Ahluwalia J (2000) *Is there an innate gaze module? Evidence from human neonates*. *Infant Behav Devel* 23: 223–229.
- Connellan J, Baron-Cohen S, Bárti A, Wheelwright S, Ahluwalia J (2000) *Sex differences in neonatal social cognition*, *Infant Behav Devel* 23: 113–118.

## GÁBOR CSUKLY (2010)

### Emotional facial expression recognition in depressed and healthy subjects

Supervisor: Lajos Simon

Emotional facial expressions are an important part of social communication and play a crucial role in interpersonal interaction. Universal recognition of facial expressions of basic emotions has been demonstrated by Ekman *et al.*'s research group in several cross-cultural studies.

The phenomenon that the literature refers to as “mood congruency” effect implies that subjects with depressed mood tend to judge positive emotions as neutral and neutral faces as negative.

We evaluated 3 clinical studies to analyze the associations between emotion recognition, depressive symptoms and psychological distress respectively. We used a virtual human—high-resolution digital copy of a living person—for presenting the basic emotions during the experiments. In the first study the subjects were all healthy volunteers (n=117) and we found that subjects with mild psychiatric symptom distress had poorer performance in affective facial recognition in general. Furthermore, the same subjects had poorer functioning especially in neutral face recognition and that they were prone to attribute negative emotions for example sadness and fear to neutral faces. We also found that the negative bias showed the most pronounced association with symptoms of depression as compared to other symptom dimension. In the second experiment data were collected from a total of 46 depressed and control subjects distributing according to a 1:1 ratio (23 depressed and 23 control subjects), who were matched by age, gender and education. We found that persons with depression, as compared to non-depressed control subjects, performed more poorly in overall and neutral facial emotion perception tasks, and the difference was more pronounced at lower intensities. Furthermore, we found that persons with depression made more errors in the direction of high arousal facial expressions (fear, anger, surprise), and recognized the low arousal facial expressions (neutral, disgust, sadness) with lower accuracy as compared to non-depressed control subjects. The subjects (n=106) in the third study were inpatients in a study of psychopathology of depression. In this study we found that those depressed patients, who recognized positive emotions and neutral facial expressions at a lower rate, had the more severe symptoms of depressions indicated by the BDI.

Overall, our findings raise the possibility that difficulties in emotion processing contribute to the severity of psychiatric symptom distress, and that, as previous studies pointed out, neutral face recognition and a negative bias in neutral face recognition can be a sensitive sign of early psychiatric disorder. Furthermore, we can conclude that the disability to accurately recognize non-emotional and emotional facial expressions along

with the tendency to make more errors in facial expression recognition to the high arousal emotions, can be two important contributing factors for the well documented social problems of patients with depression.

- Csukly G, Czobor P, Simon L, Takács B (2008) *Basic emotions and psychological distress: association between recognition of facial expressions and SCL-90 subscales. Compr Psych* 49: 177–183.
- Csukly G, Czobor P, Simon L, Takács B (2009) *Facial expression recognition in depressed subjects: the impact of intensity level and arousal dimension. J Nerv Ment Dis* 197: 98–103.
- Csukly G, Czobor P, Unoka Z, Takács B, Simon L (2009) *Depressziós betegek tüneti súlyossága és érzelem felismerési képessége közötti összefüggések. Psych Hung* 24: 68–73.

## JUDIT LAZÁRY (2010)

### Serotonin transporter gene and threatening life events are associated with depressive phenotype

*Supervisor: György Bagdy*

Although heritability of the affective disorders is well accepted, several questions are still unsolved. The phenotype associated with the disorder is still uncertain, e. g., different disorders of the affective spectrum could be observed in the family of the patient. Furthermore, genetic studies failed to provide exact explanation for these questions either. Based on neurobiological and pharmacological evidence the serotonin transporter gene is one of the most frequently investigated candidate in mood disorders and other disorders of the affective spectrum. Data on functional polymorphism located in the serotonin transporter gene (5-HTTLPR) suggested that direct association between the 5-HTTLPR and depression was not replicable. New models considering the interaction with environment factors (Gene-Environment interaction, G x E) or with other genes (Gene-Gene interaction, G x G) appeared to be more appropriate. Therefore we designed our studies in a large scale Hungarian, nonclinical population using the above models. Our results provide evidence that the association of positive affective family history and depressive symptoms are mediated by the affective temperaments. As different subgroups of affective disorders appeared in the affective family history we conclude that the heritable component of the affective disorders does not determine the given clinical phenotype, rather it is a kind of vulnerability for affective lability which is manifested by the affective temperaments. We reported first that the role of the promoter region variant (5-HTTLPR) is not exclusive, and the middle region (tagged by the SNP rs140700) of the gene has also a significant role in the G x E model. Furthermore, we discovered a significant Gene x Gene x Environment interaction between 5-HTTLPR, rs140700 and threatening life events. Haplotype analyses of the serotonin transporter gene suggested that the majority of the S allele carriers for 5-HTTLPR with multiple threatening life events expressed high depression score. Among these persons we identified, however, a subgroup with much lower depression score. In this group the above parameters are associated with the A allele-type of rs140700. These results, namely a dissimilar allele distribution of rs140700 among 5-HTTLPR S carriers provide a possible explanation for contradictory findings in previous studies. Hence, our data support the role of serotonin trans-

porter gene in the background of depression, but a more sophisticated model including G x G x E interaction of depression was suggested. In another study, interaction of 5-HTTLPR with the cannabinoid receptor 1 gene promoter was significantly associated with anxious phenotype. These results suggest that extremely high or low synaptic serotonin concentration could be associated with high anxiety score. These findings call attention to the serotonergic dysfunction in the vulnerability for affective disorders.

- Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Juhasz G, Bagdy Gy (2008) New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events and depressive phenotype. *Biol Psychiatr* 64: 498–504.
- Lazary J, Gonda X, Benko A, Gacser M, Bagdy Gy (2009) Association of affective family history with depressive phenotype is mediated by affective temperaments. *Psychiatr Res* 168: 145–152.
- Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Hunyady L, Juhasz G, Bagdy G (2009) Promoter variants of the cannabinoid receptor 1 gene (CNR1) in interaction with 5-HTTLPR affect the anxious phenotype. *Am J Med Gen* 150 B: 1118–1127.

## LÁSZLÓ MAYER (2009)

### The development of emotion-regulation and representational abilities in adopted children

*Supervisor: Judit Balázs*

**Introduction:** Few Hungarian studies are published about the prevalence of depressive symptoms and its risk factors in childhood and adolescence. Several international studies have demonstrated the association between stressful life events as risk factors of depression and depressive symptoms in childhood, as well as childhood major depression. Besides the examination of the number of stressful life events, the effect of certain specific life events were demonstrated as well.

**Aims:** In our present study we have examined beside the prevalence of depressive symptoms, the significance of stressful life events as risk factors of childhood depression, on community and clinical samples. Our aim has been to prove that there are certain stressful life events that play a more decisive role in the development of the childhood depression than others, with regards to both frequency and characteristics. We have assumed, that there exists a characteristic pattern of life events in the major depressive group, which consists of specific stressful life events.

**Methods:** For the community sample we collected data in 9 elementary schools in two regions of Hungary. We have analyzed the data of 2652 students. The clinical sample consisted of 434 children with MDD (between the ages of 7 and 14.9), the control group consisted of 724 community children. Depressive symptoms were measured by the short version of Child Depression Inventory (CDI). The diagnoses of MDD were verified on the basis of ISCA-D semistructured diagnostic interviews. We obtained information about stressful life events from questionnaires completed by parents (by the biological mother in the clinical study).

**Results:** The mean depressive score on the CDI was 3.61 in the examined community sample. 14.9% of the students had a score of 7 or higher, which implies an increased

risk of clinical depression. Examined children had an average of 2.35 stressful life events out of the 26. We have found a significant positive correlation between depressive symptoms and stressful life events. Children with major depression experienced already twice the number of stressful life events by the age of 7–9, than members of the comparison group. Teasing and abuse have greatly increased depression, regardless of age and sex (respectively  $OR=13.5$ ,  $OR=10$ ).

Several life events displayed age or sex interactions. In both genders, divorce and family argument have had a great impact on depression with the change of age.

**Conclusions:** Awareness of the associations between stressful life events and childhood and adolescent depression can help experts dealing with children in the early recognition and the effective treatment of depression.

- Mayer L, Kiss E, Baji I, Skultéti D, Vetró Á (2006) *Életesemények minőségi elemzése és kapcsolata a depressziós tünetekkel általános iskolás populációban. Psychiatr Hung* 5: 360–370.
- Mayer L, Lopez-Duran NL, George C, Baji I, Kapornai K, Kiss E, Kovacs M, Vetró Á (2009) *Characteristics of stressful life events in a clinical sample of depressed children in Hungary. J Affect Disord* 115: 207–214.
- Feng Y, Vetró Á, Kiss E, Kapornai K, Daróczy G, Mayer L, Tamás Zs, Baji I, Gáboros J, King N, Kennedy JL, Wigg K, Kovacs M, Barr CL (2008) *Association of the neurotrophic tyrosine kinase receptor 3 (NTRK3) gene and childhood-onset mood disorders. Am J Psychiatry*, 165: 610–616.

## ORSOLYA NAGY (2010)

### Neuropsychological impairments in schizophrenia: focus on interactive memory systems and attention

*Supervisor: Szabolcs Kéri*

**Background:** Assessment of neuropsychological functions greatly broadens our understanding of schizophrenia. The thesis aimed to investigate memory processes and multi-focal attention capacity. **Objectives and methods:** The purpose of Experiment 1 was to investigate dopaminergic mechanisms in feedback-guided habit learning measuring plasma levels of metabolite of dopamine, serotonin and norepinephrine. In Experiment 2, basal ganglia (BG) and medial temporal lobe (MTL) dependent learning was studied in patients with schizophrenia using acquired equivalence learning test. In Experiment 3, we investigated how patients with schizophrenia can track multiple moving targets and assessed its relationship with motion perception, sustained attention/context processing, and object and spatial working memory. **Results:** *Experiment 1:* Participant who had lower dopamine metabolite level than the median value of the whole sample committed more errors during the feedback guided habit-learning phase compared with participants who has higher plasma level than the median value. A similar phenomenon was not observed for the context-dependent phase of the task and for serotonin and norepinephrine metabolites. *Experiment 2:* Patients with schizophrenia showed a selective deficit on stimulus generalization, whereas stimulus-response learning was spared. The stimulus generalization deficit correlated with the CVLT performance (total scores from trials 1–5



and long-delay recall), but not with the n-back test performance. The number of errors during stimulus-response learning correlated with the daily chlorpromazine-equivalent dose of antipsychotics. *Experiment 3:* Results revealed that patients with schizophrenia displayed impaired performances on multiple-object tracking tasks. Specific relationship was shown among object tracking, velocity discrimination, and spatial working memory. In patients with schizophrenia, velocity discrimination and spatial working memory were the predictive factors of multiple-object tracking, whereas in healthy control subjects the single predictive factor was velocity discrimination. Only the Continuous Performance Test made significant contribution to discriminating between patients and controls. **Conclusion:** Our results suggest that dopamine plays a special role in feedback-guided cognitive sequence learning. The acquired equivalence task was the first study to show that patients with schizophrenia exhibit deficits during MTL-dependent learning, but not during BG-dependent learning within a single task. High-dose first generation antipsychotics may disrupt BG-dependent learning by blocking dopaminergic neurotransmission in the nigro-striatal system. Multiple-object tracking is impaired in schizophrenia, and that it is specifically associated with motion perception and spatial working memory.

- Nagy O, Kelemen O, Benedek G, Myers CE, Shohamy D, Gluck MA, Kéri S (2007) Dopaminergic contribution to cognitive sequence learning. *J Neural Transm* 114: 607–612.
- Nagy O, Kelemen O, Erdélyi R, Pataki M, Janka Z, Myers CE, Gluck MA, Kéri S (2005) Tanult ekvivalencia a szkizofréniában: új módszer a hippocampalis és basalis ganglionokhoz kapcsolódó memóriafunkciók mérésére [Learned equivalence in schizophrenia: novel method for the measurement of memory functions of the hippocampus and basal ganglia]. *Psychiatr Hung* 20: 363–369.
- Kéri S, Nagy O, Kelemen O, Myers CE, Gluck MA (2005) Dissociation between medial temporal lobe and basal ganglia memory systems in schizophrenia. *Schizophr Res* 77: 321–328.

## PATRÍCIA POLGÁR (2009)

### Cognitive differences between deficit and non-deficit schizophrenia

*Supervisor: István Bitter*

**Aims:** Cognitive dysfunction is a core feature in schizophrenia and has a great impact on psychosocial functioning. Still it remains unclear, whether the different diagnostic subgroups have a specific cognitive profile. The topic of my thesis was to investigate the neurocognitive characteristics of deficit (SZ-D) and non-deficit schizophrenia (SZ-ND), and to examine if the two diagnostic subgroups have a qualitative difference in cognitive functioning. Earlier studies using classic neuropsychological methods resulted in a global, quantitative difference between the two groups, therefore I chose a different approach: in the first study we used a classic, well-documented test on executive functioning, but in order to obtain more refined results, we implemented a factor analytic approach. The second study includes a new test method based on the latest results of cognitive neuroscience. **Methods:** In Study 1, 154 SZ-D, 121 SZ-ND patient and 130 healthy controls completed the WCST (Wisconsin Card Sorting Test). We performed an exploratory factor analytic study on WCST variables for the total group and each subgroup, then we

assessed the ability of the WCST factors to distinguish between the SZ-D, SZ-ND and control groups. In Study 2, we used a new method, the Kilroy-test to investigate procedural and context-dependent learning. 78 patients (45 SZ-D, 27 SZ-ND) and 30 healthy controls completed the test, which has two phases: while the training phase is dominantly related to basal ganglia circuits, the context-dependent probe phase requires intact medial-temporal lobe functioning. Thus, the two interactive memory systems can be examined separately within one test. **Results:** *Study 1:* Results of the exploratory factor analysis of the whole sample yielded 2 factors which together explained approximately 95% of the total variance. The distribution of the amount of variance explained across the 2 factors was the following: factor 1 (“general executive functioning”)=76%, and factor 2 (“non-perseverative errors”)=19%. The 2-factor solution remained stable for the three groups. Comparison of the diagnostic groups on each of the factors revealed that deficit schizophrenia patients suffer from a more severe degree of impairment on the “General executive function” factor than nondeficit schizophrenia patients. Significant group differences were detectable among each of the groups on Factor 1 ( $p < 0.05$  for all pairwise comparisons). As for Factor 2, a significant group ( $p = 0.0012$ ) difference was found, indicating poorer functioning in the nondeficit schizophrenia subgroup vs. the controls; a difference between the deficit and nondeficit subgroups approached statistical significance ( $p = 0.073$ ). *Study 2:* Results revealed that deficit and nondeficit patients were similarly impaired on the probe phase compared with controls ( $p < 0.001$ ). However, the training phase was not compromised in SZ-ND patients, but SZ-D patients showed a significant impairment compared to controls and SZ-ND patients ( $p < 0.01$ ). More severe negative symptoms were associated with more errors on the training phase.

**Discussion:** In Study 1, results revealed that both schizophrenia groups showed executive function impairment as compared to controls. SZ-D patients suffer from a more severe degree of impairment on the “General executive function” factor (conceptualization, flexibility, set shifting) than SZ-ND patients. On the other hand, non-perseverative error type (factor 2) seems to be less typical to SZ-D than to the SZ-ND patients. The results of Study 2 revealed that while context-dependent, MTL-mediated learning is uniformly impaired in schizophrenia, BG-mediated procedural learning remains relatively intact in SZ-ND patients. However, deficit syndrome is associated with prominently impaired stimulus-response reinforcement learning. In conclusion, the two diagnostic subgroups seem to differ not only in the degree of cognitive impairment, but in the characteristics as well. The deficit-syndrome can be characterized by a specific profile regarding executive function, and shows greater impairment in procedural learning.

- Polgár P, Farkas M, Nagy O, Kelemen O, Réthelyi J, Bitter I, Myers CE, Gluck MA, Kéri S (2008) How to find the way out from four rooms? The learning of “chaining” associations may shed light on the neuropsychology of the deficit syndrome of schizophrenia. *Schizophr Res* 99: 200–207.
- Polgár P, Farkas M, Nagy O, Kelemen O, Réthelyi J, Bitter I, Myers CE, Gluck MA, Kéri Sz. (2007) Kognitív készségek tanulása depresszióban: a kontextus-váltás hatása. *Psychiatr Hung* 22: 271–275.
- Farkas M, Polgár P, Kelemen O, Réthelyi J, Bitter I, Myers EC, Gluck MA, Kéri S (2008) Associative learning in deficit and non-deficit schizophrenia. *Neuroreport* 19: 55–58.

**VIKTÓRIA SIMON (2010)****Epidemiology of adult Attention Deficit Hyperactivity Disorder (ADHD)***Supervisor: István Bitter*

In spite of the growing literature of adult ADHD, relatively little is known about the prevalence and correlates of adult ADHD. Thus, the main objective of my thesis was to estimate the prevalence of adult ADHD and identify certain demographic correlates based on a meta-analysis of the available prevalence data in the literature, and based on a direct estimation on a large Hungarian community sample. For the meta-analysis we used Medline, Psyclit and Embase databases as well as hand search to find relevant publications and applied a mixed-effect meta-regression technique. In the Hungarian study, subjects between 18 and 60 years were included in the screening phase of the study (N=3529), conducted in 17 GP practices in Budapest. Out of 279 positively screened subjects 161 subjects participated in a clinical interview and filled in a self-report questionnaire to confirm the diagnosis. Beside DSM-IV diagnostic criteria, we applied four alternative diagnostic criteria: “No-onset” (DSM-IV criteria without the specific requirement for onset); Sx/full (DSM-IV “symptoms only” criteria); and Sx/reduced (DSM-IV “symptoms only” criteria with a reduced threshold for symptom count). The pooled prevalence of adult ADHD was 2.5% (95% CI: 2.1%–3.1%). Gender and mean age, interacting with each other, were significantly related to ADHD’s prevalence. Meta-regression analysis indicated that the proportion of subjects with ADHD decreases with age when males and females are equally represented in the sample. In the Hungarian study crude prevalence estimates adjusted for the specificity and sensitivity data of the screener were 1.35% in the “DSM-IV” group, 1.64% in the “No-onset” group, 3.65% in the “Sx/full” group and 4.16% in the “Sx/reduced” group. Logistic regression analysis showed that ADHD was significantly more prevalent with younger age and male gender [ $\chi^2=14.46$ ;  $p=0.0007$ ]. Prevalence estimates corrected for the “not-interviewed” subsample and adjusted for specificity and sensitivity data of the screener was 2.3% in males, 0.91% in females; 2.02% in the  $\leq 40$  years age group and 0.70% in the  $>40$  years age group, based on DSM-IV diagnostic criteria. In conclusion, prevalence rates found in the Hungarian study are somewhat lower, but still are in line with those reported in the literature. Based on our findings, prevalence of ADHD in adults declines with age in the general population. We think, however, that the unclear validity of DSM-IV diagnostic criteria for diagnosing adult ADHD can lead to reduced prevalence rate by the underestimation of the prevalence of adult ADHD.

- Simon V, Czobor P, Bálint S, Mészáros A, Bitter I (2009) Prevalence and correlates of adult ADHD—a meta-analysis. *Br. J Psychiatry* 194: 204–211.
- Bitter I, Simon V, Bálint S, Mészáros Á, Czobor P (2010) How do different diagnostic criteria, age and gender affect the prevalence of attention deficit hyperactivity disorder in adults? An epidemiological study in a Hungarian community sample. *Eur Arch Psychiatry Clin Neurosci* 260: 287–296.
- Simon V, Czobor P, Bálint S, Mészáros Á, Murai Z, Bitter I (2007) A felnőttkori figyelemhiányos/hiperaktivitási zavar (attention deficit/hyperactivity disorder—ADHD) epidemiológiai vizsgálatainak részletes áttekintése. *Psychiatr Hung* 22: 4–19.

**TAMÁS TELEK (2009)****Association of menstrual symptoms with personality dimensions and mood fluctuation***Supervisor: István Bitter*

Physiological and psychological symptoms occurring during the premenstrual period of the menstrual cycle may have an impact on everyday functioning, well-being and behaviour. Most women are affected by these changes in the psychic and physiological symptoms related to the female reproductive cycle, although symptoms reach diagnostic level only in a small part of them. Despite of this, the number of studies related to the background of premenstrual symptoms in healthy women is limited. The aim of our studies was to investigate the characteristics and personality correlates of symptom fluctuations associated with premenstrual syndromes in healthy women. In the first part of our study 63, while in the second part of our study 40 women participated. All participants were psychiatrically healthy, non-PMDD women, with regular menstrual cycles. The participants completed the PRISM (Prospective Record of the Impact and Severity of Menstrual Symptoms) calendar every evening for three cycles. In the first study participants completed the Symptom Distress Checklist-SCL-51, the State Trait Anxiety Inventory-STAI, the Zung Self-rating Depression Scale (ZSDS), the Eating Attitude Test (EAT), and the Mind and Body Cathexis Scale on three pre-defined days in the early follicular, late follicular and in late luteal phase of the cycle. In the second study we used the Temperament and Character Inventory (TCI) to assess personality dimensions. We analysed physiological and psychological symptoms based on the PRISM calendar established the volume of changes in symptom severity from the late follicular to the late luteal phase and created two groups based on the magnitude of PRISM score fluctuation. We averaged the psychometric scores obtained in the three different cycle phases and we analysed the pattern of change. We also analysed the personality profile differences in the two groups created based on the PRISM scores. We found significantly higher scores in the luteal phase on scales measuring anxiety (STAI, SCL), depression (SCL, ZSDS), somatisation (SCL), interpersonal sensitivity (SCL), and obsessive compulsive symptoms (SCL) compared to scores of the follicular phase in the whole sample. We found a significant time and grouping interaction in the case of the of SCL somatisation and ZSDS depression scores. The group experiencing more marked late luteal symptoms had significantly higher scores in the novelty seeking (NS), self-directedness (S), cooperativeness (C), self-transcendence (ST) dimensions and significantly lower scores in the harm avoidance (HA) dimension. Our results indicate that the fluctuation of physical and psychical symptoms during the menstrual cycle has a significant impact on the well-being of healthy women. The mood fluctuation related to the cycle is independent from the physical symptoms, and even in case of healthy women the severity of symptoms related to neuroticism and mood is significantly higher in the premenstrual period compared to the follicular period. Therefore, the impact of the female reproductive cycle on the psychological well-being of women should be taken into consideration in both diagnostics and research. The personality dimensions identified in the second part of our study may serve as protective factors preventing the emergence of premenstrual disorders in women experiencing a more marked fluctuation of symptoms during their

menstrual cycle. Our results add an important new aspect to our existing view about premenstrual symptomatology by extrapolating our current knowledge to the healthy population.

- Telek T, Gonda X, Lazary J, Benko A, Pap D, Vargha A, Bagdy Gy (2010) *The possible protective role of personality dimensions against PMS. Psychiatry Res* 179: 81–85.
- Gonda X, Telek T, Juhasz G, Lazáry J, Vargha A, Bagdy Gy (2008) *Patterns of mood changes throughout the reproductive cycle in healthy women without premenstrual dysphoric disorders. Prog Neuropsychopharmacol Biol Psychiatry* 12: 1782–1788.

**PROGRAM 4/2.**

**BEHAVIORAL SCIENCES**

**Coordinator:**  
**Mária KOPP M. D., Ph. D., D. Sc.**  
Behavioral Sciences Institute  
4 Nagyvárad sq, Budapest H–1089  
Tel.: +36 1 210 2930  
E-mail: [kopmar@net.sote.hu](mailto:kopmar@net.sote.hu)

**Program overview**

Behavioral sciences constitute an integrative field which bridge the paradigms of natural and social sciences. They study human behavior in a biological, psychological and social perspective, and provide an opportunity for establishing and analysing the components of healthy behavior, the psychological and social risk factors of diseases, as well as investigating the background of self-destructive conduct and the development of attitudes to protect health. They examine the regularities and the possibilities of developing human behavior from an interdisciplinary and integrative perspective relying on achievements of Medicine, Psychology, Sociology, Anthropology, Bioethics, Neuroanatomy and Neurophysiology. Nowadays, the prevention and successful treatment of diseases which impact on public health cannot rely entirely on a biomedical approach since the behavioral risk factors are highly influenced by psychological and social factors. The professional Program follows the analogous one of Johns Hopkins University (Baltimore, Maryland, USA).

***Titles of research projects***

Psychosocial and inherited factors in depression and addiction  
Examination of relationships and causal contexts of chronic stress  
(conjugal, work-related, social) and depressive, distressful  
symptoms and cardio-vascular diseases  
Possible methods for measuring and assessing health care, services  
and deliveries in Hungary  
The relationships between sleep, cognitive activity and affective  
processes  
The function and significance of the social capital in the healthcare  
system

***Supervisors***

György Bagdy  
Piroska Balog  
Éva Belicza  
Róbert Bódizs  
Péter Gaál

Development of early attachment: stress reactivity and parental care	Judit Gervai
Genetic and environmental influences on infant temperament and attachment	Judit Gervai
Relationships between health condition and personality traits among adolescents and young adults with special regard to suicidal behaviour	Ágnes Hajnal
Mental health aspects of death, dying and bereavement	Katalin Hegedűs
Behaviour redress and health psychology	Mária Kopp
Health and bioethics	József Kovács
The relationship between stress, quality of life and physical-mental health (gender and age specific perspectives).	
Stress management, health protection and the development of communicative competence	Mónika Erika Kovács
Medical anthropology: cultural and intercultural aspects of diseases and their treatment	Imre Lázár
Questions of efficiency in curative and preventive communication	Erzsébet Németh
The social and health significance of disorders in sleep and awake state	Márta Novák
Gender differences in the disorders in sleep and awake state	Márta Novák
The significance of mood and anxiety disorders in patients suffering from chronic diseases	Márta Novák
Post-traumatic stress disorder (PTSD), trauma research	Dóra Perczel-Forintos
Suicide prevention	Dóra Perczel-Forintos
Study of youth's problem behavior and mental health based on the risk and protective theory	Bettina Pikó
Science-, research-, and innovation politics and management, financing systems and their practical applications in Hungary and in European Union	Gábor Pörzse
Study of youth's problem behaviour and mental health based on the risk and protective theory	Adrienn Stauder
Eating disorders and obesity—clinical aspects and prevention	Iréna Szumska
Psychological assessment of eating disorders	Ferenc Túri

### ***Ph.D. students***

Bernadett Babusa	ft (a)
Beáta Bencsik	pt (a)
Beatrix Rafael (Bodóné)	pt
Mária Eszter Czira	ft
Szilvia Dukay-Szabó	ft
Péter Farkas	pt
Ákos Gerencsér	pt
Edmond Girasek	pt (a)
Judit Járay-Vojcek	pt
Zita Jeszenszky	pt (a)
Eszter Kovács	ft (a)
Éva Kovács	pt

### ***Supervisors***

Ferenc Túry
Adrienne Stauder
Piroska Balog
Márta Novák
Ferenc Túry
Imre Lázár
József Kovács
Péter Gaál
Imre Lázár
Mária Kopp
Bettina Pikó
Katalin Hegedűs



Csilla Zita Madarász	pt	Márta Novák
Júlia Mazzag	pt (a)	Erzsébet Németh
Ágnes Mezei	ft	Ferenc Túry
Sándor Mihály	pt	József Kovács
Katalin Fornádi (Molnárné)	pt (a)	Márta Novák
Győző Orbán	pt	Dóra Perczel- Forintos
Ágota Pap	pt	Mária Kopp
Ildikó Papp	ft	Ferenc Túry
István Péntes	pt (a)	Ferenc Túry
Piroska Sándor	ft	Róbert Bódizs
Anna Susánszky	ft (a)	Mária Kopp
Nóra Erzsébet Szabó	pt (a)	Katalin Hegedűs
Annamária Szentes	pt	Ferenc Túry
Orsolya Szent-Imrey	pt	Piroska Balog
Mónika Ditta Tóth	ft	Mária Kopp
Péter Vajer	pt	Adrienne Stauder
Márta Varga	pt	Ferenc Túry

### ***Ph. D. candidates***

Mária Eszter Czira	ft
Zoltán Cserhádi	ft
Ágnes Zsófia Kovács	ft
Krisztina László	ft
Ibolya Nikolett Nétling	pt
János Pilling	pt
Anikó Smudla	pt
Gábor Szabó	ft
Mariann Tandari-Kovács	pt
Márta Molnár (Vargáné)	pt
Rezső Zoller	ft
László Zubek	pt
Zoltán Zsinkó-Szabó	pt

### ***Ph. D. graduates***

Szilvia Ádám	pt
László Csaba Dégi	ft
Katalin Gábor	na
Zsuzsa Györfy	na
László Harmat	ft
Sándor Kalmár	na
Noémi Keresztes	ft
Barna Konkoly-Thege	ft
Sándor Lázár Alpár	pt
Tamás Martos	pt
Zsuzsanna Pluhár	na
Éva Susánszky	na

### ***Supervisors***

Márta Novák
Adrienne Stauder
Márta Novák
Mária Kopp
Erzsébet Németh
Mária Kopp
Katalin Hegedűs
Mária Kopp
Katalin Hegedűs
Márta Novák
István Mucsi
József Kovács
Imre Lázár

### ***Supervisors***

Mária Kopp
Ferenc Túry
József Kovács
Mária Kopp
Róbert Bódizs
Mária Kopp
Bettina Pikó
Mária Kopp
Róbert Bódizs
Mária Kopp
Bettina Pikó
Ágnes Hajnal

Gusztáv Stubnya	na	Gábor Pörzse
Lilla Szeifert	ft	Márta Novák
András Szentkirályi	ft	Márta Novák
Péter Torzsa	na	István Mucsi
Ágnes Zana (Tóthné)	ft	Katalin Hegedűs

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph. D. theses successfully defended in 2009 and 2010***

### **SZILVIA ÁDÁM (2009)**

#### **Work-family conflict among female and male physicians in Hungary: prevalence, stressor predictors and potential consequences on physicians' well-being**

*Supervisor: Mária Kopp*

This research explored the level, prevalence, psychosocial characteristics and antecedents of work-family conflict (WFC), as well as its relations to organizational (job satisfaction) and individual (somatic as well as psychological morbidity including burnout) strain outcomes using quantitative and qualitative techniques among physicians (N=420) in Hungary. Female physicians (N=219) reported significantly higher mean level and prevalence of WFC compared to men (N=201). The predominant form of WFC was work-to-family conflict among physicians; however, significantly more female physicians experienced family-to-work conflict and strain-based WFC than men (39% vs. 18% and 68% vs. 20%, respectively). Significantly more male physicians experienced time-based work-family conflict than women. In regression analyses, high job demands, job strain, high workload and number of children, younger age, and lack of support in the workplace predicted WFC best (adjusted R<sup>2</sup> 0.59). Content analyses of interview data (N=123) revealed significant gender differences in the provision of social (parental, spousal, peer, and organizational) support to physicians. Female physicians lacking parental, peer (i.e., access to same-sex professional role models/mentors, gender equity), or organizational support (i.e., family-friendly policies) experienced significantly higher WFC compared to appropriate control. Significantly less female physicians reported high levels of job satisfaction. Physicians reported poor somatic and psychological health and significant gender differences were identified in the prevalence of certain somatic and psychological diseases. Female physicians scored significantly higher on the emotional exhaustion subscale of the Maslach Burnout Inventory and significantly more female physicians experienced high levels of emotional exhaustion compared to male physicians. WFC emerged as a significant predictor of job dissatisfaction, and somatic as well as psychological morbidity including burnout (emotional exhaustion and depersonalization). These findings suggest that lack of social (parental, peer, and organizational) support may function as an antecedent to WFC experienced by female physicians. Furthermore, these results imply a potential path from WFC to compromised physician well-being (i.e., job dissatisfaction, poor somatic and psychological health including burnout) in

a scarcely researched population of physicians and provide further data for cross-cultural occupational stress and burnout research in a unique cultural setting with claimed centrality of the family.

- Ádám S, Györffy Z, László K (2009) *High prevalence of job dissatisfaction among female physicians: work-family conflict as a potential stressor. Clin Exp Med J 3: 453–461.*
- Ádám S, Györffy Z, Harmatta J, Túry F, Kopp M, Szényei G (2010) *A magyarországi pszichiáterek egészségi állapota. Psychiatr Hung 25: 55–61.*
- Ádám S, Salavecz G (2010) *A stressz elméleti háttere és mérése: a Selye János Lelki Egészség Program tesztbattériájának bemutatása. Mentálhigiéné és Pszichoszomatika 11: 53–80.*

## LÁSZLÓ CSABA DÉGI (2010)

### Psychosocial aspects of cancer in hospitalized adult patients

*Supervisor: Ferenc Túry*

Literature on the role of psychosocial variables in cancer process and progress is more coinciding, convincing and reliable than the literature about the impact of psychosocial factors on cancer initiation and development. However, based on recent empirical data, there are still numerous unexplained or unanswered questions in the relation, interconnection between cancer process, progress and various psychosocial aspects. Therefore, review articles underscore actuality and need for using, applying the bio–psycho–social research framework in psychooncological studies. At the same time, examination of quality of life in cancer patients has equal importance to unrevealing the bio–psycho–social etiology, risk of cancer, since it has been evidenced that quality of life is a significant prognostic factor of cancer progress and mortality, comparable with medical and treatment-related factors. Based on these theoretical perspectives we started our research on psychosocial aspects of cancer. The basic aims of our clinical study have been to explore cancer distress and cancer-related quality of life, altogether with specifics of cancer diagnosis disclosure in Romania. According to tumor location, our heterogenic, mixed sample includes 420 hospitalized adult cancer patients. Data collection was performed in four clinical settings, in the most important oncological institutions of the Transylvanian region, Romania. Results show that in our sample 16.9% of cancer patients are not aware of their oncological diagnosis, 47.5% are clinically depressed, 46.7% experience anxiety disorders and 28.1% report critically low quality of life. 85.4% of those suffering from clinical depression and 90.7% of persons with low quality of life are patients with malignant cancer diseases. 75.9% of cancer patients who report low quality of life are also facing clinically relevant depression symptoms. Moreover, almost two-thirds of cancer patients to whom cancer diagnosis was not disclosed are highly or severely depressed. Furthermore, our data demonstrate the relevant and widespread negative effects of cancer diagnosis non-disclosure, of clinical depression and of low quality of life on psychosocial functioning and vulnerability of hospitalized cancer patients. Their prevalence, on the one hand, significantly increases hopelessness, illness intrusiveness, anxiety, vital exhaustion, depression, emotion-focused coping, lacking of family support, behaviour inhibition and external locus of control and, on the other hand, decreases problem-focused coping, physical-, emotional-, social/familial- and functional

well-being, sense of coherence and self-efficacy in cancer patients. Cancer diagnosis non-disclosure, clinically significant depression and seriously decreased quality of life are more prevalent among persons with malignant tumors and among older, undereducated, widowed and socio-economically deprived cancer patients. The relevance of our clinical research should be addressed and evaluated starting from the fact that in Romania there are no previous reference data about cancer diagnosis non-disclosure, cancer distress (depression and anxiety) and cancer-related quality of life, based on multivariant statistical analyses. Also, we have no knowledge of psychooncological studies, surveys carried out on large hospitalized samples in Romania.

- Dégi LC (2009) *Non-disclosure of cancer diagnosis: an examination of personal, medical and psychosocial factors. Supportive Care in Cancer* 17: 1101–1107.
- Kállay É, Dégi LC, Vincze A (2007) *Dysfunctional attitudes, depression and quality of life in a sample of Romanian Hungarian cancer patients. J Cogn Behav Psychother* 7: 95–106.
- Dégi LC (2008) *Pszichoszociális kockázati tényezők szerepe a daganatos megbetegedésekben. Pp 557–568 in: Kopp M (szerk), Magyar lelkiállapot, 2008. Semmelweis Kiadó, Budapest.*

## KATALIN GÁBOR (2010)

### **The possibilities and significance of management training in higher education of medicine**

*Supervisor: József Kovács*

Management approach and techniques can be tools which could propel the development of Hungarian healthcare forwarded faster than the developments and inventions of medical science and technology. Having had the opportunity to teach and train several different segments of medical professionals provided me with an opportunity to focus my research on the significance and possibilities of management training. The goals of my work were: (1) Finding areas of management training which can contribute to giving skills and abilities necessary to improve the operational effectiveness of the work of medical professionals. (2) Authenticating the effect of gained knowledge and professional approach on the work of medical professionals. Our team conducted management training, and used multiple surveys to gauge the effectiveness of the course. We examined the leadership abilities of healthcare professionals, as well as the satisfaction of employees and patients, using the “Situational Leadership” management model. The results were compared between categories, as well as to statistical data describing professional efficiency. We developed an enterprise management training programme for healthcare professionals, and examined the intrinsically important entrepreneurial competence of the healthcare professionals. We conclude that the adequate shifts in leadership styles can be learned, and can increase the effectiveness of professional work, and therefore there is a need for training of this type. The propagation of the human resource management approach is also fundamentally important, since this is the prerequisite of the cost effective, responsible, motivated work. Our experience was that the key competences from an entrepreneurial perspective, such as good communication skills, motivation, conflict and change management, are absolutely essential in the healthcare work as well. In general, our students had already possessed these competences on a high level. What

most of them were completely missing were self-esteem, validation of interests, and profit-orientation. It is not sufficient to provide our students with a high level of professional knowledge. Our training must also provide them with tools and methods that can allow them to win the confidence of patients, and enables them to work effectively in cooperation with other professionals in healthcare and other adjunct fields. Based on our results and experiences, we can state that the management approach has key importance in the reorganization of the Hungarian healthcare system. The main tool of its dissemination is the medical education system which, however, cannot be efficient without working closely together with the professional and legal regulation, working out and following uniform principles. I intend to proceed with my work in this spirit.

- Gábor K, Pogány M, Dancsházy Zs, Karsai M (1998) *Human resources and quality assurance. Managem Sci* 29: 1–5.
- Gábor K, Zoltán P, Pogány M, Kárpáti Cs (2005) *Medical enterprises for a healthier population—Management knowledge in higher education and adult education with PHARE support. Nővér* 18: 13–18.
- Gábor K, Tulkán I, Helembai K, Csanadi J, Szögi S, Kántor K (2008) *At the European Union's door—A Hungarian team's experiences of participating in the Project Leonardo for better nursing care. Int J Nursing Pract* 14: 329–334.

## ZSUZSA GYÖRFFY (2009)

### Morbidity and stressor predictors among the Hungarian female physicians

*Supervisor: Mária Kopp*

There is a growing body of evidence that female physicians experience a higher degree of stress at work compared to men and that stress can lead to physical and psychological illnesses including anxiety, burnout, substance dependence or abuse. In general, health care jobs have been characterised as demanding, with high degree of responsibility, and high workload. The combination of both physical and psychosocial stressors can be perceived quite stressful. On the other hand, female physicians are expected to perform both the female (mother and/or spouse) and the professional roles. All of these stressors may cause physical and mental disorders and behavioural reactions such as smoking or drinking behaviours. The objective of our research was to study the prevalence of morbidity in a representative sample of Hungarian female physicians as well as to analyze the potential predictors of the morbidity. Data for this epidemiological study were collected from 408 female physicians using questionnaires. 818 white collar workers from a representative survey (Hungarostudy 2002) served as controls. We explored the work-family (role) conflict using qualitative techniques among female physicians. To confirm our results: 1. We found that the prevalence of chronic somatic morbidity among female physicians was significantly higher than that in the respective control groups. The female physicians have more chronic diseases, and the somatic morbidity has appeared earlier. 2. Correlation analyses confirmed a significant relationship: the chronic morbidity associated with injurious to health, sleep disorders and higher levels of work-family conflict. 3. The stressors of the physician's profession (workload, inflexible time schedules, low income, and emotional exhaustion) are associated with sleep disorders and work-family conflict. 4. According of qualitative survey, the work-family conflict was high

among female physicians. The career adjustment (i.e. change of workplace or change of specialty) made for children and short maternity leave led to reductions in perceived work.

- Győrffy Zs, Ádám Sz, Kopp M (2005) *A magyarországi orvostársadalom egészségi állapota—országos reprezentatív minta alapján. Orv Hetilap 146: 1383–1393.*
- Győrffy Zs, Mészáros E, Ádám Sz, Kopp M (2004) *Az egészségügyben dolgozó nők gyermekvállalással és terhességgel kapcsolatos mutatói országos reprezentatív minta alapján. Mentálhigiéné és Pszichoszomatika 5: 321–333.*
- László K, Győrffy Zs, Ádám Sz, Csoboth Cs, Kopp M (2008) *Work-related stress factors and menstrual pain: a nation-wide representative survey. J Psychosom Obst Gynecol 29: 133–138.*

## LÁSZLÓ HARMAT (2010)

### Investigation of the therapeutic effects of listening to music in reducing sleep problems and anxiety

Supervisor: Róbert Bódizs

Music therapy is an intervention used in several fields of medicine. Sleep has an effect on our life quality and sleeping problems contribute to several psychological and somatic diseases. Pharmacological treatment is very common in clinical sleep medicine; however, non-pharmacological treatments are also useful in curing sleeping problems. The results of the first study supported our hypothesis that listening to slow classical music at bedtime improves sleep quality. Music had a therapeutic effect. In the second study, I measured the acute physiological effects of listening to music which includes heart rate (HR) and heart rate variability (HRV), muscle tension (EMG), and respiratory rate. The results did not support my previous hypothesis that listening to music for ten minutes would decrease the physiological arousal; on the contrary, muscle tension and perceived anxiety were increased in the music group. I did not find significant changes in any of the other physiological outcome. The third study was conducted according to a very similar procedure to the first one. There were differences only in the participating subjects and the time. I used actigraphy to make an objective measurement to support our previous findings about therapeutic effects of listening to classical music on sleep quality. I did not find significant differences in the data from the actigraph neither between the groups nor within the groups. There was no therapeutic effect of listening to music and audio-books. I have concluded that either the results of the study with physiological outcomes are not consistent with those of psychological outcomes or listening to slow classical music may improve sleep quality in subjects with sleep problems, but this effect seems not to be mediated by reduced anxiety, lowered physiological arousal, neither it is reflected in a lower number of movements during sleep.

- Harmat L (2008) *A zene terápiás hatásainak alkalmazása az alvászavarok kezelésében. Lege Artis Medicinae 18: 424–425.*
- Harmat L, Takács J, Bódizs R (2008) *Music improves sleep quality in students. J Adv Nurs 62: 327–335.*
- Harmat L (2007) *Alvászavarok kezelése zenével. Komplex Med 11: 47–50.*



**SÁNDOR KALMÁR (2009)****The consequences of the self-destructive behavior with special consideration of the suicide and its prevention opportunities. Bács-Kiskun County 1995–2006**

*Supervisor: Mária Kopp*

One of the purposes of the study was to investigate the mortality of the result of the self-devastating behavior in Bács-Kiskun County from 1995 to 2006 especially suicide which has a lot of deep complicated, ramified psychic, psychiatric, biological, historical, social and cultural roots. From 1995 to 2006 the number of the population was decreasing. In 2005 the mortality was almost the highest in Europe and it was 67.15% more than in Austria. The very high mortality rate and the suicide rate have caused serious public health problems. The most serious ones are the 9.6% increasing mortality of the malignant neoplasm of trachea, bronchus and lung, the 31.9% increasing mortality of the diseases of the respiratory system and the 16.8% increasing mortality of cerebrovascular diseases. Suicide has demanded 2144 male and 614 female together 2758 victims during this time. The depression is the most essential risk factor for committing suicide. The main purpose was to investigate the effectiveness and practical experiences of the Depression Recognition and Suicide Prevention Programs in the region of Kiskunhalas and work out new, complex, efficient mortality and suicide preventive programs. In this region the annual suicide rate decreased from the 5-year-pre-intervention average of 59.7/100.000 to 49.9/100.000. The decrease was similar to the control region (Kiskunfélegyháza), but it was higher than anywhere else in the county and Hungary ( $p < 0.0003$  and  $p < 0.001$ , respectively). In rural areas, the intervention region for female suicide rate decreased by 34% and increased by 90% in the control region ( $p < 0.066$ ). The increase in antidepressant treatment was greater in the region of Kiskunhalas compared with the control region, Kiskunfélegyháza, the county, and Hungary and in females compared with males ( $p < 0.002$ ). The study underlines the importance of the depression recognition and suicide prevention programs for the General Practitioners and their nurses. Finally, the author works out several complex suggestions not only for the prevention of the high mortality but for the suicide prevention as well.

- Kalmár S, Szanto K, Rihmer Z, Sati Mazumdar, Harrison K, Mann JJ (2011) *Antidepressant prescription and suicide rates: effect of age and gender. Suicide and Life-Threatening Behaviour* 38: 363–374.
- Kalmár S (2007) *Kiskunhalason és környékén történő öngyilkosság megelőzési próbálkozások. Pp 130–141 in: Csépe A. Összefogás a depresszió ellen. Semmelweis Kiadó, Budapest.*
- Szántó K, Kalmár S, Hendin H, Rihmer Z, Mann JJ (2007) *A suicide prevention program in a region with a very high suicide rate. Arc Gen Psychiatry* 64: 914–920.

## NOÉMI KERESZTES (2009)

### Behavioral science study of youth's leisure time physical activity

Supervisor: Bettina Pikó

Sociodemographic inequalities in leisure time sports activity have been well documented. In addition to similar results from previous studies I found in my study, further scientific relationships were also proved. Gender differences in elementary school children's sports activity was not significant, in contrast to the results of high school students. The importance of socio-economic status was also proved in my examination, moreover analyzing regular sports activity we have found a "J"-formed connection; that is, persons from the highest social class reported the highest sports activity, but lower class students showed a higher activity level than lower-middle class pupils. While family structure had an influence on health risk behaviours, I did not find any significant relationships to leisure time sports activity. Findings verified the model of "good pupil, good sport-man". Results also proved that regularly active students reported better self-perceived health and fitness, better satisfaction with life, less depressive symptoms, in a word, a better quality of life. It was also justified the role of the importance of life goals in sports activity status, that is, students who were regularly active preferred less extrinsic values as life goals for their future while these goals were very important for inactive students. In my study, it was also realized that social influences might help develop preventive health behaviors. The role of these social influences, however, showed a different picture among subsamples of girls and boys. Our results suggest that during the years of early adolescence, girls' sports participation is more influenced by social variables from their peers as compared to boys. Results supported that leisure time sports activity, similar to health risk behaviors, had clear social images associated with positive attitudes. Findings indicated that prototype perceptions were influenced by social behaviors (such as, social comparison, competitiveness, LDM). The role of these effects depends on sports activity status.

- Keresztes N, Pikó B, Pluhar Zs, Page RM (2008) *Social influences in leisure time sport activity among early adolescents. J Royal Soc Promot Health* 128: 21–25.
- Keresztes N, Pikó B, Gibbons FX, Spielberger CD (2009) *Do high and low active adolescents have different prototypes of physically active peers? Psychol Rec* 59: 39–52.
- Pikó B, Keresztes N (2006) *Physical activity, psychosocial health and life goals among youth. J Commun Health* 31: 136–145.

**BARNA KONKOLY-THEGE (2009)****A health psychological aspect of logotherapy and existential analysis:  
the relationship between meaning in life and smoking***Supervisor: Mária Kopp*

Smoking is one of the biggest public health threats the world has ever faced. According to the World Health Organization, approximately 100 million deaths were caused by smoking in the past century, and if the current trends will continue up to a billion people will die in the present century as a result of their tobacco use. Therefore, an important task of health psychology is to better understand the psychosocial factors contributing to the initiation, maintenance, and cessation of smoking. Decreased level of meaning in life—as defined in Viktor Frankl's logotherapy and existential analysis—has already proved to be connected to the misuse of alcohol and illegal substances. Besides providing a review on the theoretical basis of logotherapy and the relevant previous empirical research, the aim of the present dissertation was to examine whether the level of personal meaning in life is related to cigarette use as well. Our study analyses the results of four empirical investigations being conducted at different time points (namely 2002, 2004, and 2006), on different samples (N1=171; N2=392; N3=12.668; N4=4.307) and with the utilization of different life meaning measures (the original and a revised version of the Logo-Test, the Purpose in Life Test, and the Life Meaning Subscale from the Brief Stress and Coping Inventory). According to logotherapy and existential analysis, we hypothesized that a weaker sense of meaning in life would be associated with a higher likelihood of (more intensive) nicotine use. The most important results of our investigations are as follows: (1) People experiencing less meaning in their lives are more likely to be smokers than those having a stronger sense of meaning in life—regardless of gender. (2) According to the results of our cross-lagged longitudinal study, level of meaning in life seems to directly influence smoking status among women, while in men, smoking status proved to be a determinant of life meaning. (3) Concerning smoking intensity, our results were ambiguous; however, female smokers feeling less meaning in their life seemed to use more cigarettes when compared to those smoking women having a stronger sense of existential meaning. Finally, strengths and weaknesses of the investigations, possible directions of future research and the practical applicability of the present findings were discussed in details.

- Konkoly Thege B, Martos T (2008) *Reliability and validity of the Shortened Hungarian Version of the Existence Scale. Existenzanalyse* 25: 70–74.
- Konkoly Thege B, Bachner YG, Kushnir T, Kopp M (2009) *Relationship between meaning in life and smoking status: results of a national representative survey. Addict Behav* 34: 117–120.
- Konkoly Thege B, Stauder A, Kopp M (2010) *Relationship between meaning in life and intensity of smoking. Do gender differences exist? Psychol Health* 25: 589–599.

## SÁNDOR LÁZÁR ALPÁR (2010)

### Sleep physiological correlates of autism spectrum disorders

Supervisor: Róbert Bódizs

Autism spectrum disorder (ASD) exhibits a significantly altered brain development pattern compared to the typically developing subjects, characterized by a region specific bias in brain connectivity and/or by a delay in brain maturation. NREM dependent brain oscillatory activity (EEG) is considered a sensitive indicator of individual differences in the cortico-cortical and thalamo-cortical neural connectivity as well as brain maturation. The aim of the present study was to investigate whether sleep macro- and microstructure based on an the accurate quantitative analyses of the NREM dependent EEG power spectral density in a large frequency band (0.5–45 Hz), and sleep spindle activity and phase coherence are different in Asperger syndrome (AS) compared to typically developing children and adolescents. Standard all night EEG sleep parameters were obtained from 18 un-medicated subjects with AS and 14 controls (age range: 7.5–21.5 years) after one adaptation night. Sleep latency and wake after sleep onset were increased in AS. The AS group exhibited a significant increase in the relative delta (0.5–4 Hz) band and significant decrease in the absolute power sepectrum density (PSD) of the higher frequency ranges (8–45 Hz) and in all 10 EEG derivations. Relative PSD showed a significant increase in delta and a decrease in the sigma band for frontal, and in beta for centro-temporal derivations. Sleep spindles detected on the basis of individually adjusted frequency and amplitude criteria exhibited significantly lower average amplitude and longer length compared to the control group. This difference was more emphasized over the frontal region and with respect to slow sleep spindles. Intrahemispheric coherence measures were markedly lower in AS in the frontal areas and the right hemisphere over all EEG channels. The most prominent reduction in intrahemispheric coherence was observed over the fronto-central areas in delta, theta, alpha and sigma EEG frequency bands. The present study provides the most exhausting analyses of the NREM sleep dependent EEG activity in subjects with autism spectrum disorders. Our results support the decreased brain connecivity and the frontal dysfunction hypotheses of autism.

- Lazar AS, Lazar ZI, Biro A, Gyori M, Tarnok Z, Keszei A, Stefanik K, Prekop C, Gadoros J, Halasz P, Bodizs R (2010) *Reduced fronto-cortical brain connectivity during NREM sleep in Asperger syndrome: an EEG spectral and phase coherence study. Clin Neurophysiol* 121: 1844–1854.
- Bodizs R, Kormendi J, Rigo P, Lazar AS (2009) *The individual adjustment method of sleep spindle analysis: methodological improvements, roots in the fingerprint paradigm. J Neurosci Methods* 178: 205–213.
- Lázár AS, Bódizs R (2008) *Az alvás szerkezete és mintázatai autizmus spektrumzavarban. Psychiatr Hung* 23: 109–128.

**TAMÁS MARTOS (2010)****Life goals and mental health in the Hungarian society***Supervisor: Mária Kopp*

Life goals (aspirations) represent the dynamic, future oriented side of human functioning. At the same time, they may be interpreted as personalized responses to the environmental and cultural influences. In the theses I presented the development and assessment of a shortened version of Aspiration Index. Short Aspiration Index was found a reliable and valid measure to assess the importance of extrinsic (materialistic) life goals, e.g. wealth and good appearance, the importance of intrinsic life goals, e.g. personal growth and relationships, as well as the importance of health related life goals. The data from three cross-sectional samples, among them the Hungarostudy Epidemiological Panel 2006 representative survey, was analyzed. Intrinsic aspirations and especially health aspirations were found more important than the extrinsic aspirations in every major social group of the Hungarian society. However, individual importance of life goals is not independent from sociodemographic characteristics and subjective health status. The importance of goal striving is predicted positively by higher education, higher subjective financial status, importance of religion and better self-rated health predict positively, while it is predicted negatively by age. Concerning the indices of mental health and well-being, the importance of intrinsic aspirations proved to be a positive predictor for all of them. In contrast, the importance of extrinsic aspirations predicted negatively the meaning of life, lower hostility and anomie. However, it was in positive association also with the better actual mood. These associations were also proved under the following conditions: (1) after controlling for socio-demographic background variables and self-rated health (2) after further controlling for the interaction between life goals and objective and subjective financial status. Results indicate that the support for intrinsic goal striving may contribute to the protection and maintenance of mental health and well-being, both on individual and on societal level. Moreover, it may be a vehicle of empowerment for every strata of the Hungarian society.

- Martos T (2008) *Életcélok és esélyteremtés*. Pp 47–58 in: Kopp M (szerk), *Magyar lelkiállapot 2008. Esélyerősítés és életminőség a mai magyar társadalomban*. Semmelweis Kiadó, Budapest.
- Martos T, Konkoly Thege B, Kopp M (2010) *Health aspirations in the context of age and self-rated health. Findings from a representative Hungarian sample*. *J Health Psychol* 15: 269–278.
- Martos T, Szabó G, Rózsa S (2006) *Az Aspirációs Index rövidített változatának pszichometriai jellemzői hazai mintán*. *Mentálhigiéné és Pszichoszomatika* 7: 171–192.

**ZSUZSANNA PLUHÁR (2010)****Importance of place and environment in health consciousness and image of disease of children who attend to WN Schools***Supervisor: Bettina Piko*

The environment plays a significant role in forming children's idea of disease. On the one hand, the surrounding social environment influences children's idea of disease; on the other hand, they consider most diseases, which they have suffered from, to be of environmental origin. Thus, we evaluated the relationship between the environment and the idea of disease of 9 to 11-year-old children in primary schools in four towns near each other and two primary schools in the capital. We could contribute to a better understanding of children's idea of disease and their ability to realize environmental risks. This is important, because adequate realization of environmental hazards contributes to prevent and avoid diseases in the future. Children's realization of environmental risks is influenced by the surrounding micro environment, location of their school within the town and also the economic and geographic features of the town. It has been observed that children follow expectations when specifying hazards and they interpret "hazards" as abstractions based on what they have heard and learnt, while they rely on their experiences when specifying diseases. Children, who live in different regions, realize risk in a similar way and their knowledge of diseases caused by the external environment is also mostly similar. The answers are a little diverse only in small settlements, the reason of which is the fact that people have a different life-style in such settlements. It can be stated that children who live in different regions think about isolation (lack of open-air) and its consequences rather differently. Most diverse are the answers of pupils who live in small towns. They significantly associate isolation with lack of activity in their written answers and represent it with an ill person and visible physical signs in their drawings. Children think of isolation differently according to age. Younger children think that isolation rather means decreased activity, while older children associate isolation most commonly with laziness. As it is already known, there is a close linkage between the environment and outdoor physical activities. The results of our survey have confirmed that children prefer being in an environment in which they can be active and play, and they adapt to the facilities of their environment and residence depending on the sports they choose.

- Pluhár FZ, Piko BF, Kovacs S, Uzzoli A (2008) "Air pollution is bad for my health": Hungarian children's knowledge of the role of environment in health and disease. *Health and Place* 15: 239–246.
- Pluhár FZ, Kovács S, Piko B, Uzzoli A (2008) "What do you think, what happen people, who never go out to open air?" 9–11-year-old children's concepts of the health effects of staying indoor. *Lege Artis Medicina* 1: 64–69.
- Piko BF, Keresztes N, Pluhár ZF (2006) Aggressive behavior and psychosocial health among children. *Personality and Individual Differences* 40: 885–895.



**ÉVA SUSÁNSZKY (2009)****Developments in the quality of life of young Hungarians in the last two decades**

*Supervisor: Ágnes Hajnal*

The study of the quality of life and social adaptation of the young people belongs to the future-oriented branch of the applied health sociology. The characteristics of the conditions and behaviours during the stage of growing into adulthood anticipate and forecast health problems the generations studied here may face in their future life periods. In the present study, based on the databases of the population health studies called Hungarostudies, I analyzed the changes that had occurred in the young generations' life plans, world views, expectations from and attitudes toward their broader and closer environment, coping strategies, and their health related quality of life during the past 20 years in Hungary. On the other hand, I attempted to verify a theoretical model of the post-adolescence by the means of an empirical analysis. The first data collection was carried out in 1988, the year preceding the political transition in Hungary; other surveys were carried out every seven year afterwards (1995, 2002). In the cross-sectional surveys 14,000 young people aged 18–31 represented the total Hungarian population of the same age. The study results are based on the self-report of the young adult generations measured by internationally applied test devices. The results of the 1995 study that followed the political transition in Hungary reflect the economic and value instability as well as disappointment that characterized the first phase of the new capitalism. Similarly to the older generations, the prevalence of depressive symptomology had also increased among the young adults; especially dissatisfaction showed a striking frequency. Young males found their life situation particularly depressing. There was a gradual change in the coping strategies of the young Hungarian adults; their coping potential was increasing, new techniques of adaptation came into use, and young women's coping abilities significantly improved. Cynicism, envy, and hostility in general were decreasing while trust in the family was improving. Expectations towards performance and perfection loosened; on the other hand, the ethical judgment of the society degraded and the young people became more and more withdrawing. They evaluate themselves less and less reliable. The condition of post-adolescence can be detected even among people around their thirties. Independence is significantly influenced by the social origin and the family ties.

- Susánszky É, Szántó Zs, Kopp M (2007) *A dohányzásról való leszokás motivációs hátterének vizsgálata. Lege Artis Medicinae* 17: 616–623.
- Szanto Zs, Susanszky E, Kopp M (2005) *Relationships between unfavourable health status and smoking cessation attempts in Hungary. Soc Prev Med* 50: 324–333.
- Adam S, Györffy Z, Susánszky É (2008) *Physician burnout in Hungary. A potential role for work-family conflict. J Health Psychol* 13: 847–856.

**GUSZTÁV STUBNYA (2010)****Consolidation of Semmelweis University using innovative management tools 2003–2009***Supervisor: Gábor Pörzse*

In this thesis, working on the basis of my research and own experiences, I set out those sets of market tools and management techniques which, with appropriate adaptation, may also be applied at the level of public institutions supervised and owned by the state. Working from these foundations, I define a vision of the future that lies open for one of the leading medical universities in the European Union as well as the situation and tasks of the medical university departments offering a progressive healthcare service and positioned at the pinnacle of the provision hierarchy in the current Hungarian healthcare system. I review the external health policy and financing environment necessary for a full understanding of the above activities, the Hungarian situation of progressive provision and the domestic relations of specialist nursing fundamentally required for conducting healthcare activities. The principal management measures implemented with my personal involvement at Semmelweis University in the period between 2003 and 2009 are presented and analysed and I discuss the strategic steps taken for the expansion of the university. Through the use of in-house questionnaires and media analysis I map out the impacts of measures. I summarize the experiences of management practice acquired through innovative management means, processed during the analysis, and from these I formulate statements that can be generalized and put into practice at organizations struggling with these and similar problems. On the basis of my clinical investigations I establish that the processes of biological systems and their anomalies, as well as their answers given to external interventions, can be relevant through the analysis of processes of social organizations of a similar type. Furthermore, I justify how, and with what modifications, the management tools used in the typical market environment can be applied in universities of the 21st century operating in a university environment in which the role of the state is diminishing, and which function within budgetary frameworks in non-profit forms amidst the weaknesses of the economic environment. In this thesis I prove that, even in a recessionary external environment, sustainable development is possible using these innovative management means. Through management means analysis I underpin the assertion that strategy and consolidation, as well as strategy and operational development are not merely mutually tolerant managerial concepts but rather their existence in close alliance is the only pledge of the medium- and long-term success of an organization.

- Stubnya G, Jákó K (2009) *Progresszív ellátás Magyarországon. Orvoseképzés* 4: 213–222.
- Stubnya G (2009) *Beavatkozási lehetőségek a változó külső környezetben a Semmelweis Egyetemen. IME: Informatika és menedzsment az egészségügyben* 8(1): 17–19.
- Stubnya G, Balogh Z, Csetneki J (2009) *Zur beruflichen Situation von Pflegefachkräften und Geburtshelfern in Ungarn. PflegeRecht* 9: 428–434.

**LILLA SZEIFERT (2010)****Depression and quality of life in patients with chronic kidney disease***Supervisor: Márta Novák*

Depression is one of the most common psychiatric conditions in patients with end stage renal disease. It is associated with worsened quality of life, increased morbidity and mortality, and non-compliance in patients on chronic dialysis. In our cross-sectional study, self-reported depressive symptoms were significantly less prevalent in kidney transplanted than in waitlisted dialysis patients. In a prospective cohort study, the presence and severity of self-reported depressive symptoms were associated with mortality and graft failure in patients after kidney transplantation. New information about the association between depressive symptoms and negative outcomes can further improve our understanding of the psychosomatic context of chronic kidney disease and it also may point to potentially treatable factors associated with clinical outcome. Renal transplantation is associated with better survival, improved quality of life and lower costs compared to chronic dialysis. Numerous studies have repeatedly demonstrated that inequalities appear to exist in access to transplantation for different subgroups and several barriers of the transplantation process have been identified. However, to date little attention has been focused upon the relationship of self-reported measures of subjective well-being with modulating access to transplantation. We determined that worse self-reported quality of life and more severe self-reported depressive symptoms are associated with lower odds of being wait-listed. However, severity of depressive symptoms and quality of life were not associated with receiving a kidney transplant during the follow-up period. The present results confirm previous findings indicating that certain patient groups are disadvantaged regarding waitlisting.

- Szeifert L, Molnár MZs, Ambrus Cs, Kóczy ÁB, Kovács ÁZs, Vámos EP, Keszei A, Mucsi I, Novák M (2010) Symptoms of depression in kidney transplant recipients: a cross-sectional study. *Am J Kidney Dis* 55: 132–140.
- Novak M, Molnar MZ, Szeifert L, Kovacs AZ, Vamos EP, Zoller R, Keszei A, Mucsi I (2010) Depressive symptoms and mortality in patients after kidney transplantation: a prospective prevalent cohort study. *Psychosom Med* 72: 527–534.
- Molnar MZ, Czira M, Ambrus C, Szeifert L, Szentkirályi A, Beko G, Rosivall L, Remport A, Novak M, Mucsi M (2007) Anemia is associated with mortality in kidney transplanted patients—a prospective cohort study. *Am J Transplant* 7: 818–824.

**ANDRÁS SZENTKIRÁLYI (2010)****The significance of restless legs syndrome among patients with chronic kidney disease***Supervisor: Márta Novák*

Restless legs syndrome (RLS) is associated with unpleasant sensations in the lower extremities accompanied by a strong urge to move the legs. Sleep disorders are very common in chronic kidney disease, and the prevalence of RLS is 15–20% among dialysed

patients, which is several times more frequent than in the general population. RLS is strongly associated with insomnia, which is the most prevalent sleep disorder in the dialysed population. There are some data that RLS may be associated with depression among dialysed patients and it is a significant predictor of impaired quality of life. Finally, RLS was an independent risk factor of mortality in patients on hemodialysis. There is scarcely any data from kidney transplanted (Tx) patients regarding sleep- and mood disorders.

The following hypotheses were tested:

- (1) the prevalence of insomnia is significantly lower in the Tx group than in the wait-listed dialysed (WL) group and RLS is a significant and independent predictor of the presence of insomnia;
- (2) RLS is significantly and independently associated with worse quality of life regardless of the severity of insomnia in Tx patients;
- (3) patients with RLS show more severe depressive symptoms than patients without RLS independently of the presence of insomnia;
- (4) the presence of RLS is a significant and independent risk factor of mortality in Tx patients.

In the TransQOL-HU (Transplantation and Quality of Life-Hungary Study) cross-sectional study 1067 Tx and 214 WL patients were enrolled. RLS, insomnia, quality of life and emotional distress were assessed with standard questionnaires. Tx patients were followed for four years thereafter and mortality data were collected. Socio-demographic data and the number of co-morbidities were reported by patients at enrollment. Anamnestic data on dialysis and transplantation, medication and labor parameters were collected from patients' charts. The mean age of the total sample (WL and Tx groups combined) was  $48 \pm 13$  years and 60% were male. RLS prevalence was significantly lower in the Tx group than in the WL group (Tx: 5% vs WL: 11%;  $p=0.001$ ). The prevalence of insomnia was 15% in the WL group in contrast to 8% in the Tx group ( $p<0.01$ ). In multivariate model the presence of RLS showed a significant and independent relationship with more severe insomnia (OR=1.4, 95% CI: 1.07–1.83,  $p<0.05$ ). RLS was independently and significantly associated with lower scores in each quality of life domain, which remained significant after the correction for insomnia in two domains. These were the "role physical" (OR=6.67, 95%CI: 2.94–16.67,  $p<0.001$ ) and "bodily pain" subscales (OR=2.13, 95%CI: 1.08–4.17,  $p<0.05$ ). In multivariate logistic regression model RLS was independently and significantly associated with depression even after adjustment for insomnia (OR=2.97, 95%CI: 1.59–5.58,  $p<0.001$ ). The presence of RLS was an independent, significant predictor of mortality as suggested by multivariate Cox proportional hazard model (HR=2, 95%CI: 1–3.9,  $p<0.05$ ) in Tx patients. RLS was independently and significantly associated with insomnia, depression, impaired quality of life and mortality in Tx patients. Further prospective studies are warranted to see if the therapy of RLS could improve these patients' sleep- and mood disorders, quality of life and survival.

- Szentkiralyi A, Molnar MZ, Czira ME, Deak G, Lindner AV, Szeifert L, Torzsa P, Vamos EP, Zoller R, Mucsi I, Novak M (2009) Association between restless legs syndrome and depression in patients with chronic kidney disease. *J Psychosom Res* 67: 173–180.
- Molnar MZ, Szentkiralyi A, Lindner A, Czira ME, Szeifert L, Kovacs AZ, Fornadi K, Szabo A, Rosivall L, Mucsi I, Novak M (2007) Restless legs syndrome and mortality in kidney transplant recipients. *Am J Kidney Dis* 50: 813–820.
- Molnar MZ, Novak M, Szeifert L, Ambrus C, Keszei A, Koczy A, Lindner A, Barotfi S, Szentkiralyi A, Rempert A, Mucsi I (2007) Restless legs syndrome, insomnia and quality of life after renal transplantation. *J Psychosom Res* 63: 531–597.

**PÉTER TORZSA (2009)**

**Epidemiology and significance of obstructive sleep apnoea and snoring in family practice**

*Supervisor: István Mucsi*

In our study we evaluated the sociodemographic characteristics and health behaviour of people with different types of snoring (such as loud snoring with breathing pauses and quiet snoring) in a representative sample of the Hungarian general population (Hungarostudy 2002). We investigated the impact of snoring on different aspects of quality of life and the association of snoring with accidents. We found that snoring is common in the Hungarian general population. The prevalence of loud snoring with apnoea and quiet snoring is 37% and 23% in men and 21% and 21% in women, respectively. Snoring is associated with lower education and worse financial status in the general Hungarian population. Snoring, particularly loud snoring with apnoea is strongly correlated with high-risk health behaviour. There is a significant increasing trend between the prevalence of smoking, heavy drinking and coffee consumption in non-snorers, quiet snorers and loud snorers. Male gender, smoking, presence of comorbidities and heavy drinking are independent predictors of snoring. Snoring is associated with increased daytime sleepiness and increased prevalence of accidents. Snoring, particularly loud snoring is frequently associated with an increased occurrence of depressive symptoms and worse overall health status. The prevalence of patients grading their own health status as bad was the highest among loud snorers; these patients presented with the highest level of vital exhaustion. These findings indicate a significant impairment of the quality of life. Our literature review made it clear that family physicians often do not recognize OSAS in spite of its high prevalence and clinical significance. To assess of family physicians' knowledge about and attitude to OSAS we used the OSAKA questionnaire (Obstructive Sleep Apnoea Knowledge and Attitudes) among family physicians and residents. In our study we found that Hungarian family physicians, particularly male physicians have a lack of knowledge about sleep apnoea. It was interesting that there was an inverse correlation between physicians' BMI and age versus knowledge. Knowledge about sleep apnoea among physicians working in rural practices is lower than that of those working in the capital. Knowledge scores of sleep apnoea and the number of special diplomas correlated significantly. Our result also demonstrated that the OSAKA questionnaire is suitable for the follow-up of the efficacy of the educational intervention in interdisciplinary sleep medicine. OSAS is present in 60–80% of patients with therapy-resistant hypertension. HBPM (Home Blood Pressure Monitoring) is an efficient tool for family physicians when they screen their patients for the presence of OSAS. Our study yielded that Hungarian family physicians know and use HBPM in their practice. Family physicians follow the guidelines of European Hypertension Society on the use of HBPM.

- Torzsa P, Rihmer Z, Gonda X, Szókontor N, Sebestyén B, Kalabay L (2009) Family history of suicide: a clinical marker for major depression in primary care practice? *J Affect Disord* 117: 202–204.
- Torzsa P, Novák M, Mucsi I, Ádám Á, Kalabay L (2008) A családorvos szerepe az obstruktív alvási apnoe kiszűrésében, felismerésében. *Orv Hetil* 48: 2283–2290.

- Tislér A, Dunai A, Keszei A, Fekete B, El Hadj Othmane, T, Torzsa P, Logan AG (2006) *Primary care physicians' views about the use of home/self blood pressure monitoring: nation-wide survey in Hungary. J Hypertens 24: 1729–1735.*

## ÁGNES ZANA (TÓTHNÉ) (2009)

### **The development and change of death image in Hungary. Differences in value judgement according to age and analysis of possible measurement methods. Is death still a taboo?**

*Supervisor: Katalin Hegedűs*

The aim of our research is to examine the sociological, anthropological, and psychological aspects of attitudes towards death in the Hungarian population, respectively reviewing the various approaches as a complex system. Presenting the altered death image, respectively the change tendency, analysing and interpreting the most significant anxiety generating factors according to gender, age, and occupation. For the psychometric measurement of fear of death and attitudes toward death we validated, adapted and calibrated two scales measuring fear of death and attitudes towards death. According to our findings both the Neimeyer and Moore Multidimensional Fear of Death Scale and the Lester Attitude Toward Death Scale proved valid and suitable for measuring fear of death and attitudes towards death. The scales were calibrated by using the shortened version of Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI). Among the factors measured by MFODS Fear for significant others was the most significant factor indicating fear in all groups examined. The Fear of the dying process and Fear of the dead proved to be the other two most important factors. According to age groups and gender, youth and women scored highest on fear of death. According to age groups, youth scored highest on fear of death. There is a negative correlation between fear of death and age, the level of fear of death decreasing with advanced age. Significant difference ( $p < 0.000$ ) was found between the age groups examined. Regarding gender we found further significant differences among the MFODS factors Fear for significant others, Fear of the dying process, Fear of the dead, Fear of conscious death, and Fear for the body after death, MFODS aggregate scores of fear of death and state and trait anxiety. Our hypothesis, namely that fear of death among health care workers is higher as the normal population, was not confirmed. Yet, contrary to a segment of preceding measurements, lower level of fear, respectively anxiety was found.

- Zana Á, Hegedűs K, Szabó G (2005) *Validation of the multidimensional fear of death scale on Hungarian population. Mentálhigiéné és Pszichoszomatika 7: 257–265.*
- Zana Á, Hegedűs K, Szabó G (2008) *Changes of rituals and their effects in social connections in the Hungarian Society. Death image of the Hungarian population. Pp 382–389 in: Kopp M (szerk), Magyar lelkiállapot 2008. Esélyteremtés és esélyegyenlőség. Semmelweis Kiadó, Budapest.*
- Hegedűs K, Zana Á, Szabó G (2008) *Effect of end of life education on medical students' and health care workers' death attitude. Palliat Med 22: 264–269.*



**PROGRAM 4/3.****GROUP OF RESEARCH TOPICS, MENTAL HEALTH SCIENCES****Coordinator:****Teodóra TOMCSÁNYI M.A., C.Sc.**

Institute of Mental Health

4 Nagyváradi sq, Budapest H-1089

Tel.: +36 1 266 0878; fax: +36 1 214 5685

E-mail: [tomcsanyi.teodora@t-online.hu](mailto:tomcsanyi.teodora@t-online.hu)Web: [www.mental.usn.hu](http://www.mental.usn.hu)**Titles of research projects**

Social challenges and responses, past and present. Social context and health care

Analysis of the supportive and developmental effects of family and community

Methodology of measuring mental health characteristics; effectivity testing of educational programs and curricula

The impact of postmodernity on community models of leadership training

Social and religious theories in the dimensions of normal and pathological phenomena

Lifelong learning and its worldview-related aspects

Multidisciplinary approaches to mental health and illnesses

Factors of mental health and their consequences for satisfaction and behaviour

New religious, youth, and self-help community movements

Social and mental health aspects of preventive health protection

Value, action research, human/organizational resources development

**Supervisors**

Roger Csáky-Pallavicini †

Katalin Horváth-Szabó

András Ittész

Gábor Ittész

Endre Nagy

Dávid Németh

Teodóra Tomcsányi

Miklós Tomka †

Péter Török

Szabolcs Török

Károly Varga

**Ph.D. students**

Júlia Eszter Andrási-Stahl

pt

Apollónia Noémi Kulcsár

pt (a)

Zsuzsanna Ládonyi

pt

Rita Schiffer (Nagyné)

pt (a)

Attila Pilinszki

pt

Eszter Sabadel

ft

Tímea Tésenyi (Somosiné)

pt

Gábor Török

pt (a)

Tünde Szabó

pt

**Supervisors**

Gábor Ittész

Teodóra Tomcsányi

Endre Nagy

Károly Varga

Miklós Tomka †

András Ittész

Péter Török

Dávid Németh

Károly Varga

**Ph.D. graduate**

Anikó Kézdy

pt

**Supervisors**

Katalin Horváth-

Szabó, Péter Török

## ***Abstract of Ph.D. thesis successfully defended in 2010***

**ANIKÓ KÉZDY (2010)**

### **Developmental crises in late adolescence and young adulthood. Characteristics of religiosity and its associations with mental health in a high school and university student sample**

*Supervisor: Katalin Horváth-Szabó*

Growing attention has been given to personality characteristics that enable the individual to attain psychological balance and well-being. These approaches can be especially important when working with adolescents and young adults, since the vulnerable period of identity formation and preparation for social roles presents young people with great challenges. The present research studied the relationship between religiosity and mental health in a high school and a university student sample: the associations between religiosity, coping strategies and mental health, as well as the moderating role of religious attitudes on the relationship between religious doubts and mental health were explored. The results showed a significant correlation between the strength of religious faith and emotional-adaptive coping in both samples. According to the statistical analysis, coping strategies played a mediating role in the relationship between religiosity and mental health. This result was shown in both age groups, but, supposedly due to the changes in religiosity, different aspects of religiosity, namely frequency of church attendance and inclusion of transcendence in high school, and symbolic interpretation of religious contents in university students, contributed to mental health through the coping strategies. The results confirmed the positive relationship between religious doubts and depression/anxiety. Exploration of the role of religious attitudes showed (1) that the positive relationship between religious doubts and negative mental health is stronger at higher levels of inclusion of transcendence, and (2) that symbolic interpretation of religious contents further strengthens this relationship. The religious doubts–negative mental health relationship was strongest when both inclusion of transcendence and symbolic interpretation were high. This interaction pattern was the same in both samples. Understanding these aspects of adolescent and young adult religiosity may contribute to developing more effective psychological prevention and intervention methods.

- Kézdy A, Martos T, Urbán Sz, Horváth-Szabó K (2010) *A vallásos attitűd, a megküzdés és a lelki egészség összefüggései: azonosságok és különbségek serdülő- és fiatal felnőttkorban. Mentálhigiéné és Pszichoszomatika 11: 1–16.*
- Kézdy A, Martos T, Boland V, Horváth-Szabó K (2011) *Religious doubts and mental health in adolescence and young adulthood: the association with religious attitudes. J Adolesc 34: 39–47.*
- Kovács B, Kézdy A (2008) *Religious belief and burnout. Eur J Ment Health 3: 253–265.*

---

# SCHOOL OF PH.D. STUDIES

---

## 5. SPORT SCIENCES

---

***Chairman:***

**Zsolt RADÁK Ph.D., D.Sc.**

Faculty of Physical Education and Sport Sciences

Research Institute of Sport Sciences

44 Alkotás st, Budapest H-1123

Tel.: +36 1 487 9216, +36 1 487 9218

E-mail: [radak@mail.hupe.hu](mailto:radak@mail.hupe.hu)

### General overview

Doctoral courses embrace the whole field of sports science. Naturally, the specific topics reflect the orientation of the tutors, and respectively, the technical facilities of the laboratories. It is the Faculty of Physical Education and Sport Science that manages the doctoral courses, but other institutions, above all, the National Institute for Sports Medicine, extend the facilities available in the institutes and chairs, both in regard to educational staff and technical equipment. The other faculties and institutes of Semmelweis University are considered the basis of further development in this respect.

### ***PROGRAM 5/1.***

---

### TRAINING AND ADAPTATION

***Coordinator:***

**Gábor PAVLIK M.D., Ph.D., D.Sc.**

Institute of Kinesiology and Sport Medicine

44 Alkotás st, Budapest H-1123

Tel.: +36 1 487 9285

E-mail: [pavlik@mail.hupe.hu](mailto:pavlik@mail.hupe.hu)

### Program overview

Different stress factors exert considerable impact on normal functions and pathological processes throughout the whole life span. An adequate intensity of regular physical training positively influences the whole metabolism, and thus presumably plays a beneficial role in compensating against the harmful effects of stress. In our earlier studies we extensively examined the effects of stress and stress hormones on the development of the brain and neuroendocrine system, as well as on adaptive behavior. Recently we aimed at examining the metabolic impact of prenatal stress and its role in the development of obesity. Regular training during pregnancy might counteract the negative effects of stress by influencing the development of the hypophysis-pituitary-adrenal (HPA) axis. Regular physical training in early postnatal life also could influence brain development by acting on trophic factor production, such as NGF, BDNF. Training might enhance the resistance against harmful

neonatal risk factors, such as hypoxia, NMD A and ethanol toxicity. Chronic stress produces disturbances in neuroendocrine regulation and in adaptive behavior. The possible protective effects of dietary factors and physical training are also examined. Stress has a cardinal role in the development and maintenance of drug addiction. The beneficial effects of regular training can be evaluated with behavioral studies (reinforcement, sensitization, anxiety) as well as with biochemical examinations (glucocorticoid hormones and receptors, neuropeptides: CRF, neurotensin). The projects use several different scientific techniques such as hormonal, immunocytochemical and behavioral examinations. Perinatal age requires special new methodology to develop with respect to surgical, immunocytochemical and behavioral procedures.

### ***Titles of research projects***

### ***Supervisors***

Unfolding the imperfections in lifestyle and fitness for prevention and treatment of chronic internal diseases. Physical fitness and training programs for rehabilitation	Péter Apor
The role of physical training in the neurobiology of stress response	Klára Felszeghy
The impact of physical training and dietary factors on the neurobiology of stress response at different ages	Klára Felszeghy
Motion analysis in sport sciences and the effect of (professional) sport motion	Rita Kiss
The role of physical activity and nutrition in cardiovascular diseases	Éva Martos
The importance of non-invasive cardiovascular examinations in the establishment of performance ability	Gábor Pavlik
Genetic aspects of physical exercise	József Pucsok
Different exercise protocols and oxygen uptake kinetics. Relationships between biological status (maturation status) and functional characteristics and basic motor performance. The energy costs of different exercise protocols using different types of ergometers	Tamás Szabó

### ***Ph.D. students***

### ***Supervisors***

Zsuzsanna Major	ft	Gábor Pavlik
Noémi Szakács	pt	Rita Kiss
Anna Udvardy	ft	József Pucsok

### ***Ph.D. candidates***

### ***Supervisors***

Judit G. Noé	pt	Gábor Pavlik
Álmos Zalán Gógl	ft	József Pucsok
Irén Kalabiska	pt	Gábor Pavlik
Magdolna Peresa	pt	Gábor Pavlik
Szabolcs Tóth	pt	Gábor Pavlik
Barbara Varga-Pintér	ft	Gábor Pavlik

### ***Ph.D. graduates***

### ***Supervisors***

Viktória Anna Kovács	ft	Éva Martos
Anna Szamosi	pt	Éva Martos

full-time; pt, part-time

## ***Abstracts of Ph.D. theses successfully defended in 2010***

**VIKTÓRIA ANNA KOVÁCS (2010)**

### **The role of exercise training in the prevention and treatment of childhood weight-related problems**

*Supervisor: Éva Martos*

Given the increasing prevalence of childhood obesity, its serious health consequences and the conflicting evidence about the effectiveness of interventions, our work aimed to evaluate the association between weight status, endurance capacity and obesity-related lifestyle factors among children.

The questions were analyzed first during a prospective training program, followed by a cross-sectional study. Sixty-five overweight/obese children ( $10.2 \pm 1.3$  years; BMI:  $25.3 \pm 4.1$  kg/m<sup>2</sup>) were enrolled in the 15-week exercise program (3 times per week in 60-minute sessions). In the Óbuda Study, cross-sectional data on BMI, waist circumference, blood pressure, endurance capacity, activity and dietary habits were assessed in 3,714 primary school children (7–15 years of age).

In spite of the young ages of the participants, high numbers of cardiovascular risk factors (abdominal obesity, hypertension, dyslipidaemia, insulin resistance) were found in both samples. Our results showed that for a better cardiovascular risk assessment, waist circumference should be added to the BMI measurement.

The prevalence of being overweight and obese (based on Cole criteria) among 7–15 year-old children was 14.5% and 4.6%, respectively. Endurance capacity was very low, even lower than measurements obtained 20 years ago. We found a positive correlation between extracurricular physical activity and endurance capacity. The endurance capacity of overweight/obese children was much lower than their peers of normal weight. Obesity-related dietary habits (skipping breakfast, inadequate fruit and vegetable intake, low meal frequency, high consumption of soft drinks) and inactive behaviors were found in high proportions, and the prevalence increased parallel with age. These findings highlight the importance of a healthy school environment and the teaching of healthy lifestyles at young ages.

After the 15-week program, waist circumference decreased and muscle mass increased ( $p < 0.01$ ). Some children improved their BMI category after the program. In addition, levels of LDL-cholesterol, systolic blood pressure ( $p < 0.05$ ), the presence of cardiovascular risk factors and metabolic syndrome decreased, while endurance capacity increased ( $p < 0.001$ ) after the training period. Our program offers a beneficial approach against childhood obesity and its metabolic consequences. Due to the combination of appropriate intensity, enjoyable activities and free availability, participation was high. School seems to be an ideal setting for the prevention and treatment of childhood obesity.

- Kovács VA, Fajcsák Zs, Gábor A, Martos É (2010) *Breakfast skipping is related to higher body mass index and higher waist circumference in primary school children. Acta Aliment 39: 308–316.*
- Kovács VA, Gábor A, Fajcsák Zs, Martos É (2010) *Role of waist circumference in predicting the risk of high blood pressure in children. Int J Pediatr Obes 5: 143–150.*

- Kovács VA, Fajcsák Zs, Gábor A, Martos É (2009) *School-based exercise program improves fitness, body composition and cardiovascular risk profile in overweight/obese children. Acta Physiol Hung* 96: 337–347.

## ANNA SZAMOSI (2010)

### **The connection between alterations of retinal vessels caused by hypertension and life style modification (including forced physical activity and diet) in adolescents**

*Supervisor: Éva Martos*

Connections between essential hypertension caused by alterations of vessels in the fundus of the eye and insulin resistance syndrome based on carbohydrate metabolic changes were investigated in adolescents. The effect of life modification treatment, especially everyday physical activity, was observed.

Children and adolescents (number: 822) were investigated in four periods. No pathologic alterations in the fundus of the eye were observed in children before 10 years of age. Applied ophthalmological methods were controlled in the first period. Frequency of retinopathy found in adolescents with hypertension improved by 24 hours long ambulatory blood pressure measurement and that in adolescents with so-called “white coat” hypertension was compared in the second period. Different parameters of insulin resistance syndrome, the effect of two years of life modification treatment and the frequency of retinopathy were observed in the third period. The effect of everyday physical activity on the retinopathy of adolescents having the same diet was investigated over one year in the fourth period.

Body mass index was calculated; blood pressure was measured by office and 24-hour ambulatory blood pressure monitoring; carbohydrate metabolism was detected by plasma glucose and insulin measurements and the calculation of indexes characteristic for insulin resistance; fat metabolism was investigated by serum lipid profile and the measurement of plasma thiobarbituric acid reactive system levels. The fundus of the eye was investigated by both funduscopy and a fundus camera connected to a personal computer with special software.

Methods of life modification treatment consisted of a low fat, low salt diet and a fixed schedule including a controlled daily physical exercise program, which accommodated the subject's personal family-related responsibilities.

Both funduscopy and investigations via special fundus camera showed the same pictures, but the latter method provided more objective results and opportunities for the storage and comparison of pictures obtained at different times regarding the same retinal vessel. The frequency of retinopathy was significantly increased in adolescents with essential hypertension than those with “white coat” hypertension. A connection was found between retinopathy and insulin resistance syndrome. One year long everyday physical activity caused similar alterations of the frequency of retinopathy and the mean change of plasma thiobarbituric acid reactive system levels. The first grade retinopathy was reversible.



- Szamosi A, Czinner A, Szamosi T, Sallai Á, Hatunic M, Berla Z, Tomsits E, Almássy Z, Nolen JJ (2008) Effect of diet and physical exercises treatment on insulin resistance syndrome of schoolchildren J Am Coll Nutr 27: 177–183.
- Szamosi A, Szamosi T, Angyal J, Béli T, Soós K, Martos E (2009) A szemfenéki kép változásai magas szisztolés vérnyomású serdülőkben rendszeres fizikai aktivitás után. Gyermekgyógyászat 60: 211–215.
- Szamosi A, Karádi Z, Ecsedy M, Szamosi T (2004) Magas systoles vérnyomású serdülők szemfenéki ereinek objektív vizsgálata a hypertensív retinopathia korai jeleinek kimutatására. Szemészet 141: 27–29.

## PROGRAM 5/2.

### PHYSICAL TRAINING, REGULATION AND METABOLIC TRANSPORT

#### **Coordinator:**

**Zsolt RADÁK Ph.D., D.Sc.**

Faculty of Physical Education and Sport Sciences

Research Institute of Sport Sciences

44 Alkotás st, Budapest H-1123

Tel.: +36 1 487 9216, +36 1 487 9218

E-mail: [radak@mail.hupe.hu](mailto:radak@mail.hupe.hu)

#### **Program overview**

The current program focuses on the physiological, biomechanical, and biochemical effects of acute and regular exercise on humans and laboratory animals. In addition, sport-associated injuries and the science behind regeneration are also investigated. Human gait, motions, model and individual techniques in various sport events are studied in the laboratory of biomechanics with a close collaboration of the institute of biophysics. One of the key topics of this program is the complex mechanism of the effects of exercise on aging and the role of oxidative stress in adaptive response. The effects of exercise on the function, molecular physiology of skeletal muscle and brain serve as an exciting challenge to students and professors as well. The laboratories, animal house and the core facilities of Semmelweis University, along with the expertise of the program's professors provide unique support for excellent research in sport science.

#### ***Titles of research projects***

Sports injuries of the knee

Computer simulation of limb movements and mathematical modeling of their neural control

Somatic development of 7- to 18-year-old school children

Influence of physical activity and nutrition on cellular processes of normal and pathological brain aging

Interactions between physical activity, glucose and lipid metabolism. Movement therapy of obesity and diabetes

Exercise-induced adaptation to oxidative stress and aging

#### ***Supervisors***

István Berkes

József Laczkó

János Mészáros

Csaba Nyakas

Csaba Nyakas

Zsolt Radák

<i>In vivo</i> biomechanical study of neuro-musculo-skeletal system and its mechanical, hormonal and genetic adaptation to strength exercises	József Tihanyi
The physiological, anatomical, biomechanical, performance-physiological and cultural-anthropological unity of horse and rider	Miklós Tóth
Physiological, proteomic and genetic characteristics of physical exercise and cardiovascular and metabolic risk	Miklós Tóth
The effect of lower education in somatic development	Márta Wilhelm

***Ph.D. students***

Rozália Apró	ft
Edit Bosnyák	ft
Réka Feszthammer	pt
Renató Gál	pt
Nikolett Hart	ft
Péter Horváth	pt (a)
Réka Karádi	ft
Péter Katona	ft
Bence Kopper	pt
Krisztina Marosi	ft
Orsolya Marton	ft
Zalán Négele	pt
Krisztián Gusztáv Papp	pt
Linda Sárga	ft
Kata Sótonyi	ft
Emese Trájlre	ft
Dóra Ureczky	ft

***Ph.D. candidates***

Zoltán Ács	pt
Anni Andrea Ember	ft
Anna Kacsánci	ft
Erika Koltai	ft
Zsófia Mészáros	ft
Pampakas Polydoros	it
Gergely Pánics	na
Márta Zsuzsa Ránky	ft
Sándor Sáfár	ft
Szabolcs Zsigri	pt

***Ph.D. graduates***

Andreas Costa	it
Zoltán Keresztényi	pt
Péter Osváth	na
András Prókai	na
Riccardo di Giminiani	it
Savvas Siamilis	it
Zsófia Szabó	ft
Márk Vácz	na

***Supervisors***

Márta Wilhelm
Miklós Tóth
István Berkes
József Tihanyi
Zsolt Radák
István Berkes
Miklós Tóth
József Laczkó
József Tihanyi
Csaba Nyakas
Zsolt Radák
Csaba Nyakas
Zsolt Radák
Zsolt Radák
Miklós Tóth
Miklós Tóth
József Tihanyi

***Supervisors***

Zsolt Radák
Márta Wilhelm
Csaba Nyakas
Zsolt Radák
János Mészáros
János Mészáros
István Berkes
Csaba Nyakas
József Tihanyi
Csaba Nyakas

***Supervisors***

József Tihanyi
József Laczkó
János Mészáros
János Mészáros
József Tihanyi
Zsolt Radák
Zsolt Radák
József Tihanyi

ft, full-time; pt, part-time; it, international; a, absolutorium; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**ANDREAS COSTA (2009)**

### **Impact of repeated bouts of eccentric exercise on skeletal muscle morphology and myogenic gene expression**

*Supervisor: József Tihanyi*

The purpose of this study is to determine the effects of repeated bouts of eccentric exercise for six consecutive days on the transcriptional alteration of myogenic (MyoD, Myogenin, Myf5, and Myostatin) and cell cycle (P21, Ki-67) regulatory genes, as well as on the indirect (CK and LDH activity, MAT, and DOMS) and direct (myofiber and sarcolemma damage) markers of skeletal muscle damage in a group of middle-aged untrained individuals. This was done in an attempt to determine the appropriateness of exercise continuation with damaged muscles. Fifteen healthy untrained males were recruited for this study. The exercise group (n=9) successfully completed six sets of 15 repetitions of maximum voluntary eccentric contractions, for six consecutive days, using a dynamometer (Multicont-II). The control group (n=6) remained in a sedentary state. Blood and biopsy samples were obtained from all subjects one week prior to exercise, immediately after bout 3 (day 3), and 24 hours after the last training session on day 7. Blood samples were analyzed for creatine kinase (CK) and lactate-dehydrogenase (LDH) activity. All biopsies were stained with standard haematoxylin-eosin staining and immunohistochemically using antibodies specific for fibronectin and desmin antigens. The results of our study indicated no evidence of gross myofiber damage. In addition, no sarcolemma damage and no loss of desmin were observed as stained by anti-fibronectin and anti-desmin antibody respectively. Despite the lack of sarcolemma and myofiber damage, CK and LDH activities significantly increased each time when measured. The results of mRNA gene expression showed that Myostatin mRNA expression dramatically decreased, but the expression patterns of MRFs was impaired such that, with the exception of myogenin that showed a moderate non sustained increase, MyoD and MYf5 response was minimal. Although we observed no gross myofiber and sarcolemma damage in 8 of 9 subjects, we cannot ascertain the appropriateness of exercise continuation with affected muscles prior to full recovery. This is due to the impaired expression patterns of MRFs. Under these conditions, exercise continuation may be associated with impaired muscle growth and/or regeneration.

- Costa A, Dalloul H, Hegyesi H, Apor P, Csende Z, Racz L, Vaczi M, Tihanyi J (2007) *Impact of repeated bout of eccentric exercise on myogenic gene expression. Eur J Appl Physiol* 101: 427–436.
- Costa A, Orosz Z, Apor P, Csaba N, Siamilis S, Csende Z, Racz L, Tihanyi J (2008) *Impact of repeated bouts of eccentric exercise on sarcolemma disruption in human skeletal muscle. Acta Physiol Hung* 96: 189–202.

## ZOLTÁN KERESZTÉNYI (2009)

### **Motocontrol and movement stability of the arm. Rules in the Parkinsonian and healthy controls**

*Supervisor: József Laczkó*

Biomechanical and control characteristics of the movement disturbances caused by Parkinson's disease (PD) were studied. Two types of examinations were performed. The aim of the first study was to investigate the time span within which bradykinesia recurs immediately after the termination of deep brain stimulation (DBS). DBS is an effective treatment in advanced Parkinson's disease, however, the time course of the immediate wearing-off effect of DBS is unclear. The essence of this effect is very important when positioning the electrode during the operation, since the immediate effect of DBS on the main symptoms of Parkinsonism is used to determine the optimal site of the stimulating electrodes. The second study was performed to provide an objective method for assessment of the human movement state. There are some quite precise motor exams used in clinical practice (e. g. UPDRS), but it is still a challenge to measure the patient's status objectively. In our study variances of arm movements between patients with PD and age-matched healthy controls were compared with respect to both the endpoint (external space) and arm configurations (internal space), and aimed to assess a parameter that is suitable for quantitative characterization of the stability of human limb movements. Another aim was to discern if the error compensating system responsible for proper inter-segmental coordination is also affected by PD. In our tests we applied two different motor exams: 1. fingertapping and forearm pronation-supination exams; 2. drawing arm movements performed for circle and square. We recorded the kinematical parameters via movement analyzing system and computed the amplitude, peak velocity, frequency of movements (first study) and variances of arm configurations and endpoint trajectories (second study). Our results show that the effect of DBS had completely disappeared within one minute. This means that during the operation it is necessary to wait at least one minute after the end of stimulation before performing further assessments, and to be sure that particular tests do not interfere with each other. In specifying movement variances we objectively characterized the differences in the motor performance of PDs and healthy people and found that ill-coordinated hand movements were caused by the error in the movements of individual body parts rather than by the lack of inter-segmental coordination. The presented method provides a new tool for the objective analysis of the movement state of patients, which helps to monitor the effects of drugs, the development of motor symptoms and to provide proper diagnoses and treatments.

- Keresztényi Z, Cesari P, Fazekas G, Laczkó J (2009) *The relation of hand and arm configuration variances while tracking geometric figures in Parkinson's disease—"aspects for rehabilitation". Int J Rehabil Res 32: 53–63.*
- Keresztényi Z, Valkovic P, Eggert T, Steude U, Hermsdörfer J, Laczkó J, Böetzel K (2007) *The time course of the return of upper limb bradykinesia after cessation of subthalamic stimulation in Parkinson's disease. Parkinsonism Relat Disord 13: 438–442.*

**PÉTER OSVÁTH (2010)****Human biological and lifestyle characteristics of people with disabilities and increased health risk**

*Supervisor: János Mészáros*

The basic goal of our research was to clarify the reason why only a small proportion of 25,000–26,000 blind individuals, people with special health risks and youth in Hungary do regular physical activity at an acceptable level, and to determine why only a very small number of them are members of sport clubs.

Characterising the physique, body fat content and biological development level of the blind (B1) and pathologically obese individuals, we applied internationally accepted antropometric methods (Conrad 1963; Parízkova 1961; Mészáros and Mohácsi 1983). We summarized the limiting factors of regular physical activity of the blind by analysing the answers given in a questionnaire developed by our team.

The biological maturation of pathologically obese boys, according to the WHO (1998), moderately accelerated until puberty, in accordance with the data of the international literature. This impact is partly related to the endocrine function of the huge depo fat mass. Their physique is pyknomorphic, even if we eliminate the modifying effect of the skinfolds on the body measurements.

The relative fat proportion of the blind children and pubertal boys is higher, but their lean body mass is mostly lower than their non-blind peers in the same age category. Both results are can be linked, above all, to their very low physical activity level. In spite of the fact that blindness is not linked to morphological alteration, moderate pyknomorphic constitution was measured in the sample of the B1 category blind children. The results characterizing the majority are probably the consequence of intrauterin damage or developmental disorders.

A special curriculum of physical activity for disabled people and the special sport-related areas for the blind are missing from educational programs at the university level. Thus, the insufficient knowledge of the students in this area can be one of the reasons why they have no intention of working with disabled people at a professional level. Although regular physical activity and competitive sport for blind individuals face both objective as well as subjective barriers, we believe that the role of subjective factors are stronger than the others. Realizing the importance of sport for the blind and the non-blind is a difficult task; moreover, most visually-disabled individuals have no real intention to take part in it.

- Osváth P (2004) *A fogyatékoság ügyének megjelenése a sporttudományban. Magyar Sporttud Szemle 5: 44–46.*
- Osváth P, Ramocsa G (2006) *A sport hatása a vakok életminőségére Magyarországon. Kalokagathia 44: 170–175.*
- Osvath P, Meszaros Zs, Tóth Sz, Kiss K, Mavroudes M, Ng NK, Meszaros J (2009) *Physical and physiological performances in 10-year-old obese boys. Acta Physiol Hung 96: 475–482.*

**ANDRÁS PRÓKAI (2009)****Generational differences in regard to physique and body composition in 7- to 18-year-old boys and their issues on the estimation of morphological age and prediction of young adult height***Supervisor: János Mészáros*

Generational morphological differences were analysed with the comparison of representative growth standards. Two nation-wide anthropometric investigations were carried out in 1983 (N=12778) and 2005 (13338). Beyond the descriptive and comparative analysis, a secondary aim of the study was to evaluate the differences between the estimations of morphological age (one of the valid assessments of bone age) and predicted young adult stature. The techniques used for the anthropometric characterisation and assessment of nutritional status are accepted by the Hungarian and international literature.

Consistently and significantly taller mean heights were observed in the second representative sample. However, the body mass differences were not proportionate to the mean height differences. The boys in the second investigation were heavier. Significantly more linear physique means were found at the beginning of the new millennium, but the bone-muscle development of these boys was not more favourable. Both body mass indices and weight-related fat content means indicate the significantly greater body fat content in 2005. Consequently, the prevalence of overweight and obese boys was also remarkably greater at the beginning of the new millennium. The significantly taller mean statures, heavier body mass and the unchanged plastic index means resulted in significant differences in the calculation of morphological age and prediction of final stature. The required accuracies in the estimations suggest the usage of the new standards (described by the results in the 2005 study) exclusively.

Of the observed significant generational differences, only the taller mean heights can be attributed to the positive consequences of secular growth trends. The more linear physique, the greater relative body fat content and the unchanged bone-muscle development are not the effects of a secular growth trend; they should be related to a remarkably changed lifestyle, the generally characteristic hypoactivity.

The significant generational differences between the body composition attributes suggest fat-correction in the metric and plastic indices. These corrections increase the accuracy of the estimation of morphological age, and additionally, the prediction of young adult stature. Nowadays, this information is required in sport practice, namely in regard to talent selection and education.

- Prókai A, Mészáros J, Mészáros Zs, Photiou A, Vajda I, Sziva Á (2007) Overweight and obesity in 7 to 10-year-old Hungarian boys. *Acta Physiol Hung* 94: 267–270.
- Mészáros J, Mészáros Zs, Zsidegh M, Prókai A, Vajda I, Photiou A, Mohácsi J (2006) Nemzedékenkénti növekedési különbségek és utánpótlás-nevelés. *Magy Sporttud Szemle* 7: 3–6.
- Photiou A, Anning J, Mészáros J, Vajda I, Mészáros Zs, Sziva Á, Prókai A, Ng N (2007) Body composition and physical fitness changes in Hungarian school boys (1975–2005). *Res Quart Exerc Sport* 79: 168–173.



**RICCARDO DI GIMINIANI (2010)****Effects of whole-body vibration training on muscle strength and flexibility: significance of the vibration frequency**

*Supervisor: József Tihanyi*

The aim of this investigation was to study the significance of vibration frequency in improving explosive strength, reactive strength, muscle flexibility and isometric and eccentric force in young physically active people and patients with stroke.

Three separate studies were carried out to test the acute, acute residual and chronic effect of whole body mechanical vibration (WBV). In the first and second study, eight weeks of WBV was used, either with an individually selected vibration frequency that ranged from 20 to 35 Hz, or 30 Hz frequency for each subject. In the third study a 20 Hz frequency was applied over four weeks to acute stroke patients. In the first study dynamic force was estimated by using squat jumps, countermovement jumps or 10-second rebound jumps. In the second study the flexibility of the hamstring and lower back muscles were tested during vibration, after having finished the vibration exposure, and before and after an eight-week vibration intervention. In addition to the reactive muscle strength of the extensors in lower limbs were determined before and after eight week vibration intervention. In the third study maximum isometric force and force development and maximum eccentric force and mechanical work were determined for the affected and non-affected knee extensors.

The results of the present investigation may allow us to conclude the following:

The individually selected vibration frequency based on the EMG response for WBV intervention results in greater improvement in explosive and reactive strength as compared with fixed frequencies vibration intervention;

The improvements in vertical jumps are greatest when movement or strength exertion is carried out with short angular displacement and when the muscle stretch is as fast as that which occur in rebound jumps;

Individualized whole-body vibration without superimposing other exercises is an effective method of acutely increasing lower back and hamstring flexibility (acute and residual effect);

Individualized whole-body vibration does not induce a chronic effect on hamstring flexibility;

Low vibration frequency results in significant improvement in both isometric and dynamic strength of knee extensors in persons with restricted physical activity, and the improvement is more pronounced in the affected side than in the intact side of stroke patients. This result indicates indirectly that the effect of vibration frequency depends on the physical and the neuromuscular condition of the treated muscles.

- Di Giminiani R, Tihanyi J, Safar S, Scrimaglio R (2009) *The effects of vibration on explosive and reactive strength when applying individualized vibration frequencies. J Sports Sci* 27: 169–177.
- Di Giminiani R, Manno R, Scrimaglio R (2008) *Nuove evidenze scientifiche della vibrazione indotta sulla forza esplosiva prodotta con e senza pre-stiramento. Atletica Studi, Fidal*, 3: 13–20.
- Di Giminiani R, Scrimaglio R (2008) *Le vibrazioni indotte: recenti investigazioni con l'utilizzo della frequenza di vibrazione individuale. Riv Chinesiologia* 2: 35–45.

**SAVVAS SIAMILIS (2009)****The effects of exercise, oxidants and antioxidants on neurotrophins and the oxidative damage of the spinal cord of rats***Supervisor: Zsolt Radák*

The purpose of this study was to investigate the effect of chronic enforced exercise and the administration of hydrogen peroxide ( $H_2O_2$ ), N-tert-Butyl- $\alpha$ -phenyl-nitron (PBN) on reactive oxygen species concentration (ROS), oxidative damage markers and neurotrophins (NTs) release in the cervical spinal cord of rats. Specifically, NTs investigated include the brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and the mRNA of BDNF and BDNF's tyrosine kinase receptor (TrkB) with the mitogen-activated protein kinase (MAPK) superfamily signaling molecule, extracellular signal kinase (ERK1/2). One of our major goals was to elucidate whether there is any kind of relationship (causative or associative) between ROS concentration and BDNF release, and if antioxidant treatment might attenuate exercise-induced adaptive responses. In order to test our hypothesis and examine the physiological response of the spinal cord to exercise and oxidant-antioxidant administration, thirty-six, five-month-old, healthy, male Wistar rats were used in the study. Six rats were randomly assigned to each of six groups: non-exercised controls injected with saline (NEC), non-exercised controls injected with  $H_2O_2$  (NEH), non-exercised controls injected with N-tert-butyl- $\alpha$ -phenylnitron PBN (NEP), exercised controls injected with saline (EC), exercised controls injected with  $H_2O_2$  (EH) and exercised controls injected with PBN (EP). For the determination of free radical concentration we used an Electron Paramagnetic Resonance (EPR); for the detection of BDNF and GDNF proteins we utilized the enzyme linked immunoassay (ELISA); the estimation of oxidatively modified spinal cord proteins was performed with a Western blot; and for the detection and determination of the relative mRNA expression of BDNF and TrkB we utilized RT-PCR. Results of our study indicated that the data obtained from EPR measurement revealed that the EC group had a significantly lower accumulation of free radicals as compared to the NEC group. Similarly, there was a tendency towards significantly lower free radical accumulation in the EH group as compared to NEH. NEP had a significantly lower concentration of free radical accumulation than the other non-exercised groups NEC and NEH. In contrast to the other exercised groups, the EP group had higher free radical accumulation from its respective NEP group, but no significant difference was observed. Oxidative damage of proteins as indicated by Western blot showed no differences among the pooled samples of the groups, indicating that the accumulation of free radicals was tolerated by the cells and that the increase was still in the physiological range of the redox homeostasis. BDNF protein level was significantly decreased in the EC and NEP groups as compared to the NEC and NEH groups, respectively, and in the NEP group as compared to the EH group. Interestingly, when free radical concentration and BDNF protein concentration are plotted together in a correlation plot, a significant positive correlation results, suggesting either an associative or a causative relationship. The mRNA level of BDNF receptor, TrkB, was expressed in all of the groups, but no significant differences were observed. In contrast, BDNF mRNA expression could not be detected with RT-PCR. The pattern of TrkB expression was significantly and positively correlated with the BDNF protein concentration. Exercise-induced ERK activation was observed only in the EC and EH groups while EP and non-exercised groups' BDNF

induction seemed to be independent of ERK pathway activation. The level of GDNF protein was significantly increased in the NEH group as compared to the NEC and NEP groups, suggesting that H<sub>2</sub>O<sub>2</sub> injection altered the content of GDNF.

- Radak Z, Toldy A, Szabo Z, Siamilis S, Nyakas C, Silye G, Jakus J, Goto S (2006) *The effect of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem Int 49: 387–392.*
- Siamilis S, Jakus J, Nyakas C, Costa A, Mihalik B, Falus A, Radak Z (2009) *The effect of exercise and oxidant-antioxidant intervention on the level of neurotrophins and free radicals in spinal cord of rats. Spinal Cord 47: 453–457.*

## ZSÓFIA SZABÓ (2010)

### The role of regular physical activity on proteasome complex in traumatic brain injury

*Supervisor: Zsolt Radák*

Overtraining is, by definition, a condition wherein an athlete is training excessively yet their performance deteriorates. This is a process included in the whole organism that can be associated with vegetative processes and neuroendocrine, somatic and psychical functions. Exercise increases the formation of free radical species which enhances the oxidative stress. It was hypothesised that during overtraining there is a marked increase in the generation of free radical species due to physical activity and inflammation. The aim of present study was to measure the effect of moderate, hard and strenuous exercise on certain brain functions, the antioxidant enzyme activities of liver and muscle, and the stress markers of lipids (muscle, brain) and proteins (liver, brain).

Rats were divided (Wistar, aged 5 months) into four groups and exposed to swimming: a control, a constant duration (1 hour/day, 5/weeks, for 8 weeks), an abruptly increased duration (1 hour/day, 5/weeks, for 6 weeks, then 3.5hours/day, 7/weeks for 2 weeks), and a continuously increased duration (same as the trained group, but the duration increased by 30 minutes each week) group. In the last week an open-field test was used to measure emotional stress. Memory was assessed by the passive avoidance test, and the acrobatic-locomotor (balance) ability was evaluated with the rotarod test. We used body weight (measured each week), ACTH and corticosterone levels as a marker of overtraining.

Eight weeks of swimming resulted in significantly increased emotional and behavioral stress. The size of the thymus decreased and the adrenal increased with the increased duration of exercise. The changes in body weight, ACTH and corticosterone levels of each group demonstrated an increased stress condition. The changes in antioxidant enzyme activities and levels of RCD and TBARS proved the positive effects of moderate training, but did not support our hypothesis of overtraining. We did not find any correlation between the activity of antioxidant enzymes and the rate of oxidative stress markers of lipids and proteins.

Our hypothesis that overtraining causes massive oxidative stress is not supported. Further investigations are necessary to study the possible relationships between training loads and antioxidant enzymes and oxidative repair systems.

- Radak Z, Toldy A, Szabo Z, Siamilis S, Nyakas C, Silye G, Jakus J, Goto S (2006) *The effect of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem Int 49: 387–392.*
- Szabo Z, Ying Z, Radak Z, Gomez-Pinilla F (2010) *Voluntary exercise may engage proteasome function to benefit the brain after trauma. Brain Res 1341: 25–31.*

## MÁRK VÁCZI (2010)

### Mechanical and physiological consequences of eccentric-concentric knee extensor training

*Supervisor: József Tihanyi*

**The purpose** of the present work was (1) to examine the effects of consecutive eccentric-concentric knee extensor training on neuromechanical and biochemical variables of the muscle; (2) to compare two training regimens in which knee extensor exercises were performed at different stretching lengths. **Methods:** In the first experiment ten males performed 90 eccentric-concentric contractions on Multicont II for three consecutive days (Tr1-Tr3) and after one day of recovery for four more consecutive days (Tr4-Tr7). Mean of peak torques of the training contractions (MTr), maximal isometric (M0) and eccentric (Mecc) torque, mechanical efficiency ( $\eta$ ) and integrated electric activity of the muscle, as well as plasma creatine kinase (CK) activity were determined 24, 48, and 72 hours, respectively, after Tr1, and 1 and 3 days after Tr7. In the second experiment subjects performed eccentric knee extensor training for six consecutive days with either a small (K, 60°, n=8) or large (N, 120°, n=8) range of motion. Mean of peak torques of the training contractions (Mcs) and plasma CK activity were determined during the experiment. **Results:** In the first experiment 24 hours after Tr1, M0 and Mecc decreased and CK increased significantly, and muscle soreness developed. By the end of the experiment, MTr and M0 increased significantly, while CK activity and soreness moderated. IEMG measured during isometric contraction increased 72 hours after Tr1, but changes did not correlate with changes in M0.  $\eta$  decreased continuously throughout the study. In the second experiment Mcs decreased 24 hours after E1 in both groups, then in K it started regenerating, while in N it further declined at E2. By E6, Mcs increased significantly in K, however, in N it did not return to the baseline level. Training of N produced greater increase in CK and greater development of muscle soreness compared with that of K. **Conclusions:** Consecutive eccentric-concentric knee extensor training induced acute loss of voluntary torque production. Though it is not directly supported, it is possible that neural adaptation enhanced rapid recovery and the increased static and dynamic torque in these healthy, well trained individuals. Theses early changes, however, can be considered partial training adaptations, and they did not accumulate into sport-specific adaptations, as indicated by the decreasing tendency of mechanical efficiency. Eccentric training performed at a longer stretching length induces the greater development of muscle damage and slower regeneration in knee extensors, compared with training at a shorter stretching length.

- Váczi M, Costa A, Rácz L, Tihanyi J (2009) *Effects of consecutive eccentric training at different range of motion on muscle damage and recovery. Acta Phys Hung 96: 459–468.*

- Vácz M, Tihanyi J, Hortobágyi T, Rácz L, Csente Zs, Costa A, Pucsok J (2011) Mechanical, biochemical, and EMG responses to short-term eccentric-concentric knee extensor training in humans. *J Strength Cond Res* 25: 922–932.
- Costa A, Dalloul H, Hegyesi H, Apor P, Csente Z, Rácz L, Vácz M, Tihanyi J (2007) Impact of repeated bout of eccentric exercise on myogenic gene expression. *Eur J Appl Phys* 101: 427–436.

---

## **PROGRAM 5/3.**

---

### **SPORT AND SOCIAL SCIENCES**

#### **Coordinator:**

**Gyöngyi SZABÓ FÖLDESINÉ Ph.D., D.Sc.**

Department of Social Sciences

44 Alkotás st, Budapest H-1123

Tel.: +36 1 487 9297

E-mail: [foldesi@mail.hupe.hu](mailto:foldesi@mail.hupe.hu)

#### **Program overview**

As in the political, economic, social, and cultural spheres, the tendency towards globalization has become more powerful in the sport subsystem. Although global growth has been beneficial for sport in many ways, its impact is regarded as contradictory. The fact that sport has become global in scope produced sweeping changes both on the international and national scenes, and sport institutions have had to face new challenges to which answers are expected on the basis of scientific results. The major objective of the program “Sport and Social Sciences” is to contribute to the understanding and explanation of the impact of globalization on sport as a social phenomenon, and of the mutual relationship between sport and society. The program embraces most areas of sport sciences dealing with various social issues related to sport from the perspectives of sport philosophy, sport history, sport politics, sport economy, sport sociology, sport psychology, sport pedagogy, sport management, sport marketing, and sport law. Theoretical backgrounds and methods used in the research of the different topics should satisfy the requirements of the individual disciplines, notwithstanding the promotion of a multidisciplinary approach. The program includes the study of physical education and all traditional fields of sport, that is, school sport, university sport, sport for all, elite sport, and sport for people with special needs. In addition, the investigation of new areas of contemporary sport (recreation, sport tourism, extreme sport, risk sport, etc.) as well as comparative and cross cultural studies from the aspect of social sciences is also welcomed.

***Titles of research projects***

Sport geography, sport tourism  
 Value and practice orientation of adult and old people  
 Career, prestige, and reasons for leaving the profession of educators  
 Exploration of the organic background of learning and behavioral difficulties from a neuropsychological perspective.  
 (Developmental neuropsychology)  
 Sport in modern society  
  
 The situation and role of sport among the transformed political, economic and social relations  
 Education work of sport coaches  
 Theoretical and methodological fundamentals of Physical Education Curricula  
 Scientific basics of living standards (recreation)  
 Application possibilities of new didactic methods at the different sport education  
 Links between information, communication and sport  
 Personality characteristics of athletes, developmental effects on personality and applications in pedagogy  
 Content and organizational modernization of sport-professional training in Hungary with regards to EU integration and legal harmonization  
 Event management and marketing  
 Motor performance diagnostic and its methodological basis  
 Objective analyses of elite handball teams' performance by diagnosis program  
 Psychometric examination of anxiety, coping with stress, and self-efficacy  
 Application of autogenic training in sports and at school  
 Comparison of the mood-improving effects of various sports/physical exercises  
 The role of musical styles in aerobic training  
 The impact of tai chi or yoga on the acute response to stress and electrocardiographic QT interval  
 The social impact of physical education and sport and their relationship with Hungarian society's special groups in the first half of the 20th Century  
 The effect of school physical education and student sport on averting and limiting the health damaging risk factors appearing in students' conduct  
 Relations of society and physical education and sport from ancient times to the present—lessons of social history  
 Effects of values and culture on lifestyle  
 An examination of the motor and psychological development during the sensitive period of personality development, motor learning and motor development

***Supervisors***

Miklós Bánhidi  
 József Bognár  
 József Bognár  
 Rita Földi  
 (Fodorné)  
  
 Gyöngyi Szabó  
 (Földesiné)  
 Andrea Gál  
 (Gáldiné)  
 János Gombocz  
 Pál Hamar  
  
 László Jakabházy  
 Katalin Keresztesi  
  
 Ágnes Kokovay  
 Csaba Nagykáldi  
  
 András Nemes  
  
 Mihály Nyerges  
 Károly Ozsváth  
 Csaba Ökrös  
  
 Kornél Sipos  
  
 Kornél Sipos  
 Attila Szabó  
  
 Attila Szabó  
 Attila Szabó  
  
 Sándor Szakály  
  
 Piroska Szalay  
  
 Katalin Szikora  
  
 Tímea Tibori  
 László Tóth



Problems of sports and social integration; the relationship between sports and deviant behaviour István Vingender

### ***Ph. D. students***

Anna Zsófia Alliquander	pt
Borbála Abonyi (Bácskayné)	pt
Judit Balogh	pt
Mariann Bardocz-Bencsik	pt
Betti Barthel	pt
Franciska Bartus (Sontráné)	pt
Piroska Béki	pt
Efstathios Christodoulides	it
Orsolya Czeglédi	ft
Diána Déri	pt
Károly Dobos	pt (a)
Eszter Gál	pt
Judit Gombás	ft
Dóra Görögh	ft
Erika Gulyás	pt
Noémi Gyömbér	pt
Katalin Szilvia Hájas	pt
Csaba Halmos	pt
Tamás Horváth	pt
Zsolt Horváth	pt
Norbert Kovács	pt
Dániel Lőkös	pt (a)
Katalin Nagyvárad	pt (a)
Andrea Olajos (Adorjáné)	pt
Judit Plachy (Kopkáné)	pt
Orsolya Savella	pt
Eszter Szabó	ft
Péter Szájer	ft
János Tóth	pt
Dániel Varga	pt
Orphanos Yiannakis	it

### ***Ph. D. candidates***

Júlia Ábrahám	pt
Zsuzsanna Balogh (Olvasztóné)	pt
István Barthalos	pt
Ottó Benczenleitner	pt

### ***Supervisors***

Tímea Tibori
Kornél Sipos
János Gombocz
András Nemes
Rita Földi (Fodorné)
Ágnes Kokovay
Andrea Gál (Gáldiné)
Gyöngyi Szabó (Földesiné)
Mihály Nyerges
Mihály Nyerges
Csaba Nagykáldi
Katalin Vermes
Andrea Gál (Gáldiné)
Csaba Ökrös
Mihály Nyerges
Kornél Sipos
János Gombocz
Andrea Gál (Gáldiné)
László Tóth
József Bognár
Katalin Keresztesi
Kornél Sipos
József Bognár
Pál Hamar
József Bognár
Katalin Keresztesi
László Tóth
Pál Hamar
János Gombocz
Gyöngyi Szabó (Földesiné)

### ***Supervisors***

Tímea Tibori
József Bognár
József Bognár
József Bognár

Szabolcs Levente Bertók	pt	Károly Ozsváth
Sándor Bollók	pt	Kornél Sipos
Diana Christodoulou	it	Gyöngyi Szabó (Földesiné)
Beáta Dobay	pt	Miklós Bánhidi
Tamács Dóczi	ft	Gyöngyi Szabó (Földesiné)
László Dorogi	na	József Bognár
Ágnes Huszár	pt	Pál Hamar
Zoltán Jókay	pt	József Bognár
Zsuzsa Kalmár	pt	Pál Hamar
András Péter Killyéni	pt	Katalin Szikora
Csaba Konczos	pt	Károly Ozsváth
Magdolna Kovách (Vécseyné)	pt	József Bognár
István Kun	na	László Tóth
Panayiota Kyprianou	it	Kornél Sipos
Gyöngyvér Lacza	na	László Jakabházy
Judit Lakó (Herpainé)	pt	János Gombocz
István Munkácsi	pt	Pál Hamar
Eleonóra Murányi	ft	Pál Hamar
Andrea Pécsi (Bácsalmásiné)	pt	Károly Ozsváth
Katalin Rácz	ft	Rita Földi (Fodorné)
Panayiotis Shippy	it	Gyöngyi Szabó (Földesiné)
Katalin Starhon (Gaálné)	pt	Miklós Bánhidi
Ágnes Sztankovics (Törökné)	pt	János Gombocz
Bence Ervin Takács	ft	Sándor Szakály
Éva Thiry (Pólusné)	pt	Tímea Tibori
Attila Velenczei	pt	Gyöngyi Szabó (Földesiné)

***Ph.D. graduates***

Péter Berkes	pt
Szilvia Boros	na
Gábor Gécsi	pt
Szilvia Gita (Dimitriou)	pt
Beatrix Geosits (Kissné)	pt
Miklós Koltai	pt
Kinga Majoross	ft
Nikoletta Onyestyák	ft
Szilvia Perényi	pt
László Révész	ft
István Simon	pt
János Vácz	pt
Géza Vincze	pt

***Supervisors***

Mihály Nyerges
Kornél Sipos
József Bognár
József Bognár
Teodóra Tomcsányi
József Bognár
Kornél Sipos
Sándor Szakály
Gyöngyi Szabó (Földesiné)
József Bognár
János Gombocz
Mihály Nyerges
Mihály Nyerges

ft, full-time; pt, part-time; it, international; a, absolutorium; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**PÉTER BERKES (2009)**

### **Macro-level factors affecting the sport sponsorship decision-making process at Hungarian soccer clubs and soccer sponsor companies**

*Supervisor: Mihály Nyerges*

Over the last decades, worldwide interest in soccer sponsorship has increased dramatically in terms of media coverage, consumer interest, and corporate involvement. This study contributes to the continuing work in the development of a conceptual framework to better understand soccer sponsorship. Soccer sponsorship is not developing rapidly in Hungary because soccer clubs and commercial business have not fully recognized the positive features of sport sponsorship. For sport properties there is increased pressure to seek out corporate sponsorship support to ensure ongoing organisational viability and survival. This research work was indicated by an apparent lack of comprehensive investigation, and little empirical evidence emerged regarding the marketing orientation of the sport sponsorship strategy of Hungarian professional soccer clubs. The primary purpose of the empirical part of our study was to evaluate the importance of certain factors that affect professional soccer sponsorship decision-making in Hungary. Such factors include the relationships between soccer sponsorship objectives and the effectiveness of their measurement. The secondary purpose of this study was to conduct a gap analysis in order to develop successful sponsorship strategies—strategies that will serve the development of sport sponsorship in Hungary. The research approach is inductive, and the data collection method was via a quantitative survey. For conceptualisation purposes, in order to establish validity and reliability, the survey instruments were tested by sport marketing and sponsorship theorists and practitioners. Sampling frame: questionnaires were distributed to all Hungarian professional soccer organisations (N=18) and their sponsors (N=103), utilising integrated marketing communication (N=57). Subjects were provided self-administered questionnaires (Crowley 1991; Farelly *et al.* 1997; Van Heerden 2001). The statistical analyses were executed by Statistics for Windows 6.0 Stat-Soft Inc. (2001). The significance-level was ( $p \geq 0.05$ ) and the correlation-level ( $r \geq \pm 0.61$ ). In addition, a coefficient alpha (Cronbach's alpha) test was run to ensure internal-consistency reliability of response; the Cronbach alpha scores were between 0.796 and 0.610. The main conclusion was that Western-style corporations have become involved in sponsoring Hungarian soccer clubs, but their capitalistic attitude (i. e., economic profit motives) conflict with Hungarians' distinct socialism (i. e., social profit motives). Western corporations traditionally have seen sponsorship as a marketing vehicle to differentiate brands or organizations.

- Berkes P, Nyerges M (2003) *A labdarúgás menedzselésének főbb elvei a szponzorálás tükrében. Magyar Sporttud Szemle 4: 17–21.*
- Berkes P, Nyerges M (2004) *A sportszponzorációs döntési tevékenység makro-szintű vizsgálata az élvonalbeli magyar labdarúgásban. Magyar Sporttud Szemle 2/3: 47–52.*
- Bartha Cs, Riegler E, Berkes P (2004) *Rúgáspontosság és távolságbecslés vizsgálata labdarúgóknál. Magyar Edző 1: 18–22.*

## SZILVIA BOROS (2010)

### Physical self-concept, dietary habits, trait anxiety and trait depression amongst elite athletes and physically active and obese people

Supervisor: Kornél Sipos

The aim of the study was to analyze relationships amongst physical self-concept, nutritional behaviour and trait anxiety, and trait depression in five different subgroups, such as elite rhythmic gymnasts, physically active college students, sedentary obese adolescents (BMI<30), and yoga practitioners. Four-hundred and sixty-one people participated in the study: elite rhythmic gymnasts (n=103), sedentary secondary school children (aged 15–18, n=113), physically active college students (n=84) from four different sport-groups (running, belly dancing, aerobics, other), obese people (n=101) and yoga practitioners (n=60). Anthropometrical measurements (bodyweight, height, body fat, BMI), psychological questionnaires (STAI-H, FX1, FX2, Tennessee physical self-concept subscale, STPY-H, Y1, Y2, LDM (R/ED), LDM (N/H), and a nutritional questionnaire were used. Results showed that in rhythmic gymnasts, higher physical self-concept scores indicate some unbeneficial nutritional pattern: loss of dinner (sometimes lunch), skipping of sweets and fast food. In the physically active group, there was a negative correlation between BMI and physical self-concept, and there was a positive significant correlation between physical self-concept and time spent on physical activities. Those who spent 6–8 hours per week on physical activities had a significantly higher physical self-concept score than those who had 0–2 hours per week of exercise. Those physically active people who were more satisfied with their body consumed a greater variety of food. The amount of yoga practice negatively influences meat consumption, although it positively influences bio-food consumption. Regular yoga practise may decrease trait anxiety. The obese group had significantly lower physical self-concept scores. Higher body fat and abdominal circumference correlated with lower physical self-concept. Those obese people who had a higher physical self-concept had higher readiness for physical activity scores and lower trait depression scores. An optimal weight loss program has to be based on regular physical activity, a balanced nutritional regimen and continuous psychological supervision and patient education. We suggest that (1) the optimal weight loss program be started with a strict eating regimen which can result in fast weight loss in the first week; this process can favourably increase physical self-concept; (2) the implementation of regular physical activity in lifestyle; (3) continuous nutritional-psychological-medical counselling. Yoga practise can be a strong aid, either in the treatment of obesity or in the everyday life routine of people of all ages.

- Boros Sz (2010) *A rendszeres testmozgás szerepe a betegségek megelőzésében*. Pp 241–251 in: *Legyen egyensúlyban. Tények az elhízásról és fogyásról*. Melánia Kiadó, Budapest.
- Boros Sz, Halmy L (2009) *Physical self-concept, trait depression and readiness for physical activity of obese patients*. *Phys Educ Sport* 53: 137–139.
- Boros Sz (2008) *Sporttáplálkozás*. Krea-Fitt Kft, Budapest. ISBN 978-963-06-5785-3

**GÁBOR GÉCZI (2009)****Success and talent development as indicated by motor tests and psychometric variables of U18 hockey players***Supervisor: József Bognár*

Ice hockey is known as one of the fastest and multifactorial team sports, which requires conditioning, coordination, and also athletes' psychological and mental abilities. Also, ice hockey is characterized by high intensity intermittent skating, rapid changes in velocity and duration, and also frequent body contact (Montgomery 1988). The success in this spectacular sport mainly depends not only on player efficacy, but more on team efficacy, understanding, communication, cooperation, and team performance and humbleness (Feltz & Lirgg 1998). The purpose of this dissertation was to analyze and determine motor and psychometric factors which are the greatest determining factors regarding the successful selection of the U18 National team. We analyzed the differences between the Selected and Non-selected players, comparing the results of both groups. Furthermore, we wished to provide direction to coaches in practices, so that they can force adequate skills improvement during the youth development program. All players that attended this try out were preselected and sent by club coaches to participate in the study, so altogether, 40 U18 players did all the on-ice and off-ice motor tests and filled out all psychometric measures. From the players that participated in this particular study, 20 were U18 National Team Selected members (Mage=16.45, SD=0.512) and another 20 were Non-selected players (Mage=16.62, SD=0.50). In order to answer the research questions, a number of motor and psychological tests were administered. Nineteen on-ice and off-ice motor tests were processed. Also used were the Athletic Coping Skills Inventory (ACSI-28), Perceived Motivational Climate in Sport Questionnaire-2 (PMCSQ-2), Sport Motivation Scale (SMS), State-Trait Personality Inventory (STPI-Y). Descriptive data for all variables are described by mean (M) and standard deviation (SD). In order to answer the results questions, data of Selected and Non-selected ice hockey players were statistically compared. For the comparison, an Independent T-test was conducted. Additionally, after checking for the normality and homogeneity of variance assumptions, discriminant analysis was calculated to build a predictive model of group membership. Stepwise discriminant analysis was used for differentiating motor and psychometric differences between Selected and Non-selected U18 players. The main aim of the study was to ascertain the determining factors regarding successful selection to the U18 National ice hockey team with regard to the examined participants. According to T-test, there were significant differences between Selected and Non-selected U18 players in these tests: Crossover test with puck, Passing skill drill (sec), 1500 m track, Freedom from worry and Unequal recognition. Discriminant analysis showed that the following discriminating factors were in the sample: Unequal recognition, Concentration, Peaking under pressure, BMI, 36 meter backward skating, Freedom from worry, Trait depression and Intra-team member rivalry. Further studies require an understanding of the motor and psychometric characteristics of elite young players. Hence, it is worth examining how age plays a factor in motor and psychometric variables (U16, U18, U20, adult team). Furthermore, longitudinal studies might be useful for understanding the processes and development stages of the young player, which can help the planning

process. In addition, comparisons with players from sports other than ice hockey may be useful for talent development purposes.

- Géczi G, Bognár J, Tóth L, Sipos K, Fügedi B (2008) *Competitive state anxiety, athletic coping strategies, and state-trait personality of different age groups of Hungarian national ice hockey players*. *Int J Sports Sci Coach* 2: 277–285.
- Trzaskoma-Bicsérdy G, Bognár J, Révész L, Géczi G (2007) *The coach-athlete relationship in successful Hungarian individual sports*. *Int J Sport Sci Coach* 2: 484–495.
- Tóth I, Géczi G (2006) *Slovensko sucastou viacerych hokejovych filozofií v madarsko hokeji*. *Hokejovy Tréner* 2: 28–45.

## **SZILVIA GITA (DIMITRIOU) (2009)**

### **Education of children with disabilities in integrative/inclusive settings in Hungary**

*Supervisor: József Bognár*

This study demonstrated that the education policy in Hungary now includes the practice of inclusive education. Data were obtained through an analysis of curricula in addition to the results of a questionnaire. Today, it is no longer a question of whether the inclusion is good or can be applied, but rather one of how to do it. Practices which have already been adapted ensure that this kind of development or challenge can be applied in Hungary, but there are several changes that are necessary to do so. As we revealed in our research, teachers, who play a key role in this process, are afraid of changes. First of all, this comes from a lack of education policy, because teachers are not well prepared for this new pedagogical practice, and on the other hand, teachers, schools and students would need much more help and support. In addition, the current infrastructures of the schools do not allow for participation for all, especially for children with disabilities in mainstream schools, as they are not barrier free. The central control should enforce more laws and acts on social integration and inclusion that support children with disabilities in mainstream schools. This movement cannot only be the concern of a few people; everybody should be working hard on it to make it available for everyone. Teachers must be open to the possibility of inclusion; they have to help in its realization. It is necessary to change their attitudes towards positive thinking, to improve their theoretical and practical experiences and to adapt their teaching styles. Based on the results of a curriculum analysis, we can state that not only teachers but the central education control should be changed as well. Teachers need such curricula and supplements that can be easily used and applied in the planning process, and these documents should provide real help to the problems teachers face as they implement this new pedagogical practice. There is a need for further teacher education programs, the collection and dissemination of information, and close cooperation between schools and central control so that the practice of inclusion can be fitted into the life of a school without any problems. The support of teachers, parents and students should be more active, because they would all be trying to invent something new that would help and improve their practice and knowledge. They would also help the improved and smoother dissemination of the practice of inclusion, because without it, inclusion can not be successful in Hungary.



- Gita Sz, Bognár J, Dorogi L, Kälbli K, Rigler E (2006) *Az integráció helye és szerepe a hazai pedagógiai gyakorlatban. Magyar Sporttud Szemle 6: 15–20.*
- Gita Sz, Kälbli K, Rigler E (2006) *EU policies and legislations for social integration for people with disabilities. Kalokagatia 44: 137–145.*
- Gita Sz, Kälbli K, Bicsérdy G (2004) *Sport and disability: comparative study of European countries. Magyar Sporttud Szemle 4: 41–43.*

## BEATRIX GEOSITS (KISSNÉ) (2010)

### **Sport, career choice and mental well-being among professionals in pedagogy and education**

*Supervisor: Teodóra Tomcsányi*

The purpose of this thesis was to examine the connection between sport, the choice of profession and psychical health. The primary innovation of the thesis is that such a research, which deals with and analyzes the relationship of sport, physical activity and the exposure of burnout, have not been published yet in the Hungarian specialized literature.

1. This study proves that among people who are averagely or highly exposed to burnout, higher rate do not do any sports. Among those, who are not exposed to burnout, the rate of people who do sport and not do sports is balanced. Sport can protect from burnout. A particular feature of the Western Transdanubian model is that the youths do less sport than elderly. In connection to this, it can be said that the older generation is less likely to burnout. But it is frightening that only 2 per cent of the male teachers do sports regularly, while 35 per cent of the females do. Experts in leading positions are level with each other at 50%. The protective side of sport does not play a role in the case of male teachers.
2. It can be said about the questioned Western Transdanubian teacher population, that in consideration of professional help, they have a chance to get it and they also make use of it. The high participation rate on courses and professional further trainings may reflect that the teaching profession is continuously renewed and the need for meeting the newer and newer requirements. These are some characteristics which are closely related to the teaching profession. It shows a much better situation in this region than in other parts of the country. The high participation rate on the supervision and case discussion shows that we should not focus on the certain units (students, groups of students) of our profession individually but like part of a system. Moreover, we should realize that the whole system changes if one part changes.
3. Those teachers who are tangible and interested in systematic work are the most exposed to burnout and it does not depend on the fact that the direction of their present work interest is the same as they had it in their childhood or not. They are more exposed to burnout than those ones who have other work interest priority and those ones who had appropriate direction of work interest in childhood. The study does not prove the positive connection between the direction of work interest in childhood and at present.
4. Women were more influenced by their parents. The mother's model has the biggest influence on the choice of career. Furthermore, aunts and sisters have strong impact, as well.
5. Relationship between the period spent in the profession and burnout were examined. It can be seen that while time spent in profession is growing, the exposure to burnout

does not increase at all. On the contrary, it decreases. The reasons of the resulting figures can be that teachers who are exposed to burnout are likely to give up their profession. So, the rate of professionals who are exposed to burnout is higher in the younger generation and lower in the older generation.

- Kissné Geosits B (2009) *Some important aspects of mental higienic state of teachin professionals*. Pp 304–319 in: *Research in sport sciences. Performance analysis*. Univesity of Wales Institute, Cardiff School of Sport, Cardiff. ISBN 1-901288-00-13.
- Kissné Geosits B, Teleki B (2005) *Családi életre nevelés*. Korda Könyvkiadó, Kecskemét. ISBN 9639554332.
- Geosits B, Kardos B (1999) *Kézikönyv pályaaorientációs trénereknek*. Reginnov Tanácsadó Kft., Vasvár. ISBN 9630385406.

## MIKLÓS KOLTAI (2009)

### Examining the efficiency of collective task-solving

*Supervisor: József Bognár*

Through the examination of the paradox of co-operation—competition, multiple international and national comparative analyses have been performed. In the field of team sport—where the existence of the 2 phenomena can be an obvious and probably a definite factor—there have not been any surveys carried out regarding this topic so far. Volleyball is a team sport with a unique term-system. There is no effective possession of the ball; only the teammates can make adjustments, but this presumes effective co-operation. The common task-solving of the team presumes a high level of perception-motor control, effective anticipation and communication, rapid decision-making and a game-theoretical way of thinking. The aim of the survey is to examine the competitive attitude of Hungarian front-rank women volleyball players, to define the competitive characters and to determine the relationship between certain indicators and efficiency. In addition, we conducted in-depth interviews with the most successful volleyball coaches to find out what kind of professional, methodological and pedagogical experience can be obtained from the training and competing of players. The main finding of our survey is that co-operation and competition are two important factors which are both present in volleyball. This field is not part of the training of coaches. Successful coaches apply pedagogical methods for eliminating harmful competitive attitudes. Women volleyball players in the sample have different competitive attitudes, their HCA and PDCA averages are beyond the levels published by other authors, and their attitude of mind fit the social requirements. Training factors do not have an effect on the setting of values. Efficiency is mainly influenced by the volleyball age, the high PDCA level and the fact that the player plays in the National Team. Players with the best competitive samples are among the first four in the ranking. An extra level of physical, technical and tactical skills is a basic requirement nowadays for players competing at the sport's most elite levels. Team performance can be made more efficient if trainers would consider the different competitive samples among the personal characteristics of the players when selecting them for certain positions. It is worth re-thinking the professional methodological questions of volleyball. A high-level symbiosis of cooperation and competition in volleyball is indispensable. The key factor for success is the efficiency of common task-solving.

- Koltai M (2008) *A kétféle versengési attitűd és az együttes feladatmegoldás. A versengési karakterek meghatározása csapatsportokban. Kalokagathia 1: 74–93.*
- Koltai M (2009) *Examining the efficiency of collective task-solving among Hungarian volleyball players. Int Quarterly Sport Sci 4: 21–27.*
- Gécz G, Vincze G, Koltai M, Bognár J (2009) *Psychometric measures in elite young team-sports in Hungary. Phys Cult Sport Stud Res 46: 229–242.*

## KINGA MAJOROSS (2009)

### Coeducated sporting: the relationship of couples in ballroom dancing

Supervisor: Kornél Sipos

To be successful in the world of ballroom dancing, a high-level co-operation with the dance partner is necessary. While dancing, a man and woman are in close physical contact, and a special and intimate relationship is formed between them; its frequent consequence is that partners fall in love with each other, and this influences their dancing performance. But almost none of the available literature has concentrated on the relationships between dance partners.

The research method included a questionnaire, psychological tests (measuring personality, anxiety, emotional-social intelligence and conflict management) and the Couple Rorschach Test (the dance partners had to formulate a mutual response to each of the ten Rorschach tables). 226 adult ballroom dancers (113 of which were male and 113 were female) who specialize in various dancing styles (standard and Latin American) and categories (D, C, B, A and S classes) from all over Hungary participated in the research.

The results clearly show that the partner's importance (skills, personality) and the co-operation with him or her are all essential for the dancers' success in competitions. The development of the relationship between dancers have the same stages as those of romantic relationships (marriages): from the initial harmony through the stormy period of distributing roles (dominance), to long-term, effective co-operation.

Most of the dancers (49 per cent) considered their partners good friends, but it was frequent (22 per cent) that they had become couples in real life as well (and 48 per cent had already had a romantic relationship with at least one of their dance partners), and relatively few (15 per cent) consider this relationship a work-type relationship, a kind of co-operation needed for success. Romantic relationships between dance partners have a controversial effect on performance, and there is a great danger that they will stop dancing together after breaking up.

The Couple Rorschach Test seems to be a well functioning, reliable method for mapping the relationship between dance partners and their conflicts. Partners in a good relationship communicate more with each other and show higher rates of co-operation (accepting one another's response and building on it), while partners having a bad relationship try to rely on their own responses while providing responses to the test.

- Majoross K, Bartha C, Hamar P, Puhl S (2008) *The attention capabilities of football referees as determined by Pieron's Test. Stud Univ Babes-Bolyai, Educ Artis Gymn 53: 3–13.*
- Majoross K, Hamar P, Dózsa I, Dancs H (2008) *The relationship of couples in competitive dancing. J Human Sport Exerc 3: 12–24.*

- Majoross K (2008) *Versenytáncosok partnerkapcsolatának elemzése Közös Rorschach Vizsgálattal. Alkalm Pszichol* 10: 125–143.

## NIKOLETTA ONYESTYÁK (2010)

### **The relationship between sport and politics in Hungary during the 1980s as reflected in the summer olympic games**

*Supervisor: Sándor Székely*

Since World War II through the Soviet-American cold war fray in the 1980s, sport functioning, as the catalyst of the political, social and ideological relations of nations, became a preferred tool in making adjustments to international conflicts. In socialist Hungary the main sport area which produced political questions was the Summer Olympic Games, in point of which the dependent position on the Soviet Union was determinative, but in regards to decision-making, the main Hungarian sport and political institutions' activity was important.

The main aim of the dissertation was the exploration of the Hungarian dilemmas and decision-making mechanisms related to the Summer Games of the 1980s, on the basis of the MSZMP, OTSH, MOB, home and foreign affairs authorities' archive documents, the Hungarian press, and the interviews made with top athletes and sport leaders of the time. On strategic questions, the Central and Political Committees of the Hungarian Socialist Workers' Party decided about Olympic participation, political preparations and tasks related to the games on the basis of Soviet instructions. The resolutions were forwarded towards those executive organizations which disposed of entitlement in Olympic sport questions, but the presence of MSZMP party members there fomented the implementation of the decisions.

The Hungarian Ministry of Foreign Affairs, through its delegated diplomats, could continuously collect information about foreign countries' situations, their Olympic plans, and their activities related to the Games, which were used by party authorities to make their socialist-aligned policy. Within the Ministry of Home Affairs the organizations of state security dealt with intelligence and preventive tasks in the support and defense of the political decisions.

The official Hungarian press also stood in the service of party interests; in all socialist countries it was used to confirm the correctness of the sport-related policies. Before the Olympic Games, the newspaper articles were based on Soviet rhetoric; after the decision about Los Angeles non-participation, the reports for the year's sport events were oriented by the MSZMP Political Committee's guidelines.

In the 1980s the Olympic Games, and through them, Hungarian sport, also fell prey to big political fights, but it was able to rise from its ashes, as the leaders of the world's greatest powers realized that the international political conflicts must be solved in other fields. In 1989–1990 the change of the world order and the series of system-changes created new frameworks and possibilities for international sport relations. After the boycotts of the Moscow and Los Angeles Olympic Games, Seoul and Barcelona demonstrated that sport can be a tool of rapprochement for nations and could contribute to the preparation of the divided nations' reunification and the political acknowledgement of the newly independent nations.

- Onyestyák N (2009) *Party, state security and the 1984 Olympic Games*. Pp 256–266 in: Higgins M, Dancs H, Nagyvárad K (eds), *Research in sport science*. Cardiff.
- Onyestyák N (2009) *Partido, Organos de la Seguridad del Estado en Hungría y los Juegos Olímpicos de 1980 y 1984*. *Citius Altius Fortius* 2: 101–123.
- Onyestyák N (2010) *Boycott, exclusion or non-participation? Hungary in the years of the 1920 and 1984 Olympic Games*. *Int J Hist Sport* 27: 1920–1941.

## SZILVIA PERÉNYI (2010)

### Human values of sport participant and non-participant Hungarian youth

*Supervisor: Gyöngyi Szabó (Földesiné)*

The relationship between general human values and sport participation has not yet been the focus of research using representative samples in Hungary. The aim of this dissertation was to analyse the value preferences of participant and non-participant Hungarian youth and to describe their orientation related to human values. It was assumed that there would be differences between the sub-groups in the hierarchical order and the strength of consideration given to values, which would also be reflected in the relationships to the formulated value groups, and also along the categories of socio-demographic variables. The theoretical background of the dissertation was based on the value theories of Schwartz and Inglehart and on the theories of Bourdieu, Beck and Schulze. This empirical research was conducted among the 15–29 year-old youth population of Hungary, and focused on sport participation outside of the premises of physical activity classes. Statistical analysis and data collection was completed on three national representative samples of 8000 participants each, during the beginning, middle and end of the decade from 2000–2010. Data were analyzed with the help of parametric and nonparametric tests and multidimensional statistical methods. The results showed that youth participating in sports prioritised postmodern values representing self-direction, self-actualization and personal autonomy, and gave lower preference to materialistic values than non-participants, findings which were also maintained along the categories of the socio-demographic variables, regardless of the social determinations of human values. It seems that the sport participant/non-participant dichotomies stand for the value orientation dichotomies of postmodern/modern values outlined by Inglehart, and the cultural/economic capital stated by Bourdieu. The democratisation of sport participation also resulted in connections to new value groups. Cultural capital and the sport participation variables contributed the most among the socio-demographic variables to the formulation of value groups. These results were also reflected in the higher priority given to the realisation of democratic rights and to the acceptance of societal norms, which were accompanied by a more positive image of the future, a higher ability to assume risks, and the more modern state of mind of the sport participants. Consequently, the results of this dissertation have confirmed the association between value socialisation and sport; however, the complexity of societal value transition does not allow cause and effect conclusions to be drawn.

- Perényi Sz (2010) *On the fields, in the stands, in front of TV—value orientation of youth based on participation in, and consumption of, sports*. *Eur J Sport Soc* 7: 41–52.

- Perényi Sz (2010) *The relation between sport participation and the value preferences of Hungarian youth. Sport in Society* 13: 984–1000.
- Perényi Sz (2008) *A sporttevékenység és az értékorientáció összefüggései fiataloknál. Új Ifjúsági Szemle* 21: 65–74.

## LÁSZLÓ RÉVÉSZ (2009)

### Analyses of relevant issues of talent development, selection and success in swimming

*Supervisor: József Bognár*

Within the framework of this research, we examined the features of the Hungarian swimming sports in terms of the choice of the branch of sport, selection and the nurturing of talented persons. From among the factors being the most critical for efficiency, psychic elements first of all emerged. The most important elements include the tolerance of stress during competitions and trainings, achievement motivation as well as the tolerance of stress completed by the good relationship between coach and athletes. It is an interesting outcome that the conditional abilities have not appeared dominantly, i. e. according to the coaches, these are not responsible for the main differences. After assessing the athletes (N=424), we saw a result worth making note of related to the selection of the branch of sport, for which the parents, friends and the coach have the most important role; the influence of the teachers in physical education has, however, been hardly perceivable, and only a few chose swimming under the inspiration of the PE teacher. In connection with training it can be stated that most persons have learnt to swim within the framework of courses, but swimming training in sports clubs is also dominating. In the opinion of the athletes, the skill of the coach and their diligence, the assistance by the parents and the intrinsic motivation are the most critical factors for efficiency. In relation to these choices we stated that some tests are applied to these election procedures by only 40% of the coaches (N=70); furthermore, it is not typical in Hungarian practice that these tests be carried out by the coaches on a permanent basis. Instead, a selection “based on success” is still typical. For the examination of success we used the data of the ORV survey executed in 1984 (N=351). During the analysis it was established that in the selection process the tests for branches of sports should mainly be applied, since the differences between those being successful and unsuccessful can be determined particularly by these tests. The outcomes of the anthropometric survey and the general physical abilities tests did not show any significant differences. According to the examination of the psychic factors, task-orientation is the most typical of the athletes, where the effort for improvement was the most important part, namely, the athletes are motivated for further achievement, but at the same time, it turned out that this motivation is of extrinsic origin, and the extrinsic motivation level of the athletes is higher. It is typical of the coping skills that the athletes performed above average, but their levels of coachability, self-confidence and achievement motivation were outstanding.

- Révész L, Bognár J, Géczi G (2007) *Kiválasztás, tehetség és pedagógiai értékek az úszásban. Új Pedagógiai Szemle* 4: 45–53.
- Trzaskoma-Bicsérdy G, Bognár J, Révész L, Géczi G (2007) *The coach-athlete relationship in successful Hungarian individual sports. Int J Sport Sci Coach* 2: 484–495.



- Biro M, Fugedi B, Revesz L (2007) *The role of teaching swimming in the formation of a conscious healthy lifestyle: a case report. Int J Aquat Res Educ 1: 269–285.*

## ISTVÁN SIMON (2010)

### **Investigating adapted physical education in the network of a new type of teacher–student relationship**

*Supervisor: János Gombocz*

The aim of the present research was to investigate Adapted Physical Education (APE) as a school subject in a comprehensive way from a pedagogical point of view and to search for the pedagogical tools that influence its effectiveness. By analysing the relationship between teachers and students and the factors affecting it, we would like to show professionals in the field of APE an alternative that makes effective work possible. The research was carried out in the region of Western Hungary (Sopron, Szombathely and their catchment area). Since APE has a special position in education, there are several factors that influence the work carried out in these lessons. This special position has called for a multi-faceted approach. Emphasis was put on examining the relationship between teachers and students from different sides, but external factors influencing the educational and teaching process were also analysed. The survey involved a look at the knowledge teachers, school leaders and peers of APE students possess about APE and the way they relate to this subject. The teacher–student relationship in APE lessons was examined by means of categorical observation as well as questionnaires completed by teachers and students. In summarizing the results of the study, we can state that a renewal of APE is to be achieved only if APE teachers possess positive characteristics, a calling for their job, appropriate professionalism and a large amount of love for children, and that they are supported by parents, the school leadership, teachers and peer groups. All the features listed above prevail only if there is a well-cooperating team of school leaders, school doctors, parents and APE teachers behind the work. The results of the study also point out that even if these conditions are met, APE teachers remain isolated in their work unless APE is propagated widely within professional circles and society.

- Simon I, Kajtár G (2009) *Teacher–student interactions in PE and Adapted PE lessons by the method of categorical observation. Pp 21–33 in: Hughes M, Dancs H, Nagyvaradi K (eds), Research in Sport Science, Szombathely, Cardiff.*
- Simon I, Fügedi B, Kajtár G (2009) *Special features of teaching activities and behaviour of school-children attending adapted physical education classes. Phys Educ Sport 53: 161–165.*
- Simon I, Fügedi B, Bognár J (2009) *Adapted physical education in the Hungarian educational system: a conceptual framework. J Human Sport Exerc 5: 453–461.*

## JÁNOS VÁCZI (2010)

### **Innovative Hungarian sports marketing program to increase government Funding for Sports**

*Supervisor: Mihály Nyerges*

What are the key factors from the sports industry's point of view that influence the direction of activities in the sports market? The results of our research indicated that these factors include sports facilities, sports administration, federation and club management, innovative elements (selection and youth development, sports medicine, human resources, sports science, sports information system, sports diplomacy, sports marketing, sports communicational strategy). But to do this work efficiently, we need increased financial resources. The remedies which were designed to alleviate the problems of funding either focused on the better distribution of funds, or true investment encouragement measures which never got off the ground during the implementation phase. I assume that additional funds necessary to supplement the government budget to finance Olympic sports federations can be attained by increasing the revenues from the lottery. In my opinion, the available funds supporting the Olympic federations could be increased by a new product of the lottery market called Olympic Lottery. To support my hypothesis, I used a primary data collection method by conducting seven structured expert interviews. I interviewed three types of decision makers: political decision makers, sports economists and representatives from the sports industry. By selecting the respondents, I intended to fully cover all aspects of sports financing by finding people with relevant and appropriate knowledge. The interviews confirmed that with the implementation of the Olympic Lottery program, more income could be garnered in such a way that it would serve as one of the most stable revenue sources for Olympic sports federations. According to my calculations, if the Olympic Lottery could reach the predicted 8% market share (it is the current share of Scandinavian Lottery) of Szerencsejáték Zrt., then it would mean 12.56 billion HUF of revenue for the company. The 12% gambling tax of this revenue would yield approximately 1.5 billion HUF. This sum would go directly to the Olympic sports federation via two channels. 80% of this money should be allocated, based on the financial structure and strategy developed and used by the Hungarian Olympic Committee and the State Secretariat for Sports. The remaining 20 percent would be distributed based on players' preferences, so that money would go to support the most popular sports.

- Váczi J, Berkes P (2009) *Az állami sportfinanszírozás új útjai. Magyar Sporttud Szemle* 3/4: 42–44.
- Váczi J, Berkes P (2009) *Integrating communication and marketing efforts to influence governmental sport funding: an analysis of an innovative sport-financing program in Hungary. Int J Sport Comm* 3: 12–26.
- Váczi J, Berkes P (2009) *Húzzunk sportágakat: avagy a sportfinanszírozás új útjai. Marketing és Menedzsment* 3: 38–44.

**GÉZA VINCZE (2009)**

**The impact of the 1989–1990 transition on football talent-care**

*Supervisor: Mihály Nyerges*

Football is clearly the most popular sport on the international sport scene, and probably the only one showing complete international co-operation. Football is also a popular research area of sport science; however, there is little literature on youth and talent-care. In the past few decades, Hungarian football has lost its popularity and social acceptance. According to experts, the fall of football can only be stopped through the development of talent-care. In this Ph.D. thesis, the author set out to examine the changes occurring in Hungarian football in the two decades following the 1989–90 transition with a complex approach. The main objective of the research work was to answer the following questions: What kind of characteristics can be discovered in post-transition Hungarian sport and football? How did the turn in sport policy and sport financing have an impact on talent-care? How do the training systems established in support of talent-care function concerning theory and practice? What are the attitudes of the professional elite working in football to talent-care? What opportunities are available to improve the talent-care system, and its efficiency? In this research work, three complementary methods were combined: field work, content analysis, and in-depth interviews. The research process was characterised by the unity of analysis and evaluation, and continuous interpretation. During work, the qualitative data analysis was of primary importance. The most important results of the thesis were as follows: After the transition, the governing parties did not establish new and self-invented sport policy; rather, they took over the theory and practice of the formerly failed centralized governing system. Following the transition, the in-school training of footballers ceased and is still absent, and basic elements of the game are barely taught. Physical education teachers were excluded from the talent-care system by the leading bodies, since dealing with children was displaced from schools. Most professionals regard the old, so-called dormitory-system to be better than the new system of academies. There are major differences in the local-government financing of professional football clubs. From outside, many professional football clubs seem to be money-laundering businesses, which the local governments try to avoid as much as possible. It is a dysfunctional consequence that the local government subsidy designed for talent-care is used by clubs for other purposes. The position of talent-care is usually disadvantageous; in many cases, it does not work independently of the business directing the club, which prefers the senior, professional squad. The independent talent-care clubs are in the most unfavourable situation, as local authorities regard them to be sports enterprises, and in most cases they do not support their activity. In the conclusion, it is stated that Hungarian sport had not been at all prepared for the transition, and in the following two decades, a comprehensive national sport strategy was not prepared. Even though short-term conceptions were drawn up, they did not prove to be efficient. This statement is even more valid concerning football talent-care. Senior teams were given help through politics on local as well as national levels, however, talent-care did not have political weight, and thus, it did not have promoters either. The author's suggestions based on the results of the research may give assistance in renewing the system of talent-care and in ensuring its more efficient functioning.

- Vincze G (2005) *Proclamation for Hungarian sport*. Pp 17–27 in: Földesi Szabó G, Gál A (eds), *New social conditions in sport 1990–2005*. Hungarian Society for Sport Sciences, Budapest.
- Vincze G, Fügedi B, Dancs H, Bognár J (2007) *The effect of the 1989–1990 political transition on the developing and training of football talent in Hungary*. *Kinesiology* 40: 50–60.
- Bognár J, Géczi G, Vincze G, Szabó A (2009) *Coping skills, motivational profiles, and perceived motivational climate in young elite ice hockey and soccer players*. *Int Quarterly Sport Sci* 1: 1–11.

---

# SCHOOL OF PH.D. STUDIES

---

## 6. JÁNOS SZENTÁGOTHAÏ NEUROSCIENCES

---

***Chairman:***

**Dániel BERECKZI M. D., Ph. D., D. Sc.**

Department of Neurology

6 Balassa st, Budapest H-1083

Tel.: +36 1 210 0337

E-mail: [berecki.daniel@med.semmelweis-univ.hu](mailto:berecki.daniel@med.semmelweis-univ.hu)

### **General overview**

The Neuroscience Graduate School blends the theoretical and clinical neuroscience research topics, treats the basic questions of the structure and function of the nervous system in a synthetic view as well as the normal and pathological functioning of the human brain as observed by the clinicians. The research topics as listed below and arranged in three basic science and three clinical research Programs witness the large array and variation of supply.

### ***PROGRAM 6/1.***

---

#### **NEUROMORPHOLOGY AND CELL BIOLOGY**

***Coordinator:***

**Miklós PALKOVITS M. D.,**

**member of the Hungarian Academy of Sciences**

Department of Anatomy, Histology and Embryology

58 Tűzoltó st, Budapest H-1094

Tel.: +36 1 216 0488

E-mail: [palkovits.miklos@med.semmelweis-univ.hu](mailto:palkovits.miklos@med.semmelweis-univ.hu)

### **Program overview**

This basic research program of the Neuroscience Doctoral School covers a broad spectrum of the examination of the central nervous system—from the function and differentiation of the individual neurons to the higher cortical activity. The large variety of topics shares the methodology (neuromorphology, neurochemistry, molecular cell biology, synaptology), the functional view of the topics, and the use of the rich selection of functional neuromorphological methods. The program includes research areas intended to better understand the organization of neural tissues, neural differentiation, and the plasticity of the central nervous system. Within the program research areas cover molecular genetics, experimental neuromorphology, and the studies of normal and pathological (neurodegenerative diseases) human brains. Cytological, neuromorphological and neurochemical (immunohistochemical) areas of the program are tightly connected both with the regulatory mechanisms of the autonomous nervous system (stress, pain, food uptake, thermoregulation) and with topics evaluating the higher order functions of the central nervous system (information processing, emotion, learning, motivation, memory).

***Titles of research projects***

Localization of trigeminal pain-induced stress pathways  
(trigemino-hypothalamic pathway)

Neuroanatomical and neurochemical characterization of  
bidirectional neuronal pathways between the nucleus  
accumbens and the lateral hypothalamus

Central regulation of the food intake

Morphological and *in vivo* physiological analysis of excitatory  
and inhibitory inputs in the thalamus

Subpallial systems in relation to avian learning

Comparative neuroanatomical basis of addictive behaviour in  
avian and mammalian model systems

Transforming growth factor beta proteins in the central nervous  
system

Central amylin as a novel maternal neuropeptide

The role of the TIP39-parathyroid hormone 2 receptor neuro-  
modulator system in the central control of maternal adaptations

Endocannabinoids in the brain: a novel communication channel  
between neurons

Study of synaptic plasticity in the spinal cord of normal, inflamed  
and nerve injured rats

Functional characterisation of hippocampal inhibitory neurons  
using a combined anatomical and physiological approach

Immunohistochemical monitoring of ependymal histogenesis

Position and function of G protein-coupled receptors in neuronal  
networks

Neural cell differentiation. *In vitro* cell technology

Epileptic reorganization of the human hippocampus

Pathomechanism of the epileptic cell loss: regulation of inhibitory  
processes via receptors and membrane proteins

Synaptic information processing in the olfactory bulb

Neuronal and glial architecture of the conus medullaris and filum  
terminale

Functional anatomy of pre- and postsynaptic receptors and their  
neurotransmitters in the hippocampus

Role of prolactin-releasing peptide in the central nervous system

Development and functional organization of the extracellular  
matrix in the central nervous system of the chicken and the rat

Neurobiology of tactile functions: Combined morphological and  
physiological studies on primate cerebral cortex

***Supervisors***

Miklós Palkovits

Miklós Palkovits

Miklós Palkovits

László Acsády

András Csillag

András Csillag

Árpád Dobolyi

Árpád Dobolyi

Árpád Dobolyi

Tamás Freund

Gábor Gerber

Attila Gulyás

Mihály Kálmán

István Katona

Emília Madarász

Zsófia Maglóczky

Zsófia Maglóczky

Zoltán Nusser

Miklós Réthelyi

Gábor Nyíri

Zsuzsanna Tóth

Alán Alpár

László Négyessy

***Ph.D. students***

Mária Márta Ashaber pt

Gyula Balla ft

Csaba Cserép ft

Melinda Cservenák ft

Barna Dudok ft

***Supervisors***

László Négyessy

István Katona

Gábor Nyíri

Árpád Dobolyi

István Katona



János Hanics	pt	András Csillag
Mária Rita Karlócai	ft	Zsófia Maglóczky
Margit Katalin Kerti	ft	Zoltán Nusser
Katalin Könczöl	ft	Zsuzsanna Tóth
Dávid Lendvai	pt	Alán Alpár
Guillaume Lourmet	pt	Miklós Palkovits
Máté Neubrandt	ft	Emília Madarász
Rege Sugárka Papp	ft (a)	Miklós Palkovits
Károly Imre Pócsai	ft	Mihály Kálmán
Éva Renner (Dobolyiné)	ft	Miklós Palkovits
Zita Rovó	ft	László Acsády
Tamás Varga	pt	Árpád Dobolyi
Csilla Vincze	pt	Árpád Dobolyi
Éva Rebeka Szabó	ft	Árpád Dobolyi

***Ph. D. candidates***

Ágota Ádám	pt
István Adorján	ft
Tamás László Balázs	ft
Nóra Hádinger	a
Anikó Ludányi	pt
Barbara Orsolits	ft
Eszter Szabadits	ft
Kinga Tóth	ft
Virág Takács Tresó	ft
Anita Zádori	ft
Attila Bagó	a

***Ph. D. graduates***

Miklós Antal	ft
Eszter Bálint	ft
Ágnes Bodor	ft
Csaba Boros	pt
Kristóf Giber	ft
Anikó Reichart Guttman	pt
Mihály Köllő	ft
Márk Kozsurek	ft
Károly Markó	ft
Tünde Molnár	pt
Rita Nyilas	na
Andrea Slézia	ft
Adrienn Szabó	pt

a, absolutorium; f, full-time; pt, part-time; na, not affiliated

***Supervisors***

András Csillag
Mihály Kálmán
Miklós Palkovits
Emília Madarász
István Katona
Zsuzsa Környei
Gábor Nyíri
Zsófia Maglóczky
Attila Gulyás
Emília Madarász
Árpád Dobolyi

***Supervisors***

Zoltán Nusser
András Csillag
László Acsády
Miklós Réthelyi
László Acsády
Miklós Palkovits
Zoltán Nusser
Gábor Gerber
Emília Madarász
Miklós Palkovits
István Katona
László Acsády
Mihály Kálmán

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**MIKLÓS ANTAL (2009)**

### **Functional and structural diversity of external tufted and deep short-axon cells in the main olfactory bulb**

*Supervisor: Zoltán Nusser*

To understand the cellular and synaptic mechanisms of olfactory information processing, the intrinsic properties and synaptic connectivity of the different types of nerve cells in the main olfactory bulb need to be deciphered. The main objective of my work was to identify whether heterogeneity in structural and functional properties as well as synaptic connectivity of certain nerve cell populations in the main olfactory bulb correlate with each other, resulting in well defined subpopulations of cells; or whether these measured features of the cells vary independently, resulting in a single but highly diverse population. First, I determined the active and passive electrical properties of external tufted cells (ETCs) using *in vitro* whole-cell recordings and then I correlated them to their dendritic arborization patterns. Principal component followed by agglomerative cluster analysis revealed two distinct subpopulations of ETCs based on their electrophysiological properties. Eight out of twelve measured physiological parameters exhibited significant difference between the two subpopulations, including the membrane time constant, amplitude of spike after-hyperpolarization, variance in the inter-spike interval distribution and sub-threshold resonance. Cluster analysis of the morphological properties of the cells also revealed two subpopulations, the most prominent dissimilarity between the groups being the presence or absence of secondary, basal dendrites. Finally, clustering the cells taking all measured parameters into account also indicated the presence of two subpopulations that mapped in an almost perfect one-to-one fashion to both the physiologically and the morphologically derived groups. My results demonstrate that a number of functional and structural properties of ETCs are highly predictive of one another. However, cells within each subpopulation exhibited pronounced variability, suggesting a large degree of specialization evolved to fulfill specific functional requirements in olfactory information processing.

A universal feature of neuronal microcircuits is the presence of GABAergic interneurons that control the activity of glutamatergic principal cells and each other. In the second part of my work I used a combined electrophysiological and morphological approach to investigate a rather mysterious cell population of the main olfactory bulb. Deep short-axon cells (dSACs) of the inframitral layers are GABAergic and have extensive and characteristic axonal ramifications in various layers of the bulb, based on which unsupervised cluster analysis revealed three distinct subtypes, the glomerular (GL-), external plexiform (EPL-) and granule cell layer- (GCL-) dSACs. Each dSAC subtype exhibited different electrical properties, but received similar GABAergic and glutamatergic inputs. The local axon terminals of all dSAC subtypes selectively innervated GABAergic granule and periglomerular cells and evoked GABAA receptor-mediated inhibitory postsynaptic currents. One subpopulation of dSACs (GL-dSAC) creates a novel intrabulbar projection from deep to superficial layers. Another subpopulation (GCL-dSAC) was labeled by retrogradely-transported fluorescent microspheres injected into higher olfactory areas, consti-

tuting a novel projection-cell population of the main olfactory bulb. My results reveal multiple dSAC subtypes, each specialized to influence main olfactory bulb activity by selectively innervating GABAergic interneurons, and provide direct evidence for novel intra- and extrabulbar GABAergic projections.

- Antal M, Eyre M, Finklea B, Nusser Z (2006) External tufted cells in the main olfactory bulb form two distinct subpopulations. *Eur J Neurosci* 24: 1124–1136.
- Eyre MD, Antal M, Nusser Z (2008) Distinct deep short-axon cell subtypes of the main olfactory bulb provide novel intrabulbar and extrabulbar GABAergic connections. *J Neurosci* 28: 8217–8229.

## ESZTER BÁLINT (2009)

### Morphological analysis of dorsal and ventral striatal neuronal systems of the domestic chick (*Gallus domesticus*)

Supervisor: András Csillag

The spatial relation between the dopaminergic and dopaminoceptive structures of the avian medial striatum (MSt) was observed by confocal laser scanning microscope in the domestic chick, as well as the connections in the area ventralis tegmentalis (VTA) and the substantia nigra (SN). Dopaminergic (TH+) fibres formed baskets around the dopamine- and adenosine-regulated phosphoprotein-containing (DARPP-32+) cells of MSt. DARPP-32+ varicose fibers innervated VTA and SN and were often juxtaposed to TH+ structures. Approximately 40% of the striatal projection neurons targeting VTA, and 60% of striatonigral projection neurons were DARPP-32+, as revealed by retrograde pathway tracing. The anatomical findings, in particular the abundance of juxtapositions observed in the avian brainstem and the MSt do not rule out the possibility of reciprocal circuits connecting pairs of striatal and tegmental neurons.

Quantitative electron microscopy of chick specimens double-labeled against glutamate (GLU) and DARPP-32 revealed direct synaptic connections between GLU+ terminals and DARPP-32+ dendrites in MSt. GLU+ axons synapsed on both DARPP-32+ and DARPP-32-negative dendrites, forming asymmetrical junctions. The presence of L-aspartate (ASP) in the axons arising from the arcopallium and terminating in MSt, were verified by anterograde pathway tracing combined with ultrastructural electron microscopy. ASP was present in axon terminals with clear and round vesicles and asymmetric junctions anterogradely labelled from the arcopallium. Immunolabeling against calbindin (CB), neuropeptide Y (NPY), and DARPP-32 and anterograde pathway from nucleus of the solitary tract (NTS) were used to selectively mark the putative nucleus accumbens (Ac) subdivisions of chick. Extending between rostrocaudal atlas coordinates A10.6 and A8.8 Ac can be subdivided into core and shell, the core corresponding to the ventromedial and juxtaventricular medial striatum, and the shell representing an arched region situated ventrally and ventrolaterally to the core. Immunoreactivity to both CB and NPY is more intense in the shell than in the core. Fibers from NTS predominantly terminate in the shell division. Whereas the core lies entirely within the boundary of the MSt, the shell seems partially to overlap the ventral pallidum. The remaining part of Ac lying rostral to A10.6 probably corresponds to the rostral pole of the Ac.

- Bálint E, Kitka T, Zachar G, Ádám A, Hemmings HCJr, Csillag A (2004) Abundance and location of DARPP-32 in striato-tegmental circuits of domestic chicks. *J Chem Neuroanat.* 28: 27–36.
- Bálint E, Csillag A (2007) Nucleus accumbens subregions: hodological and immunohistochemical study in the domestic chick (*Gallus domesticus*). *Cell Tissue Res* 27: 221–230.
- Csillag A, Bálint E, Ádám Á, Zachar G (2008) The organisation of the basal ganglia in the domestic chick (*Gallus domesticus*): anatomical localisation of DARPP-32 in relation to glutamate. *Brain Res Bull* 76: 183–191.

## ÁGNES BODOR (2009)

### Comparative ultrastructural analysis of inhibitory thalamic afferents

Supervisor: László Acsády

Synaptic terminals in the nervous system display variable morphology. Typically, terminals contain a single release site, but giant terminals which establish multiple synapses also exist. Our aim was to understand how the morphology of presynaptic terminals, in particular the location and number of synapses, affects signal transmission from the presynaptic to postsynaptic neuron. Here we analyzed three different GABAergic pathways that project to the thalamus using electron microscopy and three-dimensional reconstruction. In parallel with this, the relationship between the morphology of the presynaptic terminals and the postsynaptic responses was also examined using electrophysiological techniques.

Terminals formed by the axons of reticular thalamic nucleus (nRT) on the relay cells of thalamic posterior nucleus (Po) in rat were small and mostly established a single synapse per postsynaptic target. Punctum adhaerens were rare, and synapses made onto different dendrites were usually separated by astrocytic processes. In contrast, the organization of synapses in the terminals of the recently described pretecto-thalamic pathway and the well-known nigrothalamic pathway in rat and monkey were very different. In these pathways, the terminals established multiple contacts onto a single postsynaptic target, which was usually a proximal dendrite of a relay cell. Puncta adhaerentia were found in the center of the presynaptic terminal, surrounded by a ring of synapses both directly opposing the postsynaptic surface. The side of the terminal not facing the postsynaptic surface was entirely covered by a glial sheet, which rarely penetrated into the spaces between synapses. The similarity between the nigrothalamic and pretecto-thalamic terminals across species suggests evolutionarily conserved morphology, and indicates the functional importance of this morphology. Indeed, the parallel electrophysiological experiments demonstrated that the GABAergic terminals which establish multiple contacts are transmitting reliable signals, even in case of high frequency activity.

- Bodor AL, Giber K, Rovó Z, Ulbert I, Acsády L (2008) Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway. *J Neurosci* 2: 3090–3102.
- Wanaverbecq N, Bodor AL, Bokor H, Slézia A, Lüthi A, Acsády L (2008) Contrasting the functional properties of GABAergic axon terminals with single and multiple synapses in the thalamus. *J Neurosci* 28: 11848–11861.

**CSABA BOROS (2009)****The structure of the caudal end of the spinal cord  
(conus medullaris, filum terminale)***Supervisor: Miklós Réthelyi*

The neuronal and glial architecture of the transformation of the caudal end of the spinal cord (conus medullaris) and its continuation as filum terminale were studied in the rat, cat and monkey.

Proceeding caudally in the conus medullaris first disappear the ventral horns followed by the disappearance of the dorsal horns. The grey matter of the zona intermedia follows the central canal towards the filum terminale. In the dorsal horn of the rat the substantia gelatinosa (lamina II), discontinue more cranially than the dorsal horn itself.

The grey matter of the filum terminale in the rat has been subdivided into symmetrical lateral nuclei lateral to the central canal into a midline medial nucleus located dorsally from the central canal. The neuronal perikarya measure 8–15 µm in diameter in cross sections, but in longitudinally cut specimens they may measure 30 to 35 µm. Nitric oxide synthase (NOS)-, calretinin-, cholin acetyltransferase (ChAT)-, neurokinin 1 receptor (NK-1r), and substance P (SP)-immunostained neurons were detected primarily in the lateral nucleus. The axons arborizing in the filum terminale formed four groups. Calcitonin gene-related peptide (CGRP) immunostained fibers arborized exclusively in the dorsal nucleus. NOS-, SP-, and NK-1r immunostained were detected principally in the lateral nucleus, as the axons of the resident neurons. Dense vesicular glutamate transporter 2 (VGLUT2) immunostained axon arborization was found also in the lateral nucleus, large percent of the varicosities was synaptophysin positive. Fine glycine transporter 2 (GLYT2) positive varicosities were also found in the lateral nucleus. Vesicular glutamate transporter 1 (VGLUT1)- and ChAT immunostained axons arborized in the entire grey matter. Serotonin-, enkephalin-, calretinin- and NK-1r immunostained axons were seen in the dorsolateral portion of the white matter, called shoulder region. Around 35000 neurons were estimated in the cranial 5 mm long section of the filum terminale in the rat, more caudally the number of the neurons dropped to 15000 in a 5 mm long portion. The glial fibrillary acidic protein (GFAP)-immunostained processes of the astrocytes showed radial and longitudinal orientation depending on the plane of the specimens. Oligodendrocytes were located mainly in the white matter. Various kinds of axo-dendritic and a small number of axo-somatic synapses were seen under the electron microscope.

Nervous tissue continued into the filum terminale also in the cat, the neurons occurred less densely than in the rat. Unlike in the rat the processes of the astrocytes formed a dense network in the cat filum terminale. The nervous tissue is arranged as small spheres attached to the basal surface of the ependyma in the filum terminale of the monkey.

Preliminary results of tract tracing studies indicated that fine caliber nerve fibers coursed from the sacral segments to the filum terminale.

- Réthelyi M, Lukácsi E, Boros C (2004) *The caudal end of the rat spinal cord: transformation to and ultrastructure of the filum terminale. Brain Res 1028: 133–139.*
- Boros C, Lukácsi E, Horváth-Oszwald E, Réthelyi M (2008) *Neurochemical architecture of the filum terminale in the rat. Brain Res 1209: 105–114.*
- Réthelyi M, Horváth-Oszwald E, Boros Cs (2008) *Caudal end of the rat spinal dorsal horn. Neurosci Lett 445: 153–157.*

## GIBER KRISTÓF (2009)

### Comparative study of thalamic inhibitory afferent pathways with different extrareticular origin

*Supervisor: László Acsády*

Higher order (HO) nuclei of the thalamus form functional connections between distant cortical areas. The extrareticular areas anterior pretectum (APT) and zona incerta (ZI) exert powerful inhibitory effect selectively on the HO nuclei. Whether inhibitory cells form homogeneous population and whether these two diencephalic inhibitory pathways are related is however unknown. Furthermore, the existence of two extradiencephalic inhibitory input, one from the substantia nigra reticulata (SNr) and one recently described glycinergic pathway presumably from the brain stem, raise the question, whether these are organized similarly to the diencephalic pathways. These questions were investigated using anterograde and retrograde tract tracing methods combined with pre- and postembedding immunocytochemistry and *in situ* hybridization. Both APT-thalamic and APT-ZI pathways were heterogeneous in their parvalbumin content, a feature described to correlate with firing pattern. Fibres from the APT terminated selectively in the thalamus-projecting ZI region which indicate an indirect APT-ZI thalamic pathway. The direct and indirect pathways originated from two different APT cell populations, which were however mixed within the nucleus. The APT-ZI projection contained a larger GABA-negative component and a smaller GABA-positive one as shown at the level of both projecting cells and terminals. Nigrothalamic and glycinergic input innervated selectively thalamic regions dominated by calbindin-positive cells. Glycinergic fibres originated from the brain stem, and mostly from oral pontine neurons. Terminals contained GABA transmitter. Such as nigrothalamic terminals, glycinergic boutons showed the well characterized extrareticular morphology and target selectivity, but with larger heterogeneity. The APT-ZI-thalamic and the direct APT-thalamic effects are presumably synchronized in a synergistic way via recurrent collaterals. The network of these pathways characterized by heterogeneous parvalbumin- and GABA content, indicate the complex tuning of oscillations originated from cortex acting on distant HO nuclei. The output nucleus of another complex cortico-thalamic oriented network, the SNr such as the glycinergic terminals originating from the pontine nucleus, innervate the thalamus in a similar manner. Extrareticular inhibition thus plays an important role in the associative brain functions related with synchronization of distant cortical oscillations.

- Giber K, Slezia A, Bokor H, Bodor AL, Ludanyi A, Katona I, Acsady L (2008) Heterogeneous output pathways link the anterior pretectal nucleus with the zona incerta and the thalamus in rat. *J Comp Neurol* 506: 122–140.
- Bodor AL, Giber K, Rovó Z, Ulbert I, Acsády L (2008) Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway. *J Neurosci* 28: 3090–3102.



**ANIKÓ REICHART GUTTMANN (2009)****Subcortical and autonomic connections of the prefrontal cortex***Supervisor: Miklós Palkovits*

In our previous experiments, bilateral surgical transections through the caudal portion of the anterior cingulate cortex abolished the gastroprotective effect mediated by delta-opioid receptors in the lower brainstem in rats. This finding indicates a possible role of either anterior cingulate neurons or neuronal pathways passing the anterior cingulate cortex in this mechanism. During the investigations my aims were: to identify the activated cortical areas by gastric mucosal erosion; to choose and test pseudorabies virus strains suitable for transynaptic tract-tracing, and allowing the neurochemical characterization of the infected neurons; to prove the connection between the brain regions activated by gastric ulcer; and to localize the fine topography of fibers of this pathway. Using acidified ethanol induced ulcer model in rats we found increased c-fos expression in the medial prefrontal cortical areas, the central nucleus and the intercalated cells of the amygdala. Genetically engineered strains of pseudorabies virus were tested in the nervous system of rats, and those parameters were identified which allowed the detection of the virus in the infected neuron and the definition of the neurochemical characteristic of the cell. Using surgical transections combined with transneuronal labelling with the previously selected virus strain the elements of the prefrontal cortex–amygdala–vagal nerve pathway has been proved. After unilateral virus injection into the central nucleus of the amygdala infected cells in the infralimbic, prelimbic, dorsal peduncular, anterior cingulate and insular cortices have been demonstrated. To localize the fine topography of fibers between the medial prefrontal cortex and the amygdala, biotinylated dextran amine was injected into the anterior cingulate cortex. Labelled fibers entered the cingulum, passed the corpus callosum and formed an arch downwards along the external capsule to the direction of the amygdala and reached the basolateral nucleus and the intercalated cells of the amygdala. From here the information passes to the central nucleus and reaches the stomach through the dorsal motor vagal nucleus and the vagal nerve. This is one of the possible ways through the medial prefrontal cortex may exert its gastroprotective effect.

- Boldogkői Z, Reichart A, Tóth LE, Sík A, Erdélyi F, Medveczky I, Llorens-Cortes U, Palkovits M, Lenkei Z (2002) Construction of recombinant pseudorabies viruses optimized for labeling and neurochemical characterization of neural circuitry. *Brain Res Mol Brain Res* 109: 105–118.
- Boldogkői Z, Sík A, Dénes A, Reichart A, Toldi J, Gerendai I, Kovács KJ, Palkovits M (2004) Novel tracing paradigms—genetically engineered herpesviruses as tools for mapping functional circuits within the CNS: present status and future prospects. *Prog Neurobiol* 72: 417–445.
- Reichart A, Boldogkői Zs, Lenkei Zs, Medveczky I, Palkovits M (2000) Neurochemical characterization of kidney-regulating brainstem neurons by pseudirabies transneuronal labeling. *Neurobiology* 8: 391–392.
- Reichart A, Boldogkői Zs, Lenkei Zs, Medveczky I, Palkovits M (2000) Neurochemical characterization of kidney-regulating brainstem neurons identified by pseudorabies transneuronal labeling. *Eur J Neurosci* 12: 420.

**MIHÁLY KÖLLŐ (2009)****Distribution of the KV4 voltage-gated K<sup>+</sup> channel subunits on the plasma membrane of central neurons***Supervisor: Zoltán Nusser*

The primary functions of neurons are to retrieve, modify and transmit messages. These processes are determined by a spatiotemporally dispersed pattern of ionic currents at their plasma membrane. Mammalian neurons express various voltage-gated ion-selective channels, among which K<sup>+</sup> channels received special attention in the last decade. Previous studies investigated the subcellular distributions and distribution-dependent functions of voltage-gated K<sup>+</sup> channels, and showed inhomogeneities of distributions and clustering of K<sup>+</sup> channels on the neuronal plasma membrane. However, there has been controversy on the question whether the aggregation of channels is associated with chemical synapses, and what the exact functions of this clustering are.

The aim of our experiments was to determine the cellular and subcellular distributions of the KV4.2 and KV4.3 voltage-gated K<sup>+</sup> subunits in different regions of the rat brain. We aimed to reveal whether the subcellular distribution of these subunits is homogeneous or inhomogeneous in the plasma membrane of neurons, and if the distribution of the channels in the plasma membrane associates with specific subcellular components or, conversely, channel clusters are dispersed randomly.

In our experiments, we used high-resolution light- and electron microscopic immunohistochemistry. In order to verify that the studied immunosignal was due to specific antibody-antigen interactions, we performed control experiments with double immunofluorescent reactions against different epitopes and with knock-out mice.

Our experiments showed that the KV4.2 and KV4.3 A-type K<sup>+</sup> channel subunits are enriched in dense clusters on the surface of various neurons of the rat brain, and these clusters are not randomly distributed on the plasma membrane, but are associated with intercellular junctions formed between specific cell types. Furthermore, we observed specific membrane specializations at these junctions, which are different from chemical synapses. The junctions are “symmetrical” or “asymmetrical” depending whether the cells belong to the same type. Based on theoretical considerations, we suggested a few hypotheses about their functions. Further experiments in this field may reveal important information about the physiological and pathological functioning of the brain.

- Kollo M, Holderith NB, Nusser Z (2006) Novel subcellular distribution pattern of A-type K<sup>+</sup> channels on neuronal surface. *J Neurosci* 26: 2684–2691.
- Kollo M, Holderith NB, Antal M, Nusser Z (2008) Unique clustering of A-type potassium channels on different cell types of the main olfactory bulb. *Eur J Neurosci* 27: 1686–1699.

**MÁRK KOZSUREK (2009)**

**Cocaine- and amphetamine-regulated transcript (CART) peptide in spinal neuronal networks involved in nociceptive information processing**

*Supervisor: Gábor Gerber*

Cocaine- and amphetamine-regulated transcript (CART) peptide has been implicated in regulation of several physiological functions including reward, food-intake and neuroendocrine functions. The role of CART peptide in spinal nociception has been suggested by behavioural studies and dense plexus of CART-immunoreactive fibres has been described in the superficial laminae of the spinal cord, which are key areas of the pain transmission, but the anatomical evidence for the involvement of CART peptide in the spinal nociception has been completely missing.

We used antibody against CART peptide, together with markers for various types of primary afferents, interneurons and descending systems to determine the origin of the CART-immunoreactive axons in the superficial laminae of the rat spinal cord. Calcitonin gene-related peptide (CGRP), a marker for peptidergic primary afferents in the dorsal horn, was present in 72.6% and 34.8% of CART-immunoreactive axons in lamina I and II, respectively. The majority of these fibres also contained substance P (SP). The other subpopulation of CART-immunoreactive boutons in laminae I–II also expressed SP and/or somatostatin (SOM) without CGRP, but contained vesicular glutamate transporter 2, which is present mainly in excitatory interneuronal terminals. In lamina I, CART peptide was present in half of the CGRP-immunoreactive terminals and many of the CART/CGRP-ergic boutons were also immunoreactive for galanin (GAL), which is one of the key peptides in chronic pain states. Electron microscopy showed that most of the CART terminals formed asymmetrical synapses mainly with dendrites. Using retrograde tract tracing and multiple immunofluorescent labelling, the relationship between CART and/or CGRP axons and projection neurons, playing a pivotal role in forwarding painful stimuli toward supraspinal levels, were examined. The contact density analysis of CART-ergic and/or CGRP-containing fibres terminating on lamina I spinoparabrachial projection neurons showed that all examined cells received contacts, but the innervation density did not differ significantly between either of the different neurochemical or the morphological subclasses of these cells.

Our data demonstrate that the majority of CART-immunoreactive axons in the spinal dorsal horn originate from peptidergic nociceptive primary afferents, while a smaller proportion of them arises from local interneurons. The co-existence of CART with nociceptive peptides in primary afferents and excitatory interneurons suggests that the peptide can affect glutamatergic neurotransmission as well as the release and effects of SP, SOM and GAL in nociception and other sensory processes. Furthermore, we revealed a direct non-selective input from CART-immunoreactive axons to lamina I projection neurons.

These results provide anatomical bases for involvement of CART peptide in spinal pain transmission.

- Kozsurek M, Lukácsi E, Fekete Cs, Wittmann G, Réthelyi M, Puskár Z (2007) Cocaine- and amphetamine-regulated transcript peptide (CART) is present in peptidergic C primary afferents and axons of excitatory interneurons with a possible role in nociception in the superficial laminae of the rat spinal cord. *Eur J Neurosci* 26: 1624–1631.

- Kozsúrek M, Lukácsi E, Fekete Cs, Puskár Z (2009) Nonselective innervation of lamina I projection neurons by cocaine- and amphetamine-regulated transcript peptide (CART)-immunoreactive fibres in the rat spinal dorsal horn. *Eur J Neurosci* 29: 2375–2387.

## KÁROLY MÁRKÓ (2009)

### Adhesion dependent cell selection and differentiation: a synthetic integrin-ligand and its applications

Supervisor: Emília Madarász

Cell adhesion to the extracellular matrix provides the possibility for the construction of multicellular organisms and tissue genesis. It also influences cell migration, proliferation and differentiation. The synthetic analogues of ECM components can be used as cell adhesive substrates both *in vitro* and in clinical applications as well. During my Ph.D. work, we designed, synthesized and tested a series of cell adhesive peptide-conjugates. The physico-chemical properties of the peptides made them capable to absorb to any surfaces used in cell culturing. One of the peptides—AK-cyclo(RGDfC)—proved to be a highly effective adhesive substrate for a wide range of cell types. Its cyclic RGD motif—supposed to be specific for  $\alpha_v$ -integrins—also could be used for the selective sorting and long-term culturing of radial glia-like neural stem cells. Beyond their application as specific and selective adhesive substrates for cell sorting and culturing, AK-cyclo(RGDfC) and similar peptide-conjugates may be used in therapeutics on the surface of implants and prostheses.

- Markó K, Ligeti M, Mező G, Mihala N, Kutnyánszky E, Kiss É, Hudecz F, Madarász E (2008) A novel synthetic peptide polymer with cyclic RGD motifs supports serum-free attachment of anchorage-dependent cells. *Bioconjugate Chem* 19: 1757–1766.
- Szilávik V, Vág J, Markó K, Demeter K, Madarász E, Oláh I, Zelles T, O'Connell BC, Varga G (2008) Matrigel-induced acinar differentiation is followed by apoptosis in HSG cells. *J Cell Biochem* 103: 284–295.
- Jelítai M, Andarová M, Markó K, Kékesi K, Koncz P, Syková E, Madarász E (2004) The role of GABA in early neuronal development: studies on an embryonic neuroectodermal stem cell clone. *J Neurosci Res* 76: 801–811.

## TÜNDE MOLNÁR (2009)

### Comparing binding and action of Gamma-Hydroxy Butyric Acid (GHB) with succinic acid in the nucleus accumbens

Supervisor: Miklós Palkovits

$\gamma$ -Hydroxybutyrate (GHB), a naturally occurring metabolite of  $\gamma$ -amino butyric acid (GABA) with dose-dependent dangerous behavioral effects has been known recently as a recreational drug (drug of abuse). GHB metabolizes via succinate (SUC) an intermediary metabolite of the tricarboxylic/citric acid cycle. GHB can act as a weak agonist on GABAB receptor but the existence of its own specific receptor has also been reported.

My purpose was to characterize the specific target and function of GHB in the *nucleus accumbens*—the brain area responsible for the development of addiction—and to disclose its possible interaction with SUC.

Applying radioatracrer binding technique a new, GABAB-independent GHB-, and SUC-sensitive target protein was found. Further characterization of this target excluded its possible function as a transporter molecule but revealed its interaction with gap-junction ligands.

Stereospecific interaction of NCS-382—the only known GHB receptor selective ligand—with binding sites recognizing GHB and SUC disclosed identity of the high-affinity SUC binding target with the GABAB-independent GHB receptor (GHB/SUC receptor).

Substantiating the results obtained from binding experiments a GABAB-independent GHB function has been disclosed in the *nucleus accumbens*. The astroglial  $\text{Ca}^{2+}$  ion transients induced by GHB persevered in GABAB R1 subunit knock-out mice as well. It can be supposed, that the astroglial GHB/SUC receptor participate in the mobilization of intracellular  $\text{Ca}^{2+}$  ion and in the regulation of the propagation of the released  $\text{Ca}^{2+}$  ion through gap junctions. It is noteworthy that the common intermediary metabolite of the citric acid cycle SUC may play a role in chemical signalling between neural cells.

- Molnár T, Kútiné FE, Kardos J, Simon-Trompler E, Palkovits M, Emri Zs (2006) Metabolic GHB precursor succinate binds to  $\gamma$ -hydroxybutyrate receptors: characterization of human basal ganglia areas nucleus accumbens and globus pallidus. *J Neurosci Res* 84: 27–36.
- Molnár T, Kútiné FE, Kardos J, Palkovits M (2007) Characterization of specific succinate binding site in brain synaptic membranes. *Ideggyógy Sz* 60: 201–204.
- Molnár T, Barabás P, Héja L, Kútiné FE, Lasztóczy B, Szabó P, Nyitrai G, Simon-Trompler E, Hajós F, Palkovits M, Kardos J (2008) Gamma-hydroxybutyrate binds to the synaptic site recognizing succinate monocarboxylate: a new hypothesis on astrocyte–neuron interaction via the protonation of succinate. *J Neurosci Res* 86: 1566–7156.

## RITA NYILAS (2010)

### Pre- or postsynaptic distribution of distinct endocannabinoid-synthesizing enzymes at chemical synapses

Supervisor: István Katona

Endocannabinoids, such as anandamide and 2-arachidonoylglycerol (2-AG), are lipid signaling molecules acting on  $\text{CB}_1$  and  $\text{CB}_2$  cannabinoid receptors. One of the most striking effects cannabinoid receptor agonists exert in the nervous system, is antinociception in both acute and chronic pain. The critical role of 2-AG in pain regulation has been implicated however, the underlying molecular anatomical basis at the spinal level has been largely unknown. Therefore, using molecular neuroanatomical approaches, we aimed to identify sites of 2-AG synthesis and action in the dorsal spinal cord. Our results showed that dorsal horn neurons widely expressed diacylglycerol lipase- $\alpha$  (DGL- $\alpha$ ), the synthesizing enzyme of 2-AG. Moreover, dense distribution of DGL- $\alpha$  proteins and  $\text{CB}_1$  receptors was revealed, especially in the superficial layers. DGL- $\alpha$  was demonstrated to be located in a remarkable postsynaptic position at excitatory synapses formed by incoming nociceptive primary afferents, while  $\text{CB}_1$  was shown presynaptically on excitatory and inhibitory axon terminals. Furthermore, DGL- $\alpha$  in postsynaptic elements re-

ceiving nociceptive input co-localized with metabotropic glutamate receptor 5 (mGluR<sub>5</sub>), whose activation induces 2-AG biosynthesis. In an animal model of stress-induced analgesia, 2-AG-mediated retrograde suppression of nociceptive transmission at the spinal level through the mGluR<sub>5</sub>-DGL-2-AG-CB<sub>1</sub> pathway was shown. On the other hand, spinal endocannabinoids, produced upon intense nociceptive stimulation, were demonstrated *in vitro* to activate also CB<sub>1</sub> receptors located on inhibitory dorsal horn interneurons and reduce synaptic inhibition of spinal output neurons. On a behavioural level, this leads to pain sensitization called hyperalgesia, a key feature of pathological pain, revealing a novel, unexpected role of spinal 2-AG signaling in nociception.

Despite that of 2-AG, the molecular and neuroanatomical architecture of synaptic anandamide signaling still remains to be determined. In our further experiments, we aimed to uncover the cellular source and subcellular positioning of N-acylphosphatidyl-ethanolamine-hydrolyzing phospholipase D (NAPE-PLD), a biosynthetic enzyme of anandamide and its related bioactive congeners, the N-acylethanolamines (NAEs). Our data demonstrated that, in contrast to its predicted role in retrograde signaling, NAPE-PLD was concentrated presynaptically, on intracellular membrane cisternae of hippocampal excitatory axon terminals. Furthermore, the highest density of NAPE-PLD was found in mossy terminals of granule cells, which do not express CB<sub>1</sub> receptors. The remarkably different sites of NAE synthesis and action from that of 2-AG at glutamatergic synapses imply heterogeneous physiological roles for distinct endocannabinoids in the regulation of synaptic functions.

- Nyilas R, Gregg LC, Mackie K, Watanabe M, Zimmer A, Hohmann AG, Katona I (2009) Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia. *Eur J Neurosci* 29: 1964–1978.
- Pernía-Andrade JA, Kato A, Witschi R, Nyilas R, Katona I, Freund TF, Watanabe M, Filitz J, Koppert W, Schüttler J, Ji G, Neugebauer V, Marsicano G, Lutz B, Vanegas H, Zeilhofer HU (2009) Endocannabinoids and CB<sub>1</sub> receptors mediate C-fiber-dependent heterosynaptic plasticity in spinal cord. *Science* 325: 760–764.
- Nyilas R, Dudok B, Urban GM, Mackie K, Watanabe M, Cravatt BF, Freund TF, Katona I (2009) Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. *J Neurosci* 28: 1058–1063.



**ANDREA SLÉZIA (2009)*****In vivo* electrophysiological properties of first and higher order relays and the extrareticular inhibitory system**

*Supervisor: László Acsády*

The thalamus is one of the most prominent subcortical structures that relays and transmits information to the cortex. Thalamic nuclei can be divided into two separate types according to their function. First order nuclei are responsible for the information filtering and signal transmission towards the cortex, whereas higher order thalamic nuclei might play a role in more complex, integrative functions. Recently it was shown by our laboratory that the latter relays differ not only in their excitatory network properties described previously, but they also possess a different inhibitory system. Both groups of nuclei receive reticular inhibition, but there are other sources of inhibitory inputs arriving selectively to higher order thalamic nuclei which originate outside of the thalamic reticular nucleus, hence their name: extrareticular inhibitory system.

In my thesis, I focused first on the difference of the firing properties and the phase properties of the cortical slow oscillations of the first order and higher order relays by applying *in vivo* electrophysiological methods on anaesthetized rats. Then I started to examine two nuclei of the extrareticular inhibitory system. First, I examined the firing properties and the phase characteristic of the anterior pretectum (APT) and zona incerta (ZI), then I examined the effect of the chemical stimulation of the pretecto-thalamic pathway on the firing properties of higher order thalamic nuclei.

Our results show that first order and higher order thalamic relays differ in their firing characteristics and their phase properties to slow oscillations. From the three cell types we distinguished in the APT, the group of fast bursting cells was capable of transmitting a very strong inhibition to the higher order target cells. From the two groups of ZI cells, the majority of the modulated ones showed as high or even higher synchrony to the cortical slow wave oscillations as relay cells. Chemical stimulation of the APT changed the firing and phase properties of higher order relay cells.

The difference in the phase properties between these two types of thalamic nuclei might partly arise from the difference in their excitatory network properties (V. layer input), or partly from the extrareticular inhibition arriving selectively onto higher order nuclei. The neocortex has to deal with these two types of information coming from the two different thalamocortical systems.

- Slézia A, Hangya B, Bokor H, Ulbert I, Barthó P, Acsády L (2011) Heterogenous firing pattern in different relay nuclei: burst properties and phase relationship to cortical activity. *J Neurosci* 31: 607–617.
- Barthó P\*, Slézia A\*, Varga V, Bokor H, Pinault D, Buzsáki G, Acsády L (2007) Cortical control of zona incerta. *J Neurosci* 27: 1670–1681.
- Bokor H, Frére SG, Eyre MD, Slézia A, Ulbert I, Lüthi A, Acsády L (2005) Selective GABAergic control of higher-order thalamic relays. *Neuron* 45: 929–940.

## ADRIENN SZABÓ (2010)

### Connections of astroglia and connective tissue

Supervisor: Mihály Kálmán

In the central nervous system the connections to the connective tissues have a great importance, along the meninges and the vessels penetrating the brain. The aim of the study is therefore to find immunohistochemical reactions which are characteristic of the state and functional alterations of the glia-connective tissue connections, especially the gliovascular connections. The animals were albino adult and developing (E12–E20, and P0–P20) rats. To study the effect of the brain lesions, stab wounds were performed in ketamine-xylazine narcosis by sterile disposable needles. Coronal sections were cut by Vibratome, and floating sections were processed for fluorescent immunohistochemistry against: (1) laminin, one of the main component of basal lamina; (2) fibronectin; (3)  $\beta$ -subunit of the laminin-receptor dystroglycan; (4) dystrophins, which form complex with dystroglycan, especially the Dp71 splicing variant, which has not been mapped in the brain yet; (5) the  $\alpha$ V and  $\beta$ 1 subunits, the most frequent subunits of the integrin, another laminin-receptor; (6) the marker of the mature astroglia, GFAP (glial fibrillary acidic protein); (7) the nestin which is found in the immature astroglia. The new experimental results are: (1) First mapping on the distribution of the Dp71f *in situ*. Its localization showed correlations to the GFAP-expression, and to the basal lamina production by astrocytes, including reactive astrocytes. Ependyma and subventricular zone were also labeled. (2) Following lesions, correlations were found between

- a) the appearance of the laminin positivity and the disappearance of dystroglycan;
- b) the disappearance of dystroglycan and the GFAP expression in the reactive glia;
- c) the disappearance of laminin and the formation of new gliovascular connections.

(3) During development the dystroglycan immunopositivity appears between the appearance of the laminin immunopositivity and the formation of the early gliovascular connections. (4) In developing brain the disappearance of the laminin-immunopositivity and the replacement of the nestin-containing radial glia by GFAP-containing astrocytes, occur in the same time interval. There is no local coincidence, however, between them. (5) The immunoreactivity of the  $\alpha$ V integrin-subunit increases in post-lesional glial reaction. In accordance with the former data published, the laminin-immunoreactivity of the brain vessels (without antigen-exploration), and the disappearance of the  $\beta$ -dystroglycan refer to the decoupling of the gliovascular connections. Beyond the experimental data, let me emphasize a general consequence: applying histochemical markers, the vascularization and the vascular reactions following lesions or other pathological alterations can be monitored and its stages can be distinguished. It may have a great importance in the pathology, especially because the inhibition of vascularization is a possibility in the therapy of tumors, including brain tumors.

- Szabó A, Kálmán M (2004) Disappearance of the post-lesional laminin immunopositivity of brain vessels is parallel with the formation of gliovascular junctions and common basal lamina. A double-labeling immunohistochemical study. *Neuropath Appl Neurobiol* 30: 169–170.
- Szabó A, Jancsik V, Mornet D, Kálmán M (2004) Immunofluorescence mapping of dystrophin in rat brain: astrocytes contain the splice variant Dp71f but confined to subpopulations. *Anat Embryol* 208: 463–477.

• Szabó A, Kálmán M (2008) Post traumatic lesion absence of  $\beta$ -dystroglycan-immunopositivity in brain vessels coincides with the glial reaction and the immunoreactivity of vascular laminin. *Curr Neurovasc Res* 5: 206–213.

**PROGRAM 6/2.**

---

**NEUROENDOCRINOLOGY**

**Coordinator:**  
**Zsolt LIPOSITS M.D., Ph.D., D.Sc.**  
Institute of Experimental Medicine,  
Hungarian Academy of Sciences  
43 Szigony st, Budapest H-1083  
Tel.: +36 1 210 9400  
E-mail: [liposits@koki.hu](mailto:liposits@koki.hu)

**Program overview**

The course gives a deep insight into the structural organization and functional properties of neuroendocrine brain centres controlling the operation of the endocrine system. It presents the classical breakthroughs and well-established results of the discipline and also provides information on contemporary research trends and discoveries in the field of neuroendocrinology. Special attention is paid to the most recently developed research methods exploring the frontline topics of the field at molecular, cellular and system levels. The course focuses on the effects of neuroendocrine brain centres upon the pituitary-endocrine axes and *vice versa*, the wide scale genomic and non-genomic actions of peripheral hormones modulating the performance of the nervous system. The main topics of the course include (1) The functional neuroanatomy of the hypothalamo-hypophyseal system; (2) The organization and functional characteristics of the magno- and parvicellular neurosecretory systems; (3) The physiology of reproduction; (4) The mechanisms of thyroid hormone actions; (5) The neurobiology of stress and adaptation; (6) The central regulation of feeding and energy homeostasis; (7) Regulation of neuroendocrine rhythms and (8) The behavioral neurobiology of endocrine events. The course also focuses on the translational aspects of endocrine/neuroendocrine research, highlights inventions and novelties in the diagnosis and therapy of endocrine diseases. The course is in harmony with the educational and research mission of the International Neuroendocrine Federation.

***Titles of research projects***

***Supervisors***

Neuronal and hormonal control of hypothalamic regulatory mechanisms	Zsolt Liposits
Examination of the central regulatory mechanisms involved in the development of the “low T3 syndrome”	Csaba Fekete
Investigation of the molecular regulation of thyroid hormone activation in the central nervous system	Balázs Gereben
Neural mechanisms underlying abnormal aggressive behavior	József Halász

Integrating role of cannabinoids in trauma-induced behavioral deficits	József Haller
Hypothalamic integration: relationship between regulation of stress and metabolism	Krisztina Kovács
Effect of perinatal events on sensitivity to stress and on stress related behavior	Gábor Makara
Neuroendocrine, paracrine and autocrine regulatory mechanisms in the regulation of adenohypophyseal hormone secretion	György M. Nagy
Investigation of the metabolism of dopamine and norepinephrine in sympathetically innervated peripheral organs (like liver, spleen or salivary gland)	György M. Nagy
The role of new signaling mechanism(s) in the regulation of pituitary function	György M. Nagy
The role of new signaling mechanism(s) in the dopaminergic regulation of pituitary prolactin secretion	György M. Nagy
Investigation of the interaction between salsolinol and different addictive drugs (like amphetamine)	György M. Nagy
The role of vasopressin in young and adults in connection with stress-related psychiatric disorders (anxiety and depression)	Dóra Zelena
Functional and morphological studies on target cells of steroid hormones in the CNS	Imre Kalló

***Ph.D. students***

Manó Aliczki	ft
Dániel Hechtl	ft
Andrea Kádár	ft
Bernadett Pintér Küblerné	pt
Csilla Molnár	ft
Ádám Tulogdi	ft
János Varga	ft

***Supervisors***

József Haller
György Nagy
Csaba Fekete
Krisztina Kovács
Erik Hrabovszky
József Haller
Dóra Zelena

***Ph.D. candidates***

Boglárka Barsy	ft
Ágnes Judit Domokos	ft

***Supervisors***

József Haller
Gábor Makara

***Ph.D. graduates***

Tamás Füzesi	ft
Judit Menyhért	ft
Máté Tóth	ft

***Supervisors***

Csaba Fekete
Csaba Fekete
József Haller

ft, full-time; pt, part-time

## ***Abstracts of Ph.D. theses successfully defended in 2010***

**TAMÁS FÜZESI (2010)**

### **Elucidation of the central regulation of the hypophysiotropic corticotropin-releasing hormone and thyrotropin-releasing hormone-synthesizing neurons in the rat**

*Supervisor: Csaba Fekete*

The hypophysiotropic corticotropin-releasing hormone (CRH)- and thyrotropin-releasing hormone (TRH)-synthesizing neurons in the hypothalamic paraventricular nucleus (PVN) play important roles in the regulation of energy homeostasis, primarily through the regulation of the hypothalamic-pituitary-adrenocortical and hypothalamic-pituitary-thyroid axes, respectively. One of the most important regulators of the hypophysiotropic CRH neurons is neuropeptide Y (NPY). Using multiple labeling immunohistochemistry and selective lesions, we have elucidated that approximately two thirds of the NPY innervation of the hypophysiotropic CRH neurons originate from the brainstem catecholaminergic neurons, while the arcuate nucleus contributes to the remaining part of this innervation. Though acute administration of NPY stimulates the hypophysiotropic CRH neurons, during fasting the elevated levels of NPY in the arcuate nucleus are accompanied with the inhibition of CRH expression. To examine this discrepancy, we infused NPY icv. for three days, then performed quantitative in situ hybridization for CRH mRNA in the PVN. We have revealed that in contrast to the acute effect of NPY, chronic administration markedly decreases CRH mRNA levels in the PVN. According to these results, we suggest that during fasting the prolonged increase of NPY in the arcuate nucleus contribute to the inhibition of CRH gene expression.

Since the adrenergic and noradrenergic neurons of the brainstem may respond differently to various physiological stimuli, using multiple labeling immunohistochemistry we have explored the contributions of the two neuronal groups in the innervation of the hypophysiotropic TRH neurons. Both adrenergic and noradrenergic axons innervate the hypophysiotropic TRH neurons, although, there is a predominance of adrenergic fibers. We propose that the different neuropeptides, which are co-expressed in adrenergic and noradrenergic neuron groups, contribute to the specific response of brainstem catecholaminergic neurons to various stimuli.

A non-hypophysiotropic TRH neuron population, which is located in the anterior parvocellular subdivision of the PVN (aPVN) are innervated by the feeding-related neurons of the arcuate nucleus. To determine how these TRH neurons are integrated within the brain, the major projection fields of this cell group were studied by anterograde and retrograde tract-tracing methods. As this neuronal group innervated brain regions that are involved in the regulation of food intake, prolactin synthesis, locomotor activity and thermogenesis, we hypothesize that the TRH neurons in the aPVN play an important part in the maintenance of energy homeostasis.

- Füzesi T, Wittmann G, Liposits Z, Lechan RM, Fekete C (2007) Contribution of noradrenergic and adrenergic cell groups of the brainstem and agouti-related protein-synthesizing neurons of the arcuate nucleus to neuropeptide-y innervation of corticotropin-releasing hor-

*mone neurons in hypothalamic paraventricular nucleus of the rat. Endocrinol 148: 5442–5450.*

- Wittmann G, Füzesi T, Singru PS, Liposits Z, Lechan RM, Fekete C (2009) Efferent projections of thyrotropin-releasing hormone-synthesizing neurons residing in the anterior parvocellular subdivision of the hypothalamic paraventricular nucleus. *J Comp Neur 515: 313–330.*
- Füzesi T, Wittmann G, Lechan RM, Liposits Z, Fekete C (2009) Noradrenergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in rats. *Brain Res 1294: 38–44.*

## JUDIT MENYHÉRT (2010)

### Distribution and relationship of neurons containing feeding-related neuropeptides in the human hypothalamus

*Supervisor: Csaba Fekete*

The maintenance of energy homeostasis is regulated by the cooperation of the brain and the peripheral endocrine systems. The main hypothalamic sensor area of the peripheral feeding related hormones is the hypothalamic arcuate nucleus. In rodents, this nucleus contains two neuronal groups that play critical role in the regulation of energy homeostasis: the orexigenic NPY/AGRP and the anorexigenic  $\alpha$ -MSH/CART synthesizing neurons. Another, recently recognized feeding related system of the hypothalamus is the hypothalamic ghrelin-synthesizing neuronal group. To understand whether the experimental data obtained from rodents may be valid for humans, we have studied the feeding related neuronal groups of the human infundibular nucleus, the analogue of the rodent arcuate nucleus, and the ghrelin-IR system of the human hypothalamus

We have found that there is a bilateral, but asymmetric connection between the NPY and  $\alpha$ -MSH-synthesizing neurons of the human infundibulum. Three times more NPY boutons were observed on the surface of the anorexigenic  $\alpha$ -MSH cells, than the number of  $\alpha$ -MSH varicosities on the NPY neurons. The reason of this phenomenon can be due to evolutionary advantages of preferring feeding stimulating pathways contra feeding inhibiting, but may be also other compensatory mechanisms present.

In rodents, CART is described as an anorexigenic peptide. However, we have observed that CART is not synthesized in the anorexigenic  $\alpha$ -MSH neurons of the human infundibular nucleus, but rather CART is present in a population of orexigenic NPY neurons in the human hypothalamus, raising the question whether CART is orexigenic or anorexigenic peptide in the human brain.

Using single-labeling immunocytochemistry, we mapped the distribution of ghrelin-IR neuronal elements in the human hypothalamus. Ghrelin-IR axons were found in almost all feeding related nuclei of the human hypothalamus, including the paraventricular nucleus (PVN), periventricular nucleus (PeVN), the infundibular nucleus, the supraoptic nucleus (SON), VMN and also in the medial part of the hypothyseal stalk.

Our data indicate that despite the similarities of the rodent and human feeding related circuits, some aspects of this regulators system underwent major changes during the evolution.



- Menyhért J, Wittmann G, Hrabovszky E, Keller É, Liposits Z, Fekete C (2006) Interconnection between orexigenic neuropeptide Y- and anorexigenic alpha-melanocyte stimulating hormone-synthesizing neuronal systems of the human hypothalamus *Brain Res* 1076: 101–105.
- Menyhért J, Wittmann G, Hrabovszky E, Szilávik N, Keller É, Tschöp M, Liposits Z, Fekete C (2008) Distribution of ghrelin-immunoreactive neuronal networks in the human hypothalamus. *Brain Res* 1125: 31–36.
- Menyhért J, Wittmann G, Lechan RM, Keller É, Liposits Z, Fekete C (2007) Cocaine- and amphetamine regulated transcript (CART) is colocalized with the orexigenic NPY and AGRP and absent from the anorexigenic  $\alpha$ -MSH neurons in the infundibular nucleus of the human hypothalamus. *Endocrinol* 148: 4276–4281.

## MÁTÉ TÓTH (2010)

### The underlying mechanisms of pathological aggression induced by early social disturbances and glucocorticoid hypofunction

Supervisor: József Haller

Our results in male rats show that glucocorticoid hypofunction has a crucial, causal role in the etiology of cold-blooded violence and its altered autonomic profile. In order to clarify the underlying mechanisms, we investigated the neural correlates of these behavioral changes by using immunohistochemical methods. Our results show that glucocorticoid hypofunction induces functional deficits rather than global changes in neuronal activation, except for the central amygdala and PVN. While normal forms of aggression were inhibited by serotonergic and prefrontal activity, abnormal forms were not influenced by the above mentioned inhibitory mechanisms or even facilitated (in the case of prefrontal activity). In addition, the increased activity of the central nucleus of amygdala was accompanied by medial amygdaloid facilitation of abnormal attacks. In the latter case, substance-P neurotransmission plays a crucial role because substance-P receptor (NK1) antagonist or hypothalamic lesion of neurons expressing NK1 receptors can abolish the abnormal character of aggressive behavior. Our findings may provide new therapeutic implications for these agents, especially in violence associated disorders that are resistant to the available pharmacotherapies. The second part of our investigations aimed at modeling the impulsive type of abnormal aggression and at clarifying its autonomic, endocrine and neural background. Prompted by earlier human findings, we studied the effects of early social deficits. Male rats reared in social isolation from weaning showed a disorganized and hypervigilant aggressive behavior which was accompanied by exacerbated stress responses as shown by increased glucocorticoid and autonomic activation during aggressive encounters. In parallel, we found a hyperactivation of the medial amygdala and certain prefrontal subregions, which were associated with disinhibited, violent attack patterns.

- Halasz J, Toth M, Kallo I, Liposits Z, Haller J (2008) The activation of prefrontal cortical neurons in aggression—a double labeling study. *Behav Brain Res* 175: 166–175.
- Halasz J, Toth M, Mikics E, Hrabovszky E, Barsy B, Barsvari B, Haller J (2008) The effect of neurokinin1 receptor blockade on territorial aggression and in a model of violent aggression. *Biol Psychiatry* 63: 271–278.

- Toth M, Halasz J, Mikics E, Barsy B, Haller J (2008) Early social deprivation induces disturbed social communication and violent aggression in adulthood. *Behav Neurosci* 122: 849–854.

---

## **PROGRAM 6/3.**

---

### **FUNCTIONAL NEUROSCIENCE**

#### ***Coordinator:***

**Szilveszter E. VIZI M.D.,**

**member of the Hungarian Academy of Sciences**

Institute of Experimental Medicine,

Hungarian Academy of Sciences

43 Szigony st, Budapest H-1083

Tel.: +36 1 210 9400

E-mail: [esvizi@koki.hu](mailto:esvizi@koki.hu)

#### **Program overview**

The doctoral program mainly covers training and research in the application of neuropharmacological, neurochemical and neurophysiological methods to approach the function of neuronal networks mainly from the functional point of view. The major direction of neuropharmacological research is the study of the non-synaptic model of the brain, which not only defines a new mechanism of chemical transmission of nerve impulses but also explains the mechanism of action of some medications with effect on the central nervous system, and may also suggest new targets for the treatment of neuropsychiatric disorders. A further research priority is the study of the less well known connection between the nervous system and the immune system, and the identification of new neurotransmitters and modulators in the central nervous system. The neurochemical research focuses on pathological processes following hypoxia and oxidative stress in the neurons of the central nervous system, with major interest in  $\text{Na}^+$  and  $\text{Ca}^{2+}$  homeostasis, in *in situ* mitochondrial function and in changes of excitability. Neurophysiological studies are performed mostly in the area of cognitive psychophysiology, and aim to understand the central nervous system mechanisms of higher level neuronal functions with the analysis of event related cerebral potential changes.

***Titles of research projects******Supervisors***

Function of ion channels and ionotropic receptors as revealed by nonlinear optical imaging and electrophysiological methods	Szilveszter E. Vizi
Nonsynaptic action mechanisms of neuroactive substances involved in chemical transmission in animal and in isolated human (obtained from surgical biopsy) tissues	Szilveszter E. Vizi
Models of brain diseases established by drug-induced alterations in neural networks of the peripheral and central nervous system	Szilveszter E. Vizi
Mechanisms in neurodegeneration and neuroprotection	Veronika Ádám
Investigation of synchronous neuronal activities using <i>in vitro</i> electrophysiological techniques	Norbert Hájos
Non-conventional effects of monoamine uptake blocker-type anti-depressants in the central nervous system	János Kiss
A possible role of nitric oxide in the regulation of neurotransmission: study of the glutamate-monoamine interaction	János Kiss
Role of receptors and ion-channels in the integrative functions of neurons: spatial and temporal patterns of calcium signaling and membrane potential changes	Balázs Lendvai
Drugs affecting ion channels: molecular action mechanisms	Árpád Mike
The role of ATP- and adenosine-mediated signalling in the nervous system	Beáta Sperlág
Role of GABA signaling in the regulation of embryonic development	Gábor Szabó
Impact of local microanatomy on the morphology and hemodynamics of aneurysms on the circle of Willis	István Szikora
Electrophysiological analysis of evoked, spontaneous oscillations and pathological signs	István Ulbert
Mechanisms of cellular damage in the central nervous- and sensory systems; potential pharmacological targets	Tivadar Zelles
Development of 3 dimensional (3D), real-time, two-photon microscopes and new methods to measure 3 dimensional signal integration in neurons and dendrites	Balázs Rózsa
Mitochondrial $\text{Ca}^{2+}$ dynamics	Christos Chinopoulos
Mechanism of free radicals production by mitochondrial alpha-ketoglutarate enzyme complex: structure–function relations	Attila Ambrus
Thy synaptical structure of the mossy fiber tract of the hippocampus	János Szabadits
<i>In vitro</i> electrophysiological studies on synchronous population activities	Lucia Wittner

***Ph. D. graduates******Supervisors***

Máté Aller	ft	Szilveszter E. Vizi
Balázs Chiovini	ft	Balázs Rózsa
Richárd Fiáth	ft	István Ulbert
Domonkos Áron Horváth	ft	György Karmos
Tamás Horváth	pt	Szilveszter E. Vizi
Gergely Kiss	ft	Christos Chinopoulos
Zsófia Komáry	pt	Veronika Ádám

Csaba Konrád	ft	Christos Chinopoulos
Orsolya Papp	ft	Norbert Hájos
Gergely Szabó	ft	Norbert Hájos
Gergely Szalay	ft	Balázs Rózsa
Judit Veres	ft	Norbert Hájos

***Ph. D. candidates***

Richard Csercsa	ft
Cecília Csölle	ft
Attila Heinrich	pt
Andrea Heinzlmann	pt
Attila Kaszás	ft
Nóra Lenkey	ft
Miklós Mándi	pt
Márk Oláh	ft
Flóra Szabó	pt

***Supervisors***

István Ulbert
Beáta Sperlág
Beáta Sperlág
Katalin Köves
Szilveszter E. Vizi
Árpád Mike
Veronika Ádám
György Nagy
Katalin Köves

***Ph. D. graduates***

Zoltán Doleviczényi	pt
Ádám Fekete	pt
Balázs Hangya	ft
Lajos Rudolf Kozák	ft
Alíz Mayer	pt
Lilla Papp	na
Marija Svrtlih	pt
Szilárd Szabó	ft
Szilvia Vajda	ft

***Supervisors***

Balázs Lendvai
Szilveszter E. Vizi
Zsolt Borhegyi
György Karmos
János Kiss
Beáta Sperlág
Gábor Szabó
Balázs Lendvai
Veronika Ádám

f, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2010*****ZOLTÁN DOLEVICZÉNYI (2010)****The role of GABAergic mechanisms in the regulation of cochlear dopamine release**

*Supervisor: Balázs Lendvai*

Between the inner hair cells (IHC) and the afferent dendrite the glutamatergic connection bears at most importance. Ischemic conditions or noise exposure can result in excessive glutamate release, having excitotoxic effect on the afferent auditory neuron.

In the short-loop feed back between the cochlea and brainstem, dopamine (DA) is released from the lateral olivocochlear (LOC) efferent terminals, reducing the excitotoxic effect of glutamate, thus it is neuroprotective. The DA release in isolated guinea pig cochlea was studied with the *in vitro* microvolume superfusion method.

The role of metabotropic glutamate receptors (mGluR) in the cochlear neurotransmission was studied by testing their selective agonists and antagonists. To understand the cochlear serotonergic innervation we tested the recently discovered group 6 and 7 serotonin receptor antagonists for their effect on DA release.

Our results suggest the presence of GABAergic system mediated effects. Selective GABAA antagonist (Bicucullin) was used for verification, that confirmed a tonic inhibition of DA release by GABA. We demonstrated that both mGluII and 5HT<sub>6/7</sub> are localized on the inhibitory GABAergic neuronal elements. Drugs acting on these receptors interrupt the inhibitory effect of GABAergic dendrites (disinhibition), facilitating the neuroprotective DA release. Glu was shown to increase the DA release on mGluR, thus it was able to compensate its own influence in iGluR (ultra-short feed back intracochlear mechanism).

As part of the study, an *in vitro* ischemia model was used with oxygen-glucose deprivation (OGD); and the effect of OGD on DA release was investigated in itself, and in the presence of D<sub>2</sub> receptor antagonist, and DA reuptake inhibitor, respectively. We demonstrated that the reverse operation of the DA-uptake system is responsible for the DA-level increase in the organ of Corti triggered off by ischemia (OGD). We proved the presence of the D<sub>2</sub> autoreceptor receptors on guinea pig cochlea LOC efferent. The blocking of these autoreceptors decreases the release of DA.

We assume that the DA release can be supported by several intracochlear mechanisms, such as the ultra-short-loop feed-back via the mGlu receptors, the inhibition of 5HT<sub>6/7</sub> receptors and the auto-feed-back effect of the D<sub>2</sub> receptors. They may serve as a basis for new therapeutic strategies for patients with sensorineural hearing loss.

- Doleviczényi Z, Vizi ES, Gacsályi I, Pallagi K, Volk B, Hársing LG Jr, Halmos G, Lendvai B, Zelles T (2008) 5-HT<sub>6/7</sub> receptor antagonists facilitate dopamine release in the cochlea via a GABAergic disinhibitory mechanism. *Neurochem Res* 33: 2364–2372.
- Doleviczényi Z, Halmos G, Répássy G, Vizi ES, Zelles T, Lendvai B (2005) Cochlear dopamine release is modulated by group II metabotropic glutamate receptors via GABAergic neurotransmission. *Neurosci Lett* 385: 93–98.
- Halmos G, Doleviczényi Z, Répássy G, Kittel A, Vizi ES, Lendvai B, Zelles T (2005) D<sub>2</sub> autoreceptor inhibition reveals oxygen-glucose deprivation-induced release of dopamine in guinea-pig cochlea. *Neuroscience* 132: 801–809.

## BALÁZS HANGYA (2010)

### Identified, putative pacemaker neurons of the medial septum lead the hippocampal network during theta activity

Supervisor: Zsolt Borhegyi

The hippocampal formation has long been considered as a main component of mnemonic processes. The regulation of these hippocampal functions strongly depends on subcortical connections, among which the reciprocal septo-hippocampal pathway plays a prominent role. The information processing in the septo-hippocampal system is substantially different during the two major hippocampal activity states, the regular 4 to 10 Hz theta oscillation and the large amplitude irregular activity. Synchronization between the medial septum and in the hippocampus was demonstrated by a large body of research.

However, the timing of events in this process and the state-dependent changes of directional influences remains elusive.

To address these questions, we analyzed dual recordings of hippocampal local field potential and medial septal (or hippocampal) single cell activity from urethane anesthetized rats. Single cell recordings were performed by the juxtacellular technique, which made the immunocytochemical identification of the recorded neurons possible. We used novel circular statistical and information theory analysis tools to gain insight into the regulation of the septo-hippocampal communication.

Our data revealed the temporal antecedence of activity changes in a subgroup of medial septal neurons containing the calcium binding protein parvalbumin (PV) and/or the putative pacemaker channel HCN (hyperpolarization activated and cyclic nucleotide gated non-selective cation channel) compared with hippocampal interneurons and pyramidal cells. The information theory analysis revealed a dominant septo-hippocampal direction of information transfer during theta oscillation that was accompanied by a hippocampo-septal feedback influence. These data suggest the pacemaker role of medial septal PV/HCN neurons in the formation of hippocampal theta oscillation.

- Hangya B, Borhegyi Z, Szilágyi N, Freund TF, Varga V (2009) *GABAergic neurons of the medial septum lead the hippocampal network during theta activity. J Neurosci 29: 8094–8102.*
- Varga V, Hangya B, Kránitz K, Ludányi A, Zemankovics R, Katona I, Shigemoto R, Freund TF, Borhegyi Z (2008) *The presence of pacemaker HCN channels identifies theta rhythmic GABAergic neurons in the medial septum. J Physiol 586: 3893–3915.*

## **LAJOS RUDOLF KOZÁK (2010)**

### **Behavioral and neural correlates of human visual processing as assessed by psychophysics and functional magnetic resonance imaging**

*Supervisor: György Karmos*

Among our sensory modalities the visual modality is the one providing the highest information load. We live in an extremely rich visual environment; in general, one has to deal with constantly moving colored surface patches during visual information processing. In the present thesis I describe our work done on color vision, and visual motion processing. We used computer-based psychophysics to test color vision in glaucoma and Best's vitelliform macular dystrophy. We found that color vision deficiencies are present in all cone pathways in both diseases despite the common views that associate glaucoma with selective blue-yellow deficits, and Best disease with selective red-green deficits. We also found that color vision deficits correlate well with standard clinical parameters used for staging in both pathologies.

We used psychophysics and/or functional magnetic resonance imaging (fMRI) to study center-surround interactions in surface integration, the interference between different observed motion types, and the effect of learning on surface segmentation.

By using psychophysics to study how moving surfaces are integrated we found strong surround influences on central ambiguous percepts. This effect depended on the inherent integration/segmentation bias of the surrounds, the presence/absence of local cues pro-



viding disambiguation, and the presence/absence of global context interfering with selected elements of the central moving surfaces.

We showed using fMRI that the interaction of real and apparent motion may lead to additive adaptation without saturation of hMT+ responses, while the interaction of motion aftereffects and apparent motion reflects interference. Moreover, neural substrates of illusory motion aftereffects can be identified when selective attention is directed to concomitant non-motion features and are masked by concurrent apparent/real motion tasks. We found that learning results in increased detection thresholds and decreased fMRI responses for task-irrelevant motion direction compared to the task-relevant motion direction throughout the visual cortex.

- Castelo-Branco M, Faria P, Forjaz V, Kozak LR, Azevedo H (2004) *Simultaneous comparison of relative damage of chromatic pathways in ocular hypertension and glaucoma: correlation with clinical measures. Invest Ophthalmol Vis Sci 45: 499–505.*
- Campos SH, Forjaz V, Kozak LR, Silva E, Castelo-Branco M (2005) *Chromatic dysfunction in Best's macular dystrophy: a model for new quantitative strategies of phenotyping and clinical staging. Arch Ophthalmol 123: 944–949.*
- Kozak LR, Castelo-Branco M (2009) *Peripheral influences on motion integration in foveal vision are modulated by central local ambiguity and center-surround congruence. Invest Ophthalmol Vis Sci 50: 980–988.*

## ALIZ MAYER (2010)

### Non-conventional effects of antidepressants in the central and peripheral nervous system

Supervisor: János Kiss

It is generally accepted that the majority of currently used antidepressants acts through the inhibition of monoamine reuptake and the concomitant increase of extracellular monoamine levels. Previously our research group proved that these compounds might have other targets than the monoamine transporters. In this work we investigated (1) the effects of monoamine uptake inhibitor and enhancer antidepressants on the ionotropic receptors, as well as (2) we studied the similarities amongst monoamine transporters and ionotropic receptors.

(1) We have shown that two atypical antidepressants, maprotilin and tianeptine acting on monoamine uptake systems are able to inhibit the function of nACh receptors. Since tianeptine is a 5-HT reuptake enhancer, its mechanism of action cannot be explained on the basis of monoamine theory. Thus our results confirm the nAChR-theory of depression, which explains the development of depression by an overactivation of nAChRs and the mechanism of action of antidepressants by the inhibition of these receptors. Furthermore, we proved that the two most potent nAChR antagonist antidepressants, the tricyclic desipramine and the SSRI fluoxetine inhibit not only the function of NMDA receptors in the central nervous system, but also the P2X receptor's function in the peripheral nervous system in the clinically relevant, low micromolar concentration range. Our data suggest that the interaction of antidepressants with ionotropic receptors might contribute to their clinical effects, thus our results might help to better understand the neurochemical background of depression and the mechanism of action of antidepressants.

(2) Previously we have shown the monoamine uptake inhibitors behave like channel blocker type nAChR and NMDAR antagonists. In this work we found that the channel blocker antagonists of nAChRs and NMDARs, mecamylamine and MK-801 inhibit the function of the striatal DA uptake system. Our data support the idea, that the channel structures in transporters have functional significance in the process of monoamine reuptake.

- Mayer A, Szasz BK, Kiss JP (2009) *Inhibitory effect of antidepressants on the NMDA-evoked [<sup>3</sup>H]noradrenaline release from rat hippocampal slices. Neurochem Int 55: 383–388.*
- Szász BK, Mayer A, Zsilla G, Lendvai B, Vizi ES, Kiss JP (2005) *Carrier-mediated release of monoamines induced by the nicotinic acetylcholine receptor agonist DMPP. Neuroparmacology 49: 400–409.*
- Vizi ES, Rózsa B, Mayer A, Kiss JP, Zelles T, Lendvai B (2004) *Further evidence for the functional role of nonsynaptic nicotinic acetylcholine receptors. Eur J Pharmacol 500: 499–508.*

## LILLA PAPP (2010)

### The role of ATP sensitive P2 receptors in the regulation of the hippocampal neurotransmitter (noradrenaline, GABA, glutamate) release: bring into focus the P2X<sub>7</sub> receptor

Supervisor: Beáta Sperlág

The activation of P2X receptors elicits [<sup>3</sup>H] NA outflow from the rat hippocampus, and the most likely candidates responsible for this action are the homomeric P2X<sub>1</sub> or P2X<sub>3</sub> receptors. This arrangement is similar to that found recently in rat sympathetic nerve terminals, where P2X<sub>1</sub>, P2X<sub>3</sub>, and P2X<sub>2/3</sub> receptors were identified to facilitate noradrenaline release, however, the differentiating effect of the low pH was not tested. Taken into account that glutamatergic excitatory terminals are equipped with presynaptic P2X<sub>2</sub> and P2X<sub>7</sub> receptors, glutamatergic and noradrenergic transmission in the rat hippocampus could be distinctly regulated by facilitatory P2X receptors.

Our data, as a first demonstration of a change in neuronal functions in P2X<sub>7</sub>R-deficient mice, provide evidence for the involvement of the P2X<sub>7</sub>R in the modulation of transmitter release in the hippocampus. Therefore, this receptor might have important role in the regulation of extracellular transmitter levels and might participate in the neuron-glia-microglia cross talk. Taking into account the activity-dependent expression of P2X<sub>7</sub>R in the brain in pathological models such as ischemia or Alzheimer's disease, these findings have important implications, not only in the control of synaptic efficacy, but also in neurodegenerative diseases, and urge further investigation on the function of this receptor in the CNS.

We report here for the first time the increased expression of p38 MAPK enzyme following P2X<sub>7</sub>R activation in the mouse hippocampus. This interaction might play a critical role in increased glutamate release, as well as in other downstream effects upon P2X<sub>7</sub>R activation, in various physiological and pathological states (e. g. synaptic plasticity, neurodegeneration, and neuroinflammation) where P2X<sub>7</sub>Rs and p38 MAPKs are known to be both activated.

- Papp L, Balázs T, Kőfalvi A, Erdélyi F, Szabó G, Vizi ES, Sperlág B (2004) *P2X receptor activation elicits transporter-mediated noradrenaline release from rat hippocampal slices. J Pharmacol Exp Ther 310: 973–980.*

- Papp L, Vizi ES, Sperl gh B (2004) Lack of ATP-evoked GABA and glutamate release in the hippocampus of P2X7 receptor  $-/-$  mice. *Neuroreport* 15: 2387–2392.
- Papp L, Vizi ES, Sperl gh B (2007) P2X7 receptor mediated phosphorylation of p38MAP kinase in the hippocampus. *Biochem Biophys Res Commun* 355: 568–574.

## MARIJA SVIRTLIH (2010)

### Molecular components and functionality of the GABA signaling during development: a study on two model systems

Supervisor: G bor Szab 

$\gamma$ -aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS and as such regulates neuronal excitability. During development GABA acts as a trophic factor modulating processes such as proliferation, differentiation and migration in both neuronal and non-neuronal tissues. The aim of the present study was to characterize GABA signaling components and function during development in two different model systems: mouse embryonic stem (ES) cells and the ocular lens. Our results can be summarized as: (1) Undifferentiated pluripotent embryonic stem (ES) cells and differentiating lens cells express neuron-specific components necessary for an active GABA signaling system including GAD, GABAR and GABA transporters.

(2) The temporal regulation of the two known GAD genes coding for the GABA-synthesizing enzymes GAD65 and GAD67 and the upstream transcriptional regulators *Dlx2/5* follows a highly reproducible pattern in the studied *in vivo* and *in vitro* differentiation systems.

(3) Based on our expression analysis, both vesicular and membrane transporter-mediated GABA release may occur in undifferentiated ES cells and during neuronal or lens differentiation in the absence of inhibitory synapses.

(4) GABA<sub>A</sub> and GABA<sub>B</sub> receptors in ES cells and lens epithelial cells display unique composition and properties; both receptor types evoke intracellular Ca<sup>2+</sup> rise by distinct mechanisms involving both the extracellular Ca<sup>2+</sup> and intracellular Ca<sup>2+</sup> stores.

(5) Despite the synergistic effect of GABA<sub>A</sub> and GABA<sub>B</sub> receptors on the intracellular Ca<sup>2+</sup>-concentration, they exert opposite effects on cellular proliferation and/or differentiation of ES cells. This effect is in sharp contrast to the receptors' properties in immature neurons, but may be similar to these in neural stem cells.

Our findings supports the view that GABA may serve as a paracrine–autocrine signaling molecule at early stages of embryonic development affecting cell proliferation, cell-cell communications and/or differentiation by mechanism(s) similar to that in the embryonic nervous system.

Using these two model systems will allow us to uncover the general mechanism of GABA action in basic developmental processes, which could also apply to the nervous system.

- Kwakowsky A\*, Schwirtlich M\*, Zhang Q, Eisenstat DD, Erdelyi F, Baranyi M, Katarova ZD, Szabo G (2007) GAD isoforms exhibit distinct spatiotemporal expression patterns in the developing mouse lens: correlation with *Dlx2* and *Dlx5*. *Dev Dyn* 236: 3532–3544 (\*authors equally contributed).
- Kwakowsky A\*, Schwirtlich M\*, Kooy F, Abraham I, Mate Z, Katarova Z, Szabo G (2008) GABA neurotransmitter signaling in the developing mouse lens: dynamic regulation of components and functionality. *Dev Dyn* 237: 3830–3841 (\*authors equally contributed).

- Schwirtlich M, Emri Z, Antal K, Máté KZ, Katarova Z, Szabó G (2010) Activation of GABAA and GABAB receptors of distinct properties adversely affects proliferation of mouse embryonic stem (ES) cells through modulation of intracellular  $Ca^{2+}$  signalling. *FASEB J* 24: 1218–1228.

## SZILÁRD SZABÓ (2010)

### Role of synaptic and nonsynaptic nicotinic acetylcholine receptors in dendritic integration: a two-photon microscope study

Supervisor: Balázs Lendvai

The function of the hippocampus lies on a balance between excitatory and inhibitory neurons. Neurotransmitters released either from hippocampal neurons or from neuronal tracts innervating the hippocampus modulate the information processing ability of these cells. Modulation by ACh is one example: it has a direct influence on mechanisms of arousal, learning and memory. Despite its importance underlying cellular mechanisms are unclear. Memory tests performed on smokers revealed improved cognitive functions. Nicotinic ligands administered to patients suffering from neurodegenerative disorders involving nAChR degradation significantly improved their condition. It has been widely accepted that synaptic plasticity underlies memory, so we focused our attention on nAChR mediated modulation of a key player in synaptic plasticity: the vAPs. Our question: how does the activation of nAChRs modulate dendritic backreporting (vAPs)? Possible components of the phenomenon were studied in acute rat hippocampus slices that keep the brain matter's 3D structure, connections and functions intact, consequently regarded as good physiological model. Recording of intracellular  $Ca^{2+}$  levels were performed using two-photon laser scanning microscopy. We modeled nicotine buildup in smokers' brain by administering 1  $\mu$ M of nicotine via perfusion, ACh release during physiological activity with local pressure application of 500  $\mu$ M–1 mM of nicotine and choline respectively. Results: we were first in the literature to show the complex interplay between backpropagating APs and the nicotinic arm of the cholinergic system. We pointed out that nicotinic ligands facilitate both cell types (excitatory and inhibitory). Evidence was also provided that the origin of this effect is pre- or extra-synaptic in pyramidal cells and post- or extra-synaptic in interneurons. From the point of network activity, excitation of pyramidal cells facilitates information processing in the hippocampus while excitation of interneurons strengthens inhibition. Diverse combinations of these elemental effects may underlie cognitive function boost attributed to nicotine.

- Lendvai B, Szabo SI, Barth AI, Zelles T, Vizi ES (2006) Application of two-photon microscopy to the study of cellular pharmacology of central neurons. *Ad Drug Deliv Rev* 58: 841–849.
- Szabo SI, Zelles T, Lendvai B (2008) Intracellular  $Ca^{2+}$  dynamics of hippocampal interneurons following nicotinic acetylcholine receptor activation. *Neurochem Int* 52: 135–141.
- Szabo SI, Zelles T, Vizi ES, Lendvai B (2008) The effect of nicotine on spiking activity and  $Ca^{2+}$  dynamics of dendritic spines in rat CA1 pyramidal neurons. *Hippocampus* 18: 376–385.

- Makara JK, Mor M, Fegley D, Szabo SI, Kathuria S, Astarita G, Duranti A, Tontini A, Tarzia G, Rivara S, Freund TF, Piomelli D (2005) Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. *Nature Neurosci* 8: 1139–1141.

## SZILVIA VAJDA (2010)

### The role of Cyclophilin D and the adenine-nucleotide translocator in the impairment of mitochondrial functions

Supervisor: Veronika Ádám

Mitochondria are the primary source of ATP generated during oxidative phosphorylation. ATP synthesis through oxidative phosphorylation in mitochondria also requires ADP and  $\text{H}_2\text{PO}_4^-$  (Pi) that are provided by adenine-nucleotide translocase (ANT) and a phosphate carrier, respectively.

In the present work, a novel method is presented for assessing the rate of ADP/ATP translocation by the ANT in intact isolated mitochondria by exploiting the differential affinity of ADP and ATP to  $\text{Mg}^{2+}$ .

Both ANT and the  $\text{F}_0\text{F}_1\text{ATPase}$  are reversible enzymes. During conditions when mitochondrial respiration gets compromised, mitochondria switch to ATP consumers by the reversal ATP synthase antagonizing a collapse in membrane potential at the expense of ATP hydrolysis. In this work it is shown that inhibition of the respiratory chain may shift the membrane potential to a range bracketed by the reversal potential of the  $\text{F}_0\text{F}_1\text{ATPase}$  and of the ANT, the latter being more negative than the former. Moreover, we show that the matrix substrate-level phosphorylation provides ATP for the  $\text{F}_0\text{F}_1\text{ATPase}$  to maintain the membrane potential during respiratory chain inhibition.

Mitochondria have enormous capacity to retain and accumulate  $\text{Ca}^{2+}$  by forming calcium- and phosphorus-rich precipitates in the matrix. Upon the collapse of mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ) these precipitates become soluble and  $\text{Ca}^{2+}$ -release occurs. A long-standing concept explained the phenomenon by the acidification of the mitochondrial matrix. In the present work it is shown that matrix acidification cannot be the only explanation of mitochondrial  $\text{Ca}^{2+}$ -release.

- Chinopoulos C, Vajda S, Csanády L, Mándi M, Mathe K, Adam-Vizi V (2009) A novel kinetic assay of mitochondrial ATP-ADP exchange rate mediated by the ANT. *Biophys J* 96: 2490–2504.
- Vajda S, Mándi M, Konrad C, Kiss G, Ambrus A, Adam-Vizi V, Chinopoulos C (2009) A re-evaluation of the role of matrix acidification in uncoupler-induced  $\text{Ca}^{2+}$  release from mitochondria. *FEBS J* 276: 2713–2724.
- Chinopoulos C, Gerencsér AA, Mándi M, Máthé K, Törőcsik B, Dóczy J, Turiak L, Kiss G, Konrád C, Vajda S, Vereczki V, Oh RJ, Adam-Vizi V (2010) Forward operation of ANT during  $\text{F}_0\text{F}_1\text{-ATPase}$  reversal: critical role of matrix substrate-level phosphorylation. *FASEB J* 24: 2405–2416.

**PROGRAM 6/4.****CLINICAL NEUROSCIENCE*****Coordinator:*****Zoltán NAGY M.D., Ph.D., D.Sc.**

Department of Cardiovascular Surgery

68 Városmajor st, Budapest H-1122

Tel.: +36 1 467 9301

E-mail: [nagyzg@oiti.hu](mailto:nagyzg@oiti.hu)**Program overview**

The Clinical Neuroscience Program within the Neuroscience Doctoral School focuses on the research of mechanisms of neuropsychiatric disorders. Laboratories in the program apply cell biological, molecular biological, pharmacological and electrophysiological methods involving Ph.D. students. Of the three research groups the vascular neurological group (head Prof. Dr. Zoltán Nagy) performs studies using permanent or transient ischemic animal models and PC12 and endothelial cell cultures applying cell biological and neuronal apoptotic studies. The epilepsy group (head: Prof. Dr. Péter Halász) studies the mechanism of spike-and-wave epilepsy, the relationship of sleep and epilepsy, and the relationship between epileptic mechanisms and cognitive function, the temporal spike activity and memory. The pharmacological group (head: Prof. Dr. György Bagdy) studies the pharmacology of serotonin, and the relationship between serotonin and sleep disorders.

***Titles of research projects***

Regulation of sleep and circadian rhythm by neurotransmitters, neuropeptides and their receptors

The introduction of genome systems biology research into the diagnostic, prevention and therapy of neurological and psychiatric disorders

Physical activity and brain aging: effects of vascular and neurotrophic factors (animal studies)

GAP-43 signal and/or regulator protein in post-ischemic brain plasticity

Brain monitoring by 128 channels EEG after ischemic stroke

Characterization of primary and immortalized human cerebral microvessel endothel under hyperbaric oxygen conditions

Relationship of patent foramen ovale and cryptogenic stroke in the secondary prevention of stroke

***Supervisors***

György Bagdy

Mária Judit Molnár

Csaba Nyakas

Zoltán Nagy

Zoltán Nagy

Judit Skopál

Géza Szilágyi

***Ph.D. students***

Magdolna Dombóvári

pt

Gabriella Inczédy-Farkas

ft

István Kapás

pt

Zita Klára Kátai

ft

Sándor Nardai

pt

***Supervisors***

Zoltán Nagy

Mária Judit Molnár

Gábor Kovács

György Bagdy

Zoltán Nagy



Krisztián Pozsegovits	pt	Zoltán Nagy
Viktória Reményi	ft	Mária Judit Molnár
Klára Pentelényi Roszkos	ft	Mária Judit Molnár
Léna Szabó	ft	András Fogarasi

***Ph. D. candidates***

László Entz	ft
Tamás Kitka	ft

***Supervisors***

Péter Halász
György Bagdy

***Ph. D. graduates***

Loránd Erőss	na
Anikó Gál	ft
Eszter Kirilly	ft
Endre Pongrácz	na
Zsanett Tárnok	ft
Edina Amália Wappler	ft

***Supervisors***

Péter Halász
Zoltán Nagy
György Bagdy
Zoltán Nagy
Péter Halász
Zoltán Nagy

f, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****LORÁND ERŐSS (2010)****The role of invasive electrodes in the presurgical evaluation for epilepsy**

*Supervisor: Péter Halász*

A new period in the surgical treatment of epilepsies in Hungary begun in 1990. The regular use of intracranial electrodes for invasive monitoring started in the middle of the 1990s. In 1996 we introduced the foramen ovale electrode technique for semi-invasive monitoring the temporomesial structures. The aim of this thesis is to summarize 15 years of neurosurgical experience with invasive electrodes in the presurgical evaluation of patients with drug resistant epilepsies. We described in details the foramen ovale technique and reviewed our surgical, epileptological, electrophysiological experience with the FO electrode technique.

With this method we were able to expand our differential diagnostic tools of temporal and extratemporal epilepsies. We were able to lateralize in mesial temporal lobe epilepsies the seizure onset zone. It was possible to localize the seizure origin within the temporal lobe and differentiate temporomesial from temporolateral, neocortical seizures. The technique was able to give answers whether the epilepsy is unilateral or bitemporal. The method is valid to differentiate seizure onset within the temporomesial structures. With the FO technique we clarified the role of temporomesial structures of seizure propagation in temporal and extratemporal epilepsies involving the temporomesial structures. We published a new FO electrode which was designed by us and produced in Hungary. The other objective of our study was to investigate interhemispheric propagation of

mesial temporal lobe epilepsy seizures in patients undergoing long-term video-EEG monitoring with combined scalp and foramen ovale electrodes. We differentiated two types of contralateral seizure propagation which seems to present two subtypes of mesial temporal lobe epilepsies. The predominance of type I. over type II. seizures with a shorter propagation time to the contralateral side for Type I. seizures indicate a more direct and probably dominant interhemispheric pathways in mesial temporal lobe epilepsy. We assume that this pathway could be similar to the dorsal hippocampal commissure which was described in details by Gloor in 1993.

In the third part of the paper we describe the new surgical method of neuronavigation and X-ray assisted implantation of subdural electrodes which was introduced into the neurosurgical practice by us. We analyze our semiology driven protocol for invasive electrode placement, the complications with invasive electrode techniques and our surgical strategies to avoid of them.

- Eross L, Bago AG, Entz L, Fabo D, Halasz P, Balogh A, Fedorcsak I (2009) *Neuronavigation and fluoroscopy-assisted subdural strip electrode positioning: a simple method to increase intraoperative accuracy of strip localization in epilepsy surgery. J Neurosurg* 110: 327–331.
- Eross L, Entz L, Fabo D, Jakus R, Szucs A, Rasonyi G, Kelemen A, Barcs G, Juhos V, Balogh A, Barsi P, Clemens Z, Halasz P (2009) *Interhemispheric propagation of seizures in mesial temporal lobe epilepsy. Clin Neurosci* 62: 319–325.
- Cash SS, Halgren E, Dehghani N, Rossetti AO, Thesen T, Wang C, Devinsky O, Kuzniecky R, Doyle W, Madsen JR, Bromfield E, Eross L, Halasz P, Karmos G, Cserecsa R, Wittner L, Ulbert I (2009) *The human K-complex represents an isolated cortical down-state. Science* 324: 1084–1087.

## ANIKÓ GÁL (2009)

### The role of mitochondria in neurodegeneration and neuroprotection

Supervisor: Zoltán Nagy

Mitochondrial dysfunctions have been associated with a variety of clinical manifestations, including neurological, psychiatric and medical disorders. Occasionally the phenotype is modified by other polymorphisms synergistically. The key role of mitochondria in brain ischaemia is well established; therefore the influence of its function could be a beneficial neuroprotective therapeutic opportunity.

In our work we investigated: (1) the genetic variability of the mtDNA tRNA<sup>Lys</sup> gene and its neighbouring regions in 470 patients suspected to have mitochondrial disease based on their clinical symptoms; (2) the frequency of the tRNA<sup>Leu(UUR)</sup> A3243G substitution among the Hungarian population with by examining 631 patients; (3) the effect of Bcl-2 and Bcl-XL antiapoptotic genes in reducing hypoxic impairment in PC12 cells in an *in vitro* hypoxic model.

In the course of our investigation of mtDNA tRNA<sup>Lys</sup> we found one previously described (A8344G) and three novel pathogen mutations (A8332G, T8310G and T8311A), five polymorphisms and anthropological marker, and one putative susceptibility SNP for neurodegeneration (A8347C). Among 631 Hungarian patients the mtDNA A3243G mutation was found in 5 women and 1 man. The segregation analysis detected further 8 cases, there-

fore the mutation frequency in the investigated cohort was 2.22%. The delivery of anti-apoptotic genes can decrease hypoxic impairment and enhance the expression of brain plasticity proteins, namely GAP-43, nestin and c-fos.

In summary, (1) high genetic variability was found in the tRNA<sup>Lys</sup> gene and its neighbouring regions among our investigated patients; (2) a heteroplasmic A8332G mutation was found in the background of dystonia and stroke-like symptoms where the synergistic effect of the A8347C, T10463C and C14520G non-synonymous mtDNA substitutions could also be observed; (3) The mtDNS A3243G substitution was detected as the underlying cause of some multi-systematic, mitochondrial phenotypes in the Hungarian population; (4) in our *in vitro* PC12 hypoxic model the mitochondrial anti-apoptosis and cell plasticity pathways are linked, however the exact mechanism is not known exactly at the moment.

- Gál A, Szabó A, Pentelényi K, Pál Z (2008) *Maternálisan öröklődő diabetes mellitus, nagyothallás, krónikus ophthalmoplegia externa és myopathia mint az mtDNS A3243G mutáció következménye. Orv Hetilap 149: 1593–1598.*
- Gal A, Szilagyi G, Wappler E, Safrany G, Nagy Z (2008) *Bcl-2 or Bcl-XL gene therapy reduces apoptosis and increases plasticity protein GAP-43 in PC12 cells. Brain Res Bull 76: 349–353.*
- Gal A, Pentelényi K, Remenyi V, Wappler EA, Safrany G, Skopal J, Nagy Z (2009) *Bcl-2 or Bcl-XL gene therapy increases neural plasticity proteins nestin and c-fos expression in PC12 cells. Neurochem Int 55: 349–353.*

## ESZTER KIRILLY (2010)

### Time course of neuronal damage and recovery induced by MDMA: expression and distribution of serotonin transporter in the rat brain

Supervisor: György Bagdy

“Ecstasy”, 3,4-methylenedioxymethamphetamine (MDMA), an amphetamine analogue is one of the most widely used recreational drugs. In spite of the fact that neurotoxic effects of MDMA has been found in several species from rodents to non-human primates, and results increasingly point to damage also in human MDMA users, data about the sensitivity of different brain areas and the recovery after neuronal damage are scarce. Serotonin transporter (5-HTT) mRNA in the raphe nuclei also has not been examined; consequently, direct comparisons of responses of dorsal and other raphe nuclei have not been measured.

We specifically emphasized the effects of MDMA on 5-HTT protein in our research because previous studies have shown that MDMA selectively destroyed the serotonergic system both in experimental animals and humans. This protein is target of MDMA and also a marker of the established damage.

Humans with genetic predisposition for the slow metabolism of MDMA, the so-called “poor metabolizers” of debrisoquin are at higher risk. Five—9%—of the Caucasian population is considered to carry this phenotype. These studies were carried out in Dark Agouti rats, a special strain that show decreased microsomal CYP2D1 isoenzyme activity, and thus may serve as a model of vulnerable human users.

These works were designed to characterize MDMA-induced damage and recovery of the serotonergic system including sleep and morphological changes within 180 days. In our experiments we investigated the 5-HTT mRNA expression in the brainstem (dorsal and median raphe nucleus) and medullary (nucleus raphe magnus, obscurus and pallidus) raphe nuclei, 5-HTT immunoreactive (IR) fibre densities in several brain areas, and 16 functional measures of sleep in response to a single dose of  $\pm$  MDMA (15mg/kg) which is relevant to ecstasy use in humans. Studies were carried out at least at three time-points within 180 days. Furthermore, behavioural experiments were performed 21 days after MDMA treatment, because at this timepoint morphological studies indicated significant axonal damage without any sign of regeneration.

We found similar changes in 5-HTT mRNA expression in the examined raphe nuclei, namely transient increases 7 days after MDMA treatment followed by transient decreases at 21 days. Significant (20–40%), widespread reductions in 5-HTT-IR fibre density were detected in most brain areas at 7 and 21 days after MDMA administration. All cortical, but only some brainstem areas were damaged. Parallel to the neuronal damage we observed significant reductions in rapid eye movement (REM) sleep latency, increased fragmentation of sleep and increases in delta power spectra in non-REM sleep. Some of these (reductions in REM latency and increased sleep fragmentation) were consistent with those described in depression. At 180 days almost all functional changes in sleep were normalized together with 5-HTT mRNA expression in the examined raphe nuclei and the recovery of 5-HTT-IR fibre density in most brain areas. Among the exceptions hippocampus should be noted because we found a significant reduction even 180 days after MDMA treatment. Disturbances in cognitive and memory functions in previous ecstasy users could be likely consequences of the long-term damage of the hippocampus. Our results also suggest that the acute MDMA administration abolished aggressive behaviour but MDMA pretreatment and the consequent depletion of serotonergic terminals did not affect aggression.

Our findings concerning the changes detected in 5-HTT mRNA expression and fibre density indicate lasting impairment of the serotonergic system and suggest that a single use of MDMA may be associated with long-lasting cognitive, learning, memory and mood deficits and sleep disturbances particularly when a constellation of genetic vulnerability and certain environmental factors are present. Our data provide further evidence for the connection between altered serotonergic functions and sleep disturbance.

- Kirilly E, Molnar E, Balogh B, Kantor S, Hansson SR, Palkovits M, Bagdy G (2008) *Decrease in REM latency and changes in sleep quality parallel serotonergic damage after MDMA: a longitudinal study over 180 days.* *Int J Neuropsychopharmacol* 11: 795–809.
- Kirilly E, Benko A, Ferrington L, Ando RD, Kelly PAT, Bagdy G (2006) *Acute and long-term effects of a single dose of MDMA on aggression in Dark Agouti rats.* *Int J Neuropsychopharmacol* 9(1): 63–76.
- Kovács GG, Andó RD, Ádori C, Kirilly E, Benedek A, Palkovits M, Bagdy G (2007) *Single dose of MDMA causes extensive decrement of serotonergic fibre density without blockage of axonal transport in Dark Agouti rat brain and spinal cord.* *Neuropathol Appl Neurobiol* 33: 193–203.

**ENDRE PONGRÁCZ (2009)****Genetic and haemorheological blood clotting factors influencing the risk and outcome of ischaemic stroke***Supervisor: Zoltán Nagy*

**Objectives:** Several genetic polymorphisms of the haemostatic system have been examined synchronically which are in a controversial relationship with the risk factors of stroke. In addition, we studied the effect of the hyper-viscosity of the blood on the electrophysiological, subclinical signs of the brainstem in ischaemic stroke patients. Furthermore, the resistance of platelets to anti-aggregation drugs was measured in connection with mortality in stroke patients; PLA2 platelets were studied *in vitro* as well.

**Patients and methods:** The prevalence of the factor II G20210A, ACE gene I/D, platelet receptor GP IIb/IIIa (LeuPro33), Leiden mutation, MTHFR G677A and fibrinogen gene 455G/A genetic polymorphisms were studied by the PCR method in ischaemic stroke patients (group <50 years and over 50 years, n=433) and in healthy control groups. Connections have been searched for by brainstem auditory evoked potentials (BAEP) and measurement of whole blood viscosity among different groups of ischaemic stroke patients and in the control group suffering from hyperviscosity. The laboratory resistance of platelet aggregation was measured by CARAT TX optical aggregometer in a large group (n=2455) of ischaemic vascular patients. Collagen, ADP and epinephrine were used as inducers.

**Results:** PLA2 and Leiden mutation prevalence has been found to be more frequent in the stroke group compared with the control group for the first time in a Hungarian sample. Significantly more ischaemic stroke and vascular events have been found in the family anamneses of patients with the heterozygous variant of ACE gene and Leiden mutation than in patients having wild types. Hyperactivity and the aggregability of PLA1/A2 platelets may occur as a result of several mechanisms. Polymorphisms of ABCA1 gene seem to be protecting factors in ischemic stroke patients with elevated HDL cholesterol compared to the controls. The prevalence of dual genetic polymorphisms is more frequent in stroke patients compared to the controls. The factor II variant coupled with the most frequent occurrence with other polymorphisms in stroke patients. It has also been detected that the pathological BAEP patterns are bilateral in cases of those with blood hyperviscosity. The FIB-455 G/A variant goes hand in hand with elevated FIB concentration in plasma compared with the control groups. As a new result, a tight connection has been detected between Leiden mutation and the hyperviscosity of plasma compared to the patients with the wild types. Our platelet aggregation studies showed that in the event of ASA resistance, the continuation of ASA treatment leads to significantly higher mortality compared with the mortality in groups in which the therapy was changed to thienopyridines.

- Andrikovics H, Pongrácz E, Kalina Á, Szilvási A, Aslanidis C, Schmitz G, Tordai A (2006) Decreased frequencies of ABCA1 polymorphism R219K and V771M in Hungarian patients with cerebrovascular and cardiovascular diseases. *Cerebrovasc Dis* 2: 254–259.
- Bernáth IS, Bernát Z, Pongrácz E, Köves P, Szakács Z, Horváth A (2004) Effects of blood hyperviscosity on functional integrity in the brain stem: a brain stem evoked auditory potential study. *Clin Hemorheol Microcirc* 31: 123–128.
- Pongrácz E, Andrikovics H, Csornai M, Bernát IS, Nagy Z (2006) Contribution of the –455G/A polymorphism at  $\beta$ -fibrinogen gene and Leiden mutation to haemorheological parameters in ischaemic stroke patients. *Clin Hemorheol Microcirc* 35:75–82.

## ZSANETT TÁRNOK (2009)

### Childhood onset neuropsychiatric disorders risk factors in Tourette's syndrome and comorbid attention deficit hyperactivity disorder

Supervisor: Péter Halász

The main purpose of these theses was to find specific genetic and neuropsychological features in child psychiatric disorders such as Tourette's syndrome and Attention Deficit Hyperactivity Disorder. The joint investigation of these two disorders is based on the high co-morbidity rates (Freeman *et al.* 2007), the common prefrontal and basal ganglia (frontostriatal) pathology and dysregulation of dopamine neurotransmitter system. Three candidate polymorphisms of dopaminergic genes were chosen (DRD4—48 bp VNTR, DAT—40 bp VNTR, COMT Val158Met). In addition, we selected three neuropsychological tests which measure distinct, independent executive functions (cognitive inhibition, cognitive flexibility and working memory) connected to the frontostriatal system. Our hypothesis was that differences exist in the genetic background of TS and ADHD, as well as in the neuropsychological features, which would apply to specific brain areas involved in these disorders. One hundred and three children diagnosed with TS, 173 children diagnosed with ADHD and 284 healthy controls participated in our genetic analysis. Our results (Tarnok *et al.* 2007b) show that the DAT—40 bp VNTR 9-repeat variant, which connected with higher dopamine transporter density in the striatum, had a significant positive association with more severe symptoms in TS. In the ADHD group the high-activity Val-allele of the COMT Val158Met polymorphism, which plays an important role in cognitive functions connected to the prefrontal cortex, showed a significant association with the disorder itself and with good methylphenidate response. This result supports the hypodopaminergic hypothesis of ADHD. In our neuropsychological study (Tarnok *et al.* 2007a), we carefully controlled for the co-morbid conditions, age, gender, intellectual ability and drug effects, therefore, we used the data of only 164 children in the analysis. A few differences have been detected in the executive profile of the two disorder: in pure TS there was a tendency toward cognitive disinhibition, which suggests the role of medial-ventral prefrontal circuits in the pathology of tic symptoms. In pure ADHD, there was a significant short term and working memory dysfunction, which was independent of co-morbid conditions, such as learning disorder. These functions are connected to the dorsolateral prefrontal circuits of the frontostriatal system, supporting the role of these brain areas in ADHD. However, the high heterogeneity of this disorder brings other complex neurobiological and psychological models into focus, which are based on motivational, and reward-dependent theories and other aspects of inhibition.

- Tárnok Z, Bognár E, Farkas L, Aczél B, Gáboros J (2007) A végrehajtó funkciók vizsgálata Tourette szindrómában és figyelemhiányos hiperaktivitás zavarban. Pp 191–210 in: Racsmány M. (szerk), *A fejlődés zavarai és vizsgálómódszerei. Akadémiai kiadó Budapest.*
- Tarnok Z, Ronai Z, Gervai J, Kereszturi E, Gáboros J, Sasvari-Szekely M, Nemoda Z (2007) Dopaminergic candidate genes in Tourette Syndrome: association between tic severity and 3' UTR polymorphism of the dopamine transporter gene. *Am J Med Genet B, Neuropsychiatr Genet* 144: 900–905.
- Freeman RD, The Tourette Syndrome International Database Consortium (Tarnok Z) (2007) Tic disorders and ADHD: answers from a world-wide clinical dataset on Tourette Syndrome. *Eur Child Adolesc Psychiatry* 16, Suppl 1.16: I/15–I/23.



**EDINA AMÁLIA WAPPLER (2010)****Effect of acute estrogen pre-treatment on the expression of apoptotic and cerebral plasticity genes and adaptive behavior following global cerebral ischemia in gerbils at different ages. Adaptation of behavioral tests to gerbils**

*Supervisor: Zoltán Nagy*

With increasing age comes an increased risk for cerebral ischemia; however in basic research mainly young animals are used. During aging the brain undergoes several structural and functional changes, and reacts differently to ischemic brain injury compared to the young brain. Although much is known about the protective effect of acute estrogen therapy in cerebral ischemia, relatively little is known about its effect on functional outcome at different ages. In our work we investigated whether a high, single-dose (4 mg/kg b.w.ip.) of estradiol pre-treatment would be neuroprotective at different ages (4-, 9- and 18 month old) ovariectomised female gerbils following 10 min global brain ischemia. Hippocampal damage was studied in behavioral tests and histology, where TUNEL-caspase-3 cells were counted to assess cell death. To investigate the gerbils' behavior first we had to adopt some test, such as open field, Y-maze, novel object recognition and hole-board spatial learning test for which we used male animals. In addition in our estrogen treatment protocol we measured the expression of some genes involved in apoptosis (bcl-XL, bax) and in cerebral plasticity (GAP-43, synapsin-I, nestin) to investigate their role in neuroprotection. Our results are the followings: (1) We adopted successfully the open field test, the Y-maze test, the novel object recognition test and the hole-board task to Mongolian gerbils. The animals had stable behavior between 4 and 18 month of age. Brain ischemia resulted in worst functional outcome in every test. (2) Following cerebral ischemia increased number of damaged cells was detected in the female gerbils with no age effect. In behavioral tests, however, older gerbils had worst functional outcome—except reference memory in the hole-board test. Estrogen treatment preserved hippocampal cells and the ameliorated the functional outcome at all ages. (3) Due to estrogen therapy, expression of the anti-apoptotic bcl-XL and the cerebral plasticity marker synapsin-I and nestin expression increased. We can conclude: some of hippocampus-dependent memory tests are reliable in gerbils; a high, single dose of estradiol is effective at different ages, it has anti-apoptotic effect and increases cerebral plasticity.

- Nagy Z, Bori Z, Gál A, Wappler E (2007) *New targets for drug therapy in brain ischemia. Orvoscépzés 82: 1–64 (in Hungarian)*
- Wappler EA, Szilágyi G, Gál A, Skopál J, Nyakas C, Nagy Z, Felszeghy K (2009) *Adopted cognitive tests for gerbils: validation by studying ageing and ischemia. Physiol Behav 97: 107–114.*
- Wappler EA, Felszeghy K, Szilágyi G, Gál A, Skopál J, Mehra RD, Nyakas C, Nagy Z (2010) *Neuroprotective effects of estrogen treatment on ischemia-induced behavioural deficits in ovariectomized gerbils at different ages. Behav Brain Res 209: 42–48.*

---

**PROGRAM 6/5.**

---

**CLINICAL NEUROLOGICAL INVESTIGATIONS*****Coordinator:*****Imre SZIRMAI M.D., Ph.D., D.Sc.**

Department of Neurology

6 Balassa st, Budapest H-1083

Tel.: +36 1 210 0330

E-mail: [szirmaiimre@gmail.com](mailto:szirmaiimre@gmail.com)**Program overview**

This doctoral (Ph.D.) program includes clinical research projects in neurology. Full time participation in clinical research can be applied by medical students from the Hungarian, English and German faculties who accomplished their fifth year training, furthermore young doctors with certain research experience in neuroscience. Part-time participation is possible for young neurologists before their neurology special exam. Participants who are not affiliated, working on any subspecialties of neuroscience, could join to the subprograms if their topic may be suited to the clinical neurology. The clinical research program includes partially laboratory work, the research activity of participants may concern to investigate clinical patients and statistical evaluation of clinical and laboratory information. The advertised programs contain mostly unsolved questions of the clinical neurology.

Research of cerebro-vascular diseases includes clinico-pathological evaluation and classification of leukoaraiosis comparing clinical symptoms and brain imaging findings. Analysis of clinical picture and outcome of neurological deficits of elderly patients who suffered ischemic insults caused by lacunar infarctions is available. By the help of the clinical register clinical and epidemiological investigations of cerebrovascular disorders is advertised. The pathomechanism of the primary headaches is unknown. To study the biochemical and physiological causes of headaches in a clinical working group is in progress. Physiological studies involve the noninvasive measurement of blood flow velocity in the intracerebral arteries. With the help of statistical software EEG activity and Doppler flow data can be analyzed simultaneously. Neuropsychologic tests, and electrophysiological measurements can be used in patients with degenerative dementias and aphasia. Research on the movement disorders especially Parkinson disease is a prominent scientific field of the department. Projects for the research of movement and coordination will start in 2012. Many aspects of phenomenology, medical and surgical treatment are under investigation; furthermore neurosurgical research field was opened in 2011. Additionally other neurosurgical programs are also available. Psychometric methods, tremorometry and electrophysiology help the differential diagnosis of movement disorders. New program for the investigation of the basic mechanism of epilepsy was just opened. Topographic aspect and mechanism of human tremor is unknown. Subprograms provide to join to the tremor-research group. Subprogram for neuropathology is ready for the investigation of dementias and cerebrovascular diseases. Learning the instrumental diagnostic technics by the aim to improve the topography of lesions of roots and nerves and scientific analysis of the underlying diseases of peripheral nerves is possible. Research topic for the investigation of optokinetic nystagmus was planned for the approach of brainstem's pathology.

***Titles of research projects***

Disturbance of cognition, behaviour and speech in cerebrovascular disorders  
Clinicopathology of leukoaraiosis  
Investigation of optokinetic nystagmus in cortical and subcortical lesions  
Examination of the characteristics of lacunar cerebral infarcts  
Clinical and epidemiological investigations of cerebrovascular disorders  
Examination of the pathomechanism and clinical characteristics of primary headaches  
Examination of event related desynchronisation, tremor and co-ordination in Parkinson's disease  
Investigation of normal and pathologic movement regulation with the help of EEG and transcranial magnetic stimulation  
Clinico-morphological correlations in degenerative diseases of the central nervous system  
Neuropathological investigations in vascular diseases of the central nervous system  
Polygraphic investigation of the blood flow regulation in healthy subjects and patients with cognitive deficit  
Examination of neuropathies with high-resolution ultrasonography and comparison with electrophysiological findings  
3D motion analysis in movement disorders  
Analysis of efficacy and mode of action of deep brain stimulation in movement disorders  
Clinical characteristics, diagnosis, pathomechanism and therapy of headache disorders  
Modern neurosurgical treatment opportunities in central nervous system pathologies  
Electrophysiological analysis of optokinetic nystagmus  
Spontaneous and electrically evoked high frequency oscillations (ripples) in epilepsy

***Supervisors***

Imre Szirmai  
Imre Szirmai  
Imre Szirmai  
Dániel Bereczki  
Dániel Bereczki  
Dániel Berecki  
Anita Kamondi  
Anita Kamondi  
Tibor Kovács  
Tibor Kovács  
Róbert Debreczeni  
Zsuzsanna Arányi  
Gertrúd Tamás  
Loránd Erőss  
Csaba Ertsey  
Péter Banczerowski  
Szilvia Gulyás  
Dániel Fabó

***Ph. D. students***

Bence Barna Gunda ft  
Nóra Manhalter pt

***Supervisors***

Dániel Bereczki  
Dániel Bereczki

***Ph. D. candidates***

Attila Álmos Balogh na  
Róbert Debreczeni na

***Supervisors***

Péter Banczerowski  
Imre Szirmai

***Ph. D. graduates***

Zsuzsanna Farkas ft  
Szilvia Gulyás ft  
Magdolna Simó na  
Tamás Patkó pt

***Supervisors***

Anita Kamondi  
Imre Szirmai  
Zsuzsanna Arányi  
Zsuzsanna Arányi

f, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**ZSUZSANNA FARKAS (2009)**

### **Electrophysiological investigation of tremor in movement disorders**

*Supervisor: Anita Kamondi*

Tremor is a rhythmic, involuntary, oscillatory movement of any body parts. The most frequent tremor syndromes are essential (ET) and Parkinsonian (PT) tremor. The differentiation of these disorders in the initial stage may be difficult. Pathogenesis of ET and PT is unknown.

We introduced a new test system (CATSYS 2000) in Hungary to objectively assess tremor parameters, rhythmicity and maximum frequency of fast alternating hand and finger movements, reaction time and postural instability. Our data measured in healthy subjects were similar to those reported in the literature. Results of ET and PT groups suggest that decreased frequency and narrow frequency dispersion prove pathological tremor even in case of normal tremor intensity. Our method might be used to differentiate pathological tremors from physiological tremor in the early stage of the disorder.

We examined the relationship between tremor intensity and frequency in ET and PT and the side-to-side asymmetry of these parameters. We concluded that symmetric decrease of frequency parameters regardless of asymmetry of intensity is characteristic of ET, while asymmetric intensity and frequency data are the main features of PT. These results emphasize the significance of bilateral tremor measurement because side difference of parameters might help to distinguish the two most common tremor types. We proved statistically that symptoms related to side difference might be detected only by grouping bilateral data according to more/less affected side, while right/left or dominant/non-dominant division might obscure these differences.

We demonstrated that ET patients are not able to synchronize repetitive movements to extrinsic timing cues and the transition between the slow and fast working mode of rhythm production is also impaired. Our results suggest that it might be caused by the impairment of the central timing mechanisms rather than by the interference of tremulous and rhythmic movements.

We also demonstrated that valproic acid induces low frequency tremor and leads to irregularity of rhythmic movements in epileptic patients with no complaints of tremor or dysrhythmia. This effect is probably due to the involvement of both the cerebellar and basal ganglia GABAergic systems.

- Farkas Zs, Csillik A, Palvolgyi L, Takacs A, Szirmai I, Kamondi A (2006) *Komplex tremorometria az esszenciális tremor és a Parkinson-szindróma elkülönítésében. Clin Neurosci* 59: 45–54.
- Farkas Zs, Csillik A, Szirmai I, Kamondi A (2006) *Asymmetry of tremor intensity and frequency in Parkinson's disease and essential tremor. Park Rel Dis* 12: 49–55.
- Farkas Zs, Szirmai I, Kamondi A (2006) *Impaired rhythm generation in essential tremor. Mov Disord* 21: 1196–1199.

**SZILVIA GULYÁS (2010)****Eye movement related EEG changes**

*Supervisor: Imre Szirmai*

The voluntary limb movements induce complex changes in the EEG. During the movement the activity of the alpha and beta frequency range decreases in both hemispheres while after the completing the movement the beta activity increases on the contralateral side (post-movement beta synchronisation; PMBS). Our knowledge regarding the EEG changes related to ocular movements is incomplete. The aim of our study was to investigate the EEG alterations induced by different eye movements to determine those cortical areas, which play a role in the control of these types of ocular movements.

The EEG was registered during a complex saccade task consisting of two saccadic movements, as well as during subcortical, inattentive optokinetic nystagmus (OKN). The EEG was analysed using mathematical methods.

After the complex prosaccadic test with a latency of about 1100 ms significant beta power increase was detected, which was similar to the PMBS induced by voluntary limb movements (1). However, these EEG changes appeared more rostral corresponding with the localization of the frontal eye field (FEF, Br 8). This suggests that the primary cortical center of saccadic eye movements is the FEF. The ocular PMBS was similar on both sides irrespective of the direction of the saccadic movements (1). The reason for this is that because of the conjugated eye movements the FEF is activated simultaneously in both hemispheres. The ocular PMBS could not be observed after the first saccade but only after the second one (1). This phenomenon, which has also been described in connection with limb movement, suggests that the development of ocular PMBS is related to the consolidation of planned eye movement performance rather than to a single motor action. During OKN we found bilateral significant alpha power reduction and increased beta power (2). This is in accordance with the classical observations, that is the visual information processing desynchronises the EEG alpha activity. However, at the beginning of the inattentive OKN periods we found significant alpha power increase over the parieto-occipital cortex (2), similar to the paradox synchronisation, which occurs during increased attention. Our results suggest that in the generation of inattentive OKN, which has been considered subcortical in origin, cortical structures are also playing role.

- Gulyás S, Szirmai I, Kamondi A (2009) Post-movement beta synchronisation after complex prosaccade task. *Clin Neurophysiol* 120: 11–17.
- Gulyás S, Pálvölgyi L, Kamondi A, Szirmai I (2007) EEG correlates of subcortical optokinetic nystagmus. *Clin Neurophysiol* 118: 551–557.

## TAMÁS PATKÓ (2009)

### Molecular and clinical study of the vestibular function

Supervisor: Zsuzsanna Arányi

In our studies we approached the vestibular system and vestibular compensation from two sides: molecular and clinical. In our *molecular studies* we found, that unilateral removal of one labyrinth does not affect the abundance of mRNAs coding for the subunits SK1, SK2 and SK3 of the calcium-dependent potassium channels, and for the subunits Na  $\alpha$ I and Na  $\alpha$ III and Na  $\beta$ 1–3 of the sodium channels in the deafferented vestibular neurons during the first month following the lesion. Our data therefore do not support the idea of a widespread dynamic modulation of the expression of ion channels by vestibular activity in the vestibular system of adult rats. In our *clinical studies* we investigated 170 patients suffering from unilateral vestibular schwannoma. We confirmed here previous data in smaller series showing that the VEMP showed abnormal findings in about 80% of acoustic neuroma patients. In addition, we showed for the first time in a large number of patients that the response to the VEMP test induced by high level clicks and to 500Hz STB may be different. In particular, a normal or reduced response could be evoked by STB even in the absence of clicks-VEMP on the affected side whereas the reverse was not true. We also studied patients suffering from multiple sclerosis, where we found 40% of the patients with abnormal VEMP. We found, that VEMP abnormalities show the strongest correlation with demyelinating MRI lesions in the brainstem and a weaker correlation with the duration of the disease. The clinical signs of vestibular dysfunction do not seem to affect the chances of obtaining abnormal results. Although the sensitivity of VEMP in detecting abnormality in MS patients is relatively low, as compared to other evoked potential modalities, its significance lies in that it is an easily and quickly performed electrophysiological method assessing the function of central vestibular pathways.

- Patko T, Vidal PP, Vibert N, Tran Ba Huy P, De Waele C (2003) Vestibular evoked myogenic potentials in patients suffering from an unilateral acoustic neuroma: a study of 170 patients. *Clin Neurophysiol* 114: 1344–1350.
- Patko T, Vassias I, Vidal PP, De Waele C (2003) Modulation of the voltage-gated sodium- and calcium-dependent potassium channels in rat vestibular and facial nuclei after unilateral labyrinthectomy and facial nerve transection: an in situ hybridization study. *Neuroscience* 117: 265–280.
- Patko T, Simo M, Aranyi Z (2007) Vestibular click-evoked myogenic potentials: sensitivity and factors determining abnormality in patients with multiple sclerosis. *Mult Scler* 13: 193–198.



**MAGDOLNA SIMÓ (2010)****Current role of evoked potentials in the neurological diagnostic process**

*Supervisor: Zsuzsanna Arányi*

Evoked potentials are sensitive neurophysiological techniques, which have a crucial role in the detection of clinically silent lesions of the nervous system. In our study, we set out to assess the new and changing role of evoked potentials in cervical spondylotic myelopathy or in suspected cervical spondylotic myelopathy, and in multiple sclerosis (MS). Cervical spondylotic myelopathy is the most common form of myelopathy in middle-aged and elderly individuals; since the widespread use of MR it has become a frequent radiological diagnosis.

We performed somatosensory (SEP) and motor (MEP) evoked potentials in patients, where MR of the cervical spine showed cervical spondylotic spinal compression. We found that in patients with nonspecific, non-confirmative and mild symptoms, possibly suggestive of myelopathy, MEP verified subclinical myelopathy with almost 100% sensitivity; SEP proved to be less sensitive. In cases of subclinical myelopathy (i. e. positive evoked potentials), surgical intervention is needed, whereas patients with normal evoked potentials should be followed. Currently, the diagnosis of multiple sclerosis is usually possible based on clinical symptoms and MR results. We carried out motor and sensory evoked potentials in patients with optic neuritis, which is a common presenting symptom of MS. Based on the McDonald criteria, the diagnosis of MS was established in all patients within a relatively short period of time (on the average two years); however, the diagnosis of clinically definitive MS (i. e. at least two clinical relapses from different lesions) was confirmed in only 16% of patients with normal evoked potentials, but in 67% of patients with abnormal SEPs and MEPs. The results of evoked potential examinations correlated well with MR findings and the clinical diagnosis of MS. Based on our results, it can be stated that today SEP and MEP examinations have mainly a predictive role by anticipating the development of clinically definitive MS following the first clinical episode, as opposed to their former diagnostic role in the detection of clinically silent lesions. With respect to their predictive role, evoked potentials have been shown to be more sensitive than MR. The results of vestibular evoked myogenic potentials (VEMP) in patients with multiple sclerosis have shown that VEMP is a simple and quick method to detect silent lesions in central vestibular pathways, commonly affected in MS. In some cases, it proved to be more sensitive than MR.

- Simó M, Szirmai I, Arányi Zs (2004) *Superior sensitivity of motor over somatosensory evoked potentials in the diagnosis of cervical spondylotic myelopathy. Eur J Neurol* 11: 621–626.
- Simó M, Barsi P, Arányi Zs (2008) *Predictive role of evoked potential examinations in patients with clinically isolated optic neuritis in light of the revised McDonald criteria. Mult Scler* 14: 472–478.
- Patkó T, Simó M, Arányi Zs (2007) *Vestibular click-evoked potentials: sensitivity and factors determining abnormality in patients with multiple sclerosis. Mult Scler* 13: 193–198.

**PROGRAM 6/6.****BIOLOGICAL PSYCHIATRY*****Coordinator:*****Gábor FALUDI M.D., Ph.D., D.Sc.**

Kútvölgyi Clinical Centre

4 Kútvölgyi st, Budapest H-1125

Tel.: +36 1 355 1122

E-mail: [faludi@kut.sote.hu](mailto:faludi@kut.sote.hu)**Program overview**

The aim of the “Biological Psychiatry” program is to study the theoretical and practical aspects of brain and mental sciences, utilising and integrating knowledge from different disciplines in understanding pathopsychological functions and therapeutic response, and to contribute with its results to everyday practice in mentalhygiene and psychiatry. One challenge for biological psychiatry is to integrate our psychopathological knowledge about brain functional changes with our present knowledge about the relationship between brain and behavior and brain structure. Research in the Biological Psychiatry program targets psychiatric disorders from neurobiological, neurochemical, genetic and neurocognitive aspects, building on knowledge from cooperation with other disciplines and experience from clinical observation and effective treatment of patients. Presently the program includes 7 Ph.D. themes offering the study of neurobiological, clinical and therapeutic aspects of adult and geriatric psychiatric disorders. The tutors are well-known and internationally acknowledged theoretical and clinical experts.

***Titles of research projects***

Biochemical background of mood and anxiety disorders

Interrelationship between biogenic amines and nociceptin/  
nocistatin systemGenetic correlats of behavioural phenotypes in major depression  
and their relationship to response to antidepressant medicationGenetic correlates of major depressive behaviour and endo-  
phenotypeGenetic and clinical aspects of bipolar disorders with special  
respects of suicide behaviourRoles for the dopaminergic and serotonergic systems in mood  
and anxiety disorders***Supervisors***

Kornélia Tekes

Kornélia Tekes

Gábor Faludi

Xénia Gonda

Zoltán Rihmer

Anna Székely

***Ph.D. students***

Gabriella Balogh

ft

Zsuzsa Halmai

ft

***Supervisors***

Gábor Faludi

Andrea Székely

***Ph.D. candidates***

Péter Döme

na

Zoltán Makkos

na

***Supervisors***

Zoltán Rihmer

Gábor Faludi

**Ph. D. graduates**

Károly Mirnics  
Annamária Rihmer  
Andrea Sárosi

na  
pt  
na

**Supervisors**

Gábor Faludi  
Gábor Faludi  
Gábor Faludi

f, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2010***

**KÁROLY MIRNICS (2010)**

**Gene expression changes in schizophrenia**

*Supervisor: Gábor Faludi*

Schizophrenia is a complex and devastating brain disorder that affects 1% of the population and ranks as one of the most costly disorders to afflict humans. The etiology of schizophrenia remains elusive but appears to be multifaceted, with genetic, nutritional, environmental and developmental factors all implicated. The development of novel tools of functional genomics enables us to approach questions addressing the etiology of complex psychiatric disorders from a novel, hypothesis-free, data-driven angle. As the prefrontal cortex dysfunction is a hallmark of the disease, using various DNA microarray platforms we performed a transcriptome profiling of the human postmortem prefrontal cortex of subjects with schizophrenia and matched controls. Data were validated by real-time quantitative PCR and *in situ* hybridization. Our results show that (1) transcripts encoding proteins involved in the regulation of presynaptic function were decreased in all subjects with schizophrenia, in a subject-specific pattern (Mirnics K *et al.* 2000. *Neuron* 28: 53–67); (2) there is a highly specific pattern of metabolic alterations in the PFC of subjects with schizophrenia (Middleton FA *et al.* 2002. *J Neurosci* 22: 2718–2729); (3) the majority of 14-3-3 gene family exhibited statistically significant decreases in expression in schizophrenia (Middleton FA *et al.* 2005. *Neuropsychopharmacol* 30: 974–983); (4) there was a systemic repression of GABA transcripts in diseased subjects (Hashimoto T *et al.* 2008. *Mol Psychiatry* 13: 147–161) and (5) expression of genes related to immune and chaperone function was increased in individuals with the disease (Arion D *et al.* 2007. *Biol Psychiatry* 62: 711–721). Furthermore, we established RGS4 as a gene critically involved in the pathophysiology of schizophrenia (Mirnics K *et al.* 2001. *Mol Psychiatry* 6: 293–301; Chowdari KV *et al.* 2002. *Hum Mol Genet* 11, 1373–1380). These data suggest that schizophrenia is a coordinated dysfunction of multiple transcriptional networks, and allows identification of convergence points within these disrupted networks (“disease-related molecular hubs”). We believe that this novel information will ultimately lead to identification of new, knowledge-based therapeutic targets for schizophrenia.

- Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P (2000) Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* 28: 53–67.
- Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P (2002) Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *J Neurosci* 22: 2718–2729.

- Middleton FA, Peng L, Lewis DA, Levitt P, Mirnics K (2005) Altered expression of 14-3-3 genes in the prefrontal cortex of subjects with schizophrenia. *Neuropsychopharmacol* 30: 974–983.

## ANNAMÁRIA RIHMER (2010)

### Psychotic and psychosocial characteristics of suicide attempters with a special focus on affective temperaments, childhood abuses and gender differences

*Supervisor: Gábor Faludi*

In our present study we have investigated the psychiatric and psychosocial characteristics of 150 nonviolent suicide attempters (106 females and 44 males) admitted at the Department of Toxicology at the Erzsébet Hospital, Capital Local Government in 2003. A particular attention have been paid to affective temperament-types, childhood physical and sexual abuse and gender differences. Our protocol involved collecting demographic data, the Hungarian version of the MINI Neuropsychiatric Interview the TEMPS-A temperament scale with 110 items the shortened version of the Bernstein Childhood Psychical and Sexual Abuse Scale and the Gotland Male Depression Scale. Our findings confirm previous Hungarian and international data concerning the connection of suicidal behaviour and psychiatric disorders, undesirable psychosocial circumstances and negative life events (1, 2). Moreover, our study provides new results regarding the strong association of the affective temperament types, the male type of depression, the seriously traumatic early life events and furthermore to their predisposing role in connection with suicidal behaviour. While the attempters scored significantly higher on the depressive, cyclothymic, irritable and anxious subscales ( $p < 0.01$ ) and and depressive, cyclothymic and irritable temperaments were significantly more frequent among nonviolent suicide attempters ( $p < 0.01$ ), than in controls, hyperthymic temperament seems to be have a protective role (3). Suicide attempters, experiencing physical and/or sexual abuse in their childhood showed significantly higher total scores ( $p < 0.05$ ) on cyclothymic and irritable temperament subscales. The Gotland Male Depressive syndrome was equally very common and equally serious both in males and females who made nonviolent suicide attempt (4). However, regardless of gender, it was significantly more severe ( $p < 0.01$ ) among those who were victims of both physical and sexual childhood abuse.

- Rihmer A, Rihmer Z, Jekkel É, Kárteszi M, Csiszér N, Farkas Á (2006) Psychiatric characteristics of 100 nonviolent suicide attempters in Hungary. *Int J Psychiat Clin Pract* 10: 69–72.
- Rihmer A, Gonda X, Balazs J, Faludi G (2006) The importance of depressive mixed states in suicidal behaviour. *Neuropsychopharmacol Hung* 8: 15–18.
- Rihmer A, Rózsa S, Rihmer Z, Gonda X, Akiskal KK, Akiskal HS (2009) Affective temperaments, as measured by TEMPS-A, among nonviolent suicide attempters. *J Affect Disord* 116: 18–22.
- Rihmer A, Rutz W, Rihmer Z, Gonda X, Webb R, Kapur N (2009) Is male-type depression specific for females, too? P 223 in: 9th World Congress of Biological Psychiatry, Paris, Abstract book.

**ANDREA SÁROSI (2010)****Cognitive and psychogenetic vulnerability markers of depression***Supervisor: Gábor Faludi*

The clinical symptoms of major depression are paralleled by typical neurocognitive deficits. The relation of STin2—one of the polymorphisms of the serotonin transporter gene—to major depressive disorder (MDD) is less widely investigated. The P2RX7 gene has been suggested as a novel candidate gene for major depressive disorder and bipolar depression. The aim of the present study was to measure the neurocognitive functions of major depressive patients and healthy controls, and identify vulnerability markers of the disease. The frequency of STin2 polymorphism and its effect on the neurocognition was investigated in major depression. We investigated the effect of P2RX7 Gln460Arg SNP on depression and anxiety using dimensional scales, since quantitative assessment could facilitate the detection of small genetic effects was investigated. The gender differences in neurocognitive impairment in patients with major depressive disorder were also studied. Relative to controls, patients with depression were significantly impaired on most of the neurocognitive tasks, but not in visuo-spatial function, which may suggest intact hippocampal function in depression. We found a significantly higher frequency of the STin2 10/10 genotype in the MDD patients' group compared to controls. Our results suggest that the presence of STin2.10 and absence of STin2.12 may be considered a possible genetic endophenotype for cognitive dysfunction detected in major depressive disorder. A significant association was found between the P2RX7 polymorphism and the severity of depression and anxiety. A significant interaction of clinical status and the P2RX7 polymorphism was also found. Our results support the association between depressive disorder and the G-allele of the Gln460Arg polymorphism of the P2RX7 gene.

Depressed women performed significantly worse on tests of cognitive interference and on the test of visual recall threshold compared to depressed men. In the light of neuro-imaging studies our results suggest that the lateralisation of hippocampal function may play an important role in the background of gender differences.

- Dömötör E\*, Sárosi A\*, Balogh G, Székely A, Héjjas K, Sasvári-Székely M, Faludi G (2007) *Neurokognitív endofenotípus és STin2 polimorfizmus asszociáció vizsgálata major depresszióban. Neuropsychopharmacol Hung 9: 53–62 (\*Authors equally contributed).*
- Hejjas K, Székely A, Domotor E, Halmai Z, Balogh G, Schilling B, Sarosi A, Faludi G, Sasvari-Szekely M, Nemoda Z (2008) *Association between depression and the Gln460Arg polymorphism of P2RX7 gene: a dimensional approach. Am J Med Genet B Neuropsychiatr Genet 150B: 295–299.*
- Sarosi A, Gonda X, Balogh G, Domotor E, Székely A, Hejjas K, Sasvari-Szekely M, Faludi G (2008) *Association of the STin2 polymorphism of the serotonin transporter gene with a neurocognitive endophenotype in major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 32: 1667–1672.*

---

## SCHOOL OF PH.D. STUDIES

---

### 7. MOLECULAR MEDICINE

---

**Chairman:****József MANDL M.D.,****member of the Hungarian Academy of Sciences**

Department of Medical Chemistry,

Molecular Biology and Pathobiochemistry

37–47 Tűzoltó st, Budapest H–1094

Tel./fax: +36 1 266 2755

E-mail: [jozsef.mandl@eok.sote.hu](mailto:jozsef.mandl@eok.sote.hu)**General overview**

The postgraduate school of molecular medical sciences serves for both biomedical basic research and primary training of researchers starting their careers in the fields of clinical research. One of the major drawbacks of biomedical research is that there is no efficient connection between the basic and clinical research. Therefore, five main Programs involve applied theoretical knowledge together with clinical research.

**PROGRAM 7/1.**

---

**CELLULAR AND MOLECULAR PHYSIOLOGY****Coordinator:****Erzsébet Ligeti M.D.,****member of the Hungarian Academy of Sciences**

Department of Physiology

37–47 Tűzoltó st, Budapest H–1094

Tel.: +36 1 266 9180/ext. 60457, +36 1 266 6504

Fax: +36 1 266 6504

E-mail: [erzsebet.ligeti@eok.sote.hu](mailto:erzsebet.ligeti@eok.sote.hu)**Program overview**

The Program provides opportunity to receive training in the field of physiology. The professors of the Program offer courses and individual training for the Ph.D. students on their respective scientific research areas. Training courses include continuous basic methodical and scientific training for small groups of students. Individual training focuses on research under the supervision of training advisors aimed at understanding physiological regulatory mechanisms at the cellular level using electrophysiological, molecular biological, biochemical, cell biological and physiological methods.



***Titles of research projects***

Structure-function of the CFTR chloride channel  
 Structure-function of the TRPM2 cation channel  
 Investigation of two-pore domain potassium channels  
 Molecular chaperones and biological networks  
 Receptor mediated regulation of type 2P potassium channels  
 Investigation of reactive oxygen producing enzymes in mammalian cells  
 Charge compensation mechanisms of reactive oxygen species forming enzymes  
 From stem cells to liver: ABC transporters during hepatic differentiation  
 Regulation of G protein-coupled receptors  
 Molecular basis of angiotensin receptor function  
 Molecular basis of regulation of the circadian rhythm  
 Role and regulation of Rho family GTPase activating proteins (GAPs)  
 Role of NADPH oxidase in antibacterial defense  
 The role of positional information (position along the body-axes) in the early differentiation of neural cells  
 Investigation of proteins involved in the differentiation and function of osteoclasts  
 Analysis of signal transduction of the immune system in knockout mouse  
 Role of oxygen radicals in the physiological effect of angiotensin II and other Ca<sup>2+</sup> mobilizing hormones  
 The molecular and physiological role of inositol lipids

***Supervisors***

László Csanádi  
 László Csanádi  
 Gábor Czirják  
 Péter Csermely  
 Péter Enyedi  
 Miklós Geiszt  
 Miklós Geiszt  
 László Homolya  
 László Hunyady  
 László Hunyady  
 Krisztina Káldi  
 Erzsébet Ligeti  
 Erzsébet Ligeti  
 Emília Madarász  
 Attila Mócsai  
 Attila Mócsai  
 András Spät  
 Péter Várnai

***Ph.D. students***

Roland Csépanyi-Kömi	pt (a)
Krisztina Ella	ft
László Sándor Erdélyi	ft
László Fülöp	ft
Pál Gyombolai	ft
Dávid Sándor Győri	ft
Kata Kenesei	ft
István Kovács	ft
Miklós Kovács	ft
Robin Dezső Palotai	ft
Márton Ákos Lőrincz	ft
Bence Szalai	ft
Csaba István Timár	ft
Balázs Tóth	ft
Dániel Tóth	ft

***Supervisors***

Erzsébet Ligeti  
 Krisztina Káldi  
 László Hunyady  
 Erzsébet Ligeti  
 László Hunyady  
 Attila Mócsai  
 Emília Madarász  
 Miklós Geiszt  
 Attila Mócsai  
 Péter Csermely  
 Erzsébet Ligeti  
 László Hunyady  
 Erzsébet Ligeti  
 László Csanádi  
 Péter Várnai

***Ph. D. candidates***

Balázs Enyedi	ft
Norbert Gyöngyösi	ft
Zsuzsanna Kertész	ft
Péter Koncz	pt
Tamás Németh	ft
Zalán Péterfi	ft
Gergő Szanda	ft

***Supervisors***

Miklós Geiszt
Krisztina Káldi
Attila Mócsai
András Spät
Attila Mócsai
Miklós Geiszt
András Spät

***Ph. D. graduates***

Ágnes Donkó	ft
Magdolna Krisztina Lévy	ft
Gábor Turu	pt

***Supervisors***

Miklós Geiszt
Erzsébet Ligeti
László Hunyady

a, absolutorium; ft, full-time; pt, part-time

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****ÁGNES DONKÓ (2010)****Examination of the function of peroxidases and NADPH-oxidases**

*Supervisor: Miklós Geiszt*

Lactoperoxidase (LPO) is expressed by different mucosal surfaces, where it contributes to the host defense mechanisms by producing hypothiocyanate. The reaction requires H<sub>2</sub>O<sub>2</sub>, which is produced by Duox enzymes. During fertilization in sea urchin eggs ovoperoxidase contributes to the hardening of fertilization envelope through dityrosine bridges, which is a common feature of mammalian peroxidases. Our aim was to study the dityrosine forming activity of LPO. We concluded that the dityrosine-forming activity of LPO is suitable for the quantification of H<sub>2</sub>O<sub>2</sub>. With the LPO-catalized dityrosine formation we successfully measured the H<sub>2</sub>O<sub>2</sub>-production of glucose-oxidase and of Nox 4 expressing FreeStyle 293F cells. Using human plasma as a model we showed that LPO crosslinks proteins through dityrosine bridges and that LPO crosslinks itself to the protein complexes as well, which could enhance its antibacterial activity. Peroxidasin (PXDN) is the latest recognized member of the mammalian peroxidase family and so far little is known about its function. Since the peroxidase activity of PXDN has not been characterized yet, we expressed PXDN in COS-7 cells and detected peroxidase activity. In COS-7 cells PXDN localized into the endoplasmatic reticule. Conflicting data were published regarding the expression pattern of PXDN, hence we studied in Northern blot experiments. We detected PXDN in different tissues, including heart, skeletal muscle, colon, spleen, kidney, liver, small intestine, placenta and lung. We showed for the first time that urothelial cells produce H<sub>2</sub>O<sub>2</sub> in a calcium dependent manner. Examining knockout urothelial cells we proved that H<sub>2</sub>O<sub>2</sub> is produced by Duox1. For the activation of H<sub>2</sub>O<sub>2</sub>-production of Duox1 we used different calcium-mobilizing stimuli such as

thapsigargin, ATP and GSK 1016790A a TRPV4 specific agonist. Future studies are needed to clarify the possible function of H<sub>2</sub>O<sub>2</sub>-production by urothelial cells in host defence or in signal transduction.

- Donkó Á, Péterfi Z, Sum A, Leto T, Geiszt M (2005) Dual oxidases. *Philos Trans R Soc Lond B Biol Sci* 360: 2301–2308.
- Donkó Á, Orient A, Szabó PT, Németh G, Vántus T, Kéri G, Órfi L, Hunyady L, Buday L, Geiszt M (2009) Detection of hydrogen peroxide by lactoperoxidase-mediated dityrosine formation. *Free Radic Res* 43: 440–445.
- Péterfi Z, Donkó Á, Orient A, Sum A, Prókai Á, Molnár B, Veréb Z, Rajnavölgyi É, Kovács KJ, Müller V, Szabó AJ, Geiszt M (2009) Peroxidasin is secreted and incorporated into the extracellular matrix of myofibroblasts and fibrotic kidney. *Am J Pathol* 175: 725–735.

## MAGDOLNA KRISZTINA LÉVAY (2010)

### Regulation of p190RhoGAP by phospholipids and phosphorylation

Supervisor: Erzsébet Ligeti

The Rho family GTPases are critical regulators of the actin cytoskeleton and are required for cell adhesion, migration, and polarity. Rho GTPases are regulated through the action of a large family of GTPase activating proteins (GAPs) that stimulate their relatively weak intrinsic GTP-hydrolyzing activity. Among the regulatory proteins of the Rho GTPases is the p190RhoGAP, a 190 kDa GTPase activating protein. Previous studies have shown that different phospholipids, such as PS and PI are able to “switch” the GTPase substrate preference for the p190RhoGAP: inhibit its RhoGAP activity and promote the RacGAP activity. At the beginning of my work it was known, that p190RhoGAP is regulated by tyrosin phosphorylation. During my Ph.D. work I investigated the regulatory effect of phospholipids, and two serine/threonine kinases: PKC $\alpha$  and GSK-3 $\beta$ . We demonstrated, that the binding of p190RhoGAP to phospholipids is controlled by electrostatic interactions. We determined that a small polybasic peptide stretch N terminal to the GAP domain of p190RhoGAP is a common site for both the phospholipid binding and PKC $\alpha$  phosphorylation. p190RhoGAP can be phosphorylated by PKC $\alpha$  at serine 1221, threonine 1226, and serine 1236 amino acids within the polybasic region. PKC $\alpha$ -mediated phosphorylation of S1221 and T1226 of p190RhoGAP prevents the binding and substrate specificity regulation by phospholipids. Transfection of COS-7 cells with mutant forms of p190RhoGAP either unable to bind to phospholipids or unable to become phosphorylated induced distinct morphological changes. Together, these findings reveal a novel GAP regulatory mechanism in which PKC $\alpha$  phosphorylation indirectly alters GTPase substrate preference by affecting the interaction with acidic phospholipids. Our observations provide a potential mechanism of Rac/Rho antagonism described in several cellular functions. In the second part of my experiments the phosphorylation of p190RhoGAP through GSK-3 $\beta$  was investigated. Both p190RhoGAP and GSK-3 $\beta$  have been implicated previously in cell migration. Three closely spaced amino acids within the p190RhoGAP terminal region undergo phosphorylation by GSK-3 $\beta$ . These phosphorylations are dependent on priming by a second kinase. The GSK-3 $\beta$  mediated phosphorylation reduces both RhoGAP and RacGAP activity of p190RhoGAP. We identified that p190RhoGAP can be phosphorylated by PKC $\alpha$  and GSK-3 $\beta$  in different parts of the molecule, and we de-

monstrated that phosphorylation by the two different kinases regulates the RhoGAP and RacGAP activity of p190RhoGAP differently.

- Lévay M, Settleman J, Ligeti E (2009) Regulation of the substrate preference of p190RhoGAP by protein kinase C mediated phosphorylation of a phospholipid binding site. *Biochemistry* 48: 8615–8623.
- Jiang W, Betson M, Mulloy R, Foster R, Lévay M, Ligeti E, Settleman J (2008) p190A Rho-GAP is a glycogen synthase kinase-3-beta substrate required for polarized cell migration. *J Biol Chem* 283: 20978–20988.

## GÁBOR TURU (2009)

### Regulation of CB1R by AT1-angiotensin and other Gq-coupled receptors

Supervisor: László Hunyady

Endocannabinoid system is involved in many physiological and pathophysiological regulations and conditions. Endocannabinoids act on CB1-, CB2- and other, presently less characterized receptors. Our knowledge on the regulation of the two main endocannabinoids, anandamide and 2-arachidonoyl glycerol is based on experiments made on neuronal tissues. The regulation of endocannabinoid production in other tissues is poorly understood. Our aim was to investigate, whether stimulation of AT1 angiotensin-, and other Gq/11-coupled receptors leads to activation of CB1 receptors, expressed in the same or in neighboring cells in non-neural tissues. CB1 receptor activation was measured by energy transfer between the heterotrimeric Go-protein subunits, or by  $\beta$ -arrestin2 translocation to the receptors in CHO cells. We have shown, that stimulation of AT1 angiotensin receptors with angiotensin II led to activation of the CB1 receptor expressed in the same and neighboring cells. Activation was blocked by diacylglycerol lipase inhibitor, suggesting the role of the 2-arachidonoyl glycerol in the CB1 receptor activation. Stimulation of AT1 receptor led to production of 2-arachidonoyl glycerol in CHO cells. We have also shown, that CB1 receptor was activated, when AT1 was expressed in other cell types, and also when other Gq/11-coupled receptors were stimulated. Tetrahydro-lipstatin, a diacylglycerol lipase inhibitor inhibited tonic activity of the CB1 receptor, suggesting that endogenously produced endocannabinoids are involved in basal G-protein activation.

- Turu G, Szidonya L, Gáborik Z, Buday L, Spät A, Clark AJL, Hunyady L (2006) Differential  $\beta$ -arrestin binding of AT1 and AT2 angiotensin receptors. *FEBS Letters* 580: 41–45.
- Turu G, Simon A, Gyombolai P, Szidonya L, Bagdy G, Lenkei Z, Hunyady L (2007) The role of diacylglycerol lipase in constitutive and angiotensin AT1 receptorstimulated cannabinoid CB1 receptor activity. *J Biol Chem* 282: 7753–7757.

**PROGRAM 7/2.****PATHOBIOCHEMISTRY*****Coordinator:*****József MANDL M.D.,****member of the Hungarian Academy of Sciences**

Department of Medical Chemistry,

Molecular Biology and Pathobiochemistry

37–47 Tűzoltó st, Budapest H–1094

Tel./fax: +36 1 266 2755

E-mail: [jozsef.mandl@eok.sote.hu](mailto:jozsef.mandl@eok.sote.hu)**Program overview**

Pathobiochemistry showed a remarkably dynamic progress in the past decades. The Program has two roles: (1) it outlines the etiology and pathogenesis of different pathological conditions, (2) it aims to help the detailed knowledge of certain important fields of pathology. In planning the program the following viewpoints were considered: in diseases different mechanisms of pathological regulation can develop, reflecting changes in extracellular signals or signal transduction.

***Titles of research projects******Supervisors***

Comparative examination of vascular gene polymorphisms in clinical pictures

György Acsády

Study of genetic variation in the development of drug addiction and other psychiatric disorders

Csaba Barta

Transport systems in the endoplasmic reticulum

Gábor Bánhegyi

Neuronal scaffold proteins

László Buday

Small G-proteins in cell function

László Buday

Investigation of signalling pathways of receptor tyrosine kinases

László Buday

Fate of glucuronides in the hepatic endoplasmic reticulum

Miklós Csala

Redox metabolism in the endoplasmic reticulum

Miklós Csala

Protein processing and quality control in the endoplasmic reticulum

Miklós Csala

Changes in molecular chaperons during aging

Péter Csermely

Functional changes of chaperons in pathologic conditions

Péter Csermely,

Csaba Sóti

Characteristics of calcium transporters

Ágnes Enyedi

Genetical and immunological factors in rheumathological conditions

Pál Géher

Separation technique methods with big efficiency the proteomic basis biomarker in research

András Guttman

Application of the specialities of separation techniques in QSAR studies

Miklós Idei

Investigation of transporter-drug interactions in human and rat hepatocytes

Katalin Jemnitz

The genetic background of gynaecological clinical pictures

József Gábor Joó

Changes of extracellular and adhesic molecules in human uterus

Anna Kádár

Estrogen receptor polymorphism, lipoproteins and coagulation factors	István Karádi
Effects of anti-cancer and anti-inflammatory peptides—signal transduction therapy	György Kéri
Rational drug design of kinase inhibitor agents	György Kéri
Cellular signalling therapy with kinase inhibitors	György Kéri
Transporter-drug interactions in human and rat hepatocytes	Katalin Jemnitz
Cell dependent thrombolysis	Kraszimir Kolev
Study of calcium transport systems in various cancer cells	Tünde Kovács
The role of pathobiochemical factors in the development and progression of inflammatory bowel diseases	Péter László Lakatos
Role of leucocytes in fibrinolysis	Raymund Machovich
Effect of thrombus matrix on thrombolysis.	Raymund Machovich
Effect of matrix on thrombolysis	
Molecular mechanisms of endoplasmic reticulum stress	József Mandl
Hypoxia signalling, expression of O <sub>2</sub> sensitive genes	József Mandl
Regulation of glucuronidation	József Mandl
Determination of hydrophobicity of the new selective tyrosine inase inhibitor molecules. Modelling the relationship between structure and biological activity	György Mészáros
Selection and application of protein specific aptamers	Tamás Mészáros
Genetic polymorphisms in monoamine neurotransmitter systems: association analyses and functional study	Zsófia Nemoda
Design, synthesis and structure—biological activity correlation studies of anticancer and antimicrobial agents	László Őrfi
The role of informatics and combinational chemistry in the design and synthesis of novel potential drugs	László Őrfi
The molecular pharmacology of the signal transmission therapies affecting the regulation of cell death following molecular farmacodiagnosics	István Peták
Analysis of small and large-scale copy number variations	Zsolt Rónai
Pathobiochemistry of pancreatic digestive enzymes	Miklós Sahin-Tóth
Membrane transporter proteins of human stem cells and their changes during cell differentiation	Balázs Sarkadi
Association between the structure and function of human ABC transporter proteins	Balázs Sarkadi
Studies on ABC transporters in malignant tumors	Balázs Sarkadi
Genetical risk factors in complex hereditary diseases	Mária Sasvári
Cross-talk between signaling pathways regulating proliferation, differentiation and cell death of B-lymphocytes	Gabriella Sármay
Identification of new citrullinated epitopes recognized by B-cells in rheumatoid arthritis: possibilities for antigen specific targeting of B-cells	Gabriella Sármay
Signal integration coopeartion between signalling pathways regulating the growth differentiation and death of B-cells	Gabriella Sármay
The role of protein denaturation and stress response in aging	Csaba Sóti



Simultaneous application of quantitative molecular genetic measurements and high capacity cell sorting in malignant disorders of the myeloid system

Biosynthesis of nitric oxide, its relation to oxidative stress and their roles in the pathobiochemistry of human placenta

Characterization of ABCG-type transporters

The role of NAK ATPase in the pathomechanism of diabetes mellitus

UV-induced tumorigenesis in skin: molecular biological mechanisms, its regulation and pathobiochemical events

Effects of UV radiation on the skin: carcinogenesis, oxidative processes, and dermo-epidermal alterations

### ***Ph.D. students***

Mehmet Alper Arslan	it (a)
Melinda Bence	ft
Gábor Bógel	pt (a)
Márton Dávid Gyurkó	ft
Laura Konta	pt
Nguyen Minh Tu	ft (a)
Réka Gabriella Nagy-Kovács	pt (a)
Szilvia Krisztina Nagy	ft
Nóra Németh	ft
Diána Papp	ft (a)
Szabolcs József Pesti	ft
Katalin Révész	ft
Zsolt Rottenberger	pt (a)
Mónika Szabó	pt
Péter Szelényi	crc (a)
Kristóf Zsolt Szalay	ft
Kornélia Szebényi	ft
Roopesh Udupa	it
Andrea Vereczkei	ft

### ***Ph.D. candidates***

Abdul Rahman Omar	ft
Zsófia Balogh	ft
Edina M. Komlódi-Pásztor	ft
Zsuzsanna Literáti-Nagy	pt
Éva Magyar	pt (a)
Nóra Meggyesi	ft
Polett Ribiczey (Szentirmai)	pt
László Seres	crc
Gábor Viktor Szabó	pt (a)
Anikó Szilvási	crc
Anna Tanka-Salamon	ft

Attila Tordai,  
Hajnalka Andrikovics

Miklós Tóth

András Váradi,  
Balázs Sarkadi  
Ágota Vér

Norbert Wikonkál

Norbert Wikonkál

### ***Supervisors***

Csaba Sóti  
Mária Sasvári  
László Buday  
Csaba Sóti  
Miklós Csala  
Csaba Sóti  
Zsolt Rónai  
Tamás Mészáros  
Zsolt Rónai  
Csaba Sóti  
László Buday  
Miklós Csala  
Krasimir Kolev  
Katalin Jemnitz  
József Mandl  
Péter Csermely  
Balázs Sarkadi  
László Buday  
Mária Sasvári

### ***Supervisors***

Mária Sasvári  
Tamás Mészáros  
András Váradi  
József Mandl  
Miklós Csala  
Attila Tordai  
Tünde Kovács  
László Homolya  
György Acsády  
Balázs Sarkadi  
Rajmund  
Machovich

Zsóka Weiszhár	pt (a)	László Romics
Nikolett Wohner	pt	Krasimir Kolev
Zsuzsanna Eszter Wolf	pt	László Romics
<b><i>Ph.D. graduates</i></b>		<b><i>Supervisors</i></b>
Annamária Balázs	ft	László Buday
Ildikó Kasza	crc	Balázs Sarkadi
Ákos Kertész	crc	Gabriella Sármay
György Paragh	ft	Norbert Wikonkál
Ferenc Pintér	crc	István Peták
Gyöngyi Rábai	ft	Krasimir Kolev
Klára Rosta	pt	Ágota Vér
Gábor Sobel	pt	Anna Kádár
Flóra Mária Szeri	ft	András Váradi,
		Balázs Sarkadi
Dávid Varga-Szabó	pt	Ágnes Enyedi
Edit Várkondi	crc	István Peták

a, absolutorium; ft, full-time; pt, part-time; it, international; crc, Cooperation Research Centre

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

### **ANNAMÁRIA BALÁZS (2009)**

#### **Investigation of Caskin1 Scaffold Protein**

*Supervisor: László Buday*

Caskin1 was cloned in 2002, as a Cask binding protein and it is expressed in the post-synaptic density (PSD) of the neurons. There are some well-defined protein-protein interaction domains in the N-terminal part of the protein and the C-terminal contains a large proline-rich region which is uncharacterised. The function of Caskin1 is not well defined, but its structure suggests that it is a scaffold protein, it may form several interactions and may have a role in the organization of the large number of proteins in the PSD. To test our hypothesis, we took advantage of the yeast two hybrid screening and obtained several interacting partners. Out of them, we verified the connection between Caskin1 and Abi2 using *in vitro* and *in vivo* methods and antibodies developed in our laboratory. We showed that Abi2 binds to the proline-rich region of Caskin1 with its SH3 domain. Abi2 participates in the signalling pathway of Abl tyrosine kinase and Rac. It may have an inhibitory effect on the transforming activity of Abl kinases and in the dynamic rearrangement of the actin cytoskeleton. Abi2 knock-out mice was shown to have serious problems in the nervous system, showing its important role. In the absence of Abi2, secondary lens fiber orientation and migration were defective in the eye. Loss of Abi2 also resulted in cell migration defects in the neocortex and hippocampus, abnormal dendritic spine morphology and density, and severe defects in both short-term and long-term memory. Neuron specific binding partner of Abi2 has not been identified so far,

that is why our results are so important, showing the interaction between Abi2 and Caskin1. In collaboration with the laboratory of Peter Tompa, we showed that the C-terminal proline-rich region of Caskin1 is unstructured. It means that this part of the protein does not have secondary and tertiary structure. Some secondary ordering can be seen at the contact site of Abi2. Using bioinformatical prediction programs, we examined 74 scaffold protein and showed that their structure considerably disordered. We suppose that the well defined interaction domains are linked by shorter or longer unstructured regions. Finally we found, that protein-kinase A and protein-kinase C can phosphorylate the proline-rich region of Caskin1. This phosphorylation may regulate the function, localization, and/or the half life of the protein.

- Balazs A, Csizmok V, Buday L, Rakacs M, Kiss R, Bokor M, Udupa R, Tompa K, Tompa P (2009) High levels of structural disorder in scaffold protein as exemplified by a novel neuronal protein, Caskin1. *FEBS J* 276: 3744–3356.
- Illés A, Enyedi B, Tamás P, Balázs A, Bogel G, Melinda, Lukács, Buday L (2006) Cortactin is required for integrin-mediated cell spreading. *Immunol Lett* 04: 124–130.

## ILDIKÓ KASZA (2010)

### Investigation of the function, cell surface expression and regulation of ABCA1 by using *in vitro* model systems

Supervisor: Balázs Sarkadi

The ABCA1 membrane protein plays a pivotal role in cellular lipid homeostasis. As an initial step of the reverse cholesterol transport pathway ABCA1 participates in the interactions of amphiphilic apolipoproteins with cholesterol and phospholipids to generate nascent HDL particles, thus removing excess cellular cholesterol. Mutations in ABCA1 cause Tangier disease, a disorder characterized by HDL deficiency, hypercholesterolemia, cholesterol deposition in macrophages, and premature atherosclerosis. The dysfunction of ABCA1 leads not only to disturbed cholesterol homeostasis but also to impaired phagocytosis and platelet functions presumably through mediating exofacial exposure of phosphatidylserine. The major goal of my study was to acquire new insight into the function, cell surface expression and regulation of ABCA1 by using various *in vitro* model systems. Investigating the role of ABCA1 in cell surface translocation of phosphatidylserine, we examined the effect of the wild type ABCA1 and a mutant variant carrying a missense mutation identified in a patient with Scott Syndrome, a rare bleeding disorder. Our results revealed that expression of wild type ABCA1 restored the defective phosphatidylserine translocation, a phenotype observed in the particular Scott Syndrome patient. This is the first demonstration of the contribution of ABCA1 in the cell surface exposure of phosphatidylserine. Our results confirm the association between ABCA1 dysfunction and a bleeding disorder. To investigate the regulation of trafficking and cell surface expression of ABCA1 we developed a model system which is suitable for monitoring the plasma membrane level of the protein. By using these model cells the effects of several pharmaceuticals with known cholesterol-lowering or HDL level-raising effects were tested. This approach revealed unknown effect of several drugs, including ezetimibe, on ABCA1. Our findings demonstrate the applicability of a novel and reliable

model system for studying cellular routing of ABCA1, opening up new opportunities to identify unknown interaction of drugs with this key transporter of cholesterol metabolism.

- Kasza I, Hegyi Z, Szabó K, Andrikovics H, Németh K, Váradi A, Sarkadi B, Homolya L (2009) *Model system for the analysis of cell surface expression of human ABCA1. BMC Cell Biol* 21(10): 93.
- Albrecht C, McVey JH, Elliott JI, Sardini A, Kasza I, Mumford AD, Naoumova RP, Tuddenham EG, Szabo K, Higgins CF (2005) *A novel missense mutation in ABCA1 results in altered protein trafficking and reduced phosphatidylserine translocation in a patient with Scott syndrome. Blood* 106: 542–549.

## ÁKOS KERTÉSZ (2009)

### **B cell signal modulating effect of cell membrane permeable phosphopeptides based on the sequences of Gab1 adaptor protein**

*Supervisor: Gabriella Sármay*

Reversible phosphorylation of protein tyrosine residues plays a critical role in signaling cascades triggered by growth factors, cytokine and antigen receptors: BCR and TCR. These activation events controlled by the opposing activities of protein tyrosine kinases (PTKs) and phosphatases (PTPs) eventually lead to diverse cellular response such as proliferation, survival, apoptosis, differentiation or migration. Adaptor proteins play important regulatory role in downstream signal transduction from the cell membrane towards the nucleus, providing a surface to form multimolecular signaling complex. Grb2-associated binder 1 (Gab1) is a scaffolding/adaptor protein involved in the signal transduction pathways of growth factors, cytokines, and antigen receptors. Gab1 adaptor protein has several tyrosine residues, which are phosphorylated upon ligand mediated tyrosine kinase activation and binds signaling molecules with SH2 domains, such as SHP-2 tyrosine phosphatase and phosphatidylinositol 3-kinase (PI 3-K). Gab1/SHP-2 contact is essential for cell growth, in addition, SHP-2 and PI3-K regulate signals leading to proliferation and cell survival, thus Gab1/SHP-2 and Gab1/PI3-K interactions might be attractive targets for the therapy of malignant cell growth. We hypothesized that phosphopeptides interacting with SH2 domains may disrupt protein-protein interaction, thereby influencing intracellular signaling. Our main goal is to design cell membrane permeable phosphopeptides corresponding to the SH2 domain binding sequences of Gab1 that would selectively regulate the B cell response, ultimately inhibiting the growth of B cell tumours. In order to transport the phosphopeptides into living cells they were conjugated to membrane-permeable carrier, namely octanoyl-oligoarginin (OR8). With this strategy the *ex vivo* effect of cell membrane-penetrating phosphopeptides was analyzed. We have shown that phosphopeptides corresponding to the SHP-2 binding motif of Gab1 (OR8-GDLLDpe) modulated tyrosine phosphorylation of intracellular proteins and reduced Erk phosphorylation triggered by anti-IgM in BL41 Burkitt-lymphoma cell line. Furthermore, OR8-GDLLDpe attenuated the BCR induced interaction between Gab1 and SHP2. The PI3-K binding sequence of Gab1, OR8-ELPNpe decreased the anti-IgM induced Akt phosphorylation and inhibited the PI3-K dependent mast cell degranulation in RBL-2H3 cells.

- Kertesz A, Takacs B, Varadi G, Toth GK, Sarmay G (2006) Design and functional activity of phosphopeptides with potential immunomodulating capacity, based on the sequence of Grb2-associated binder 1. *Ann N Y Acad Sci* 1091: 437–444.
- Kertész A, Váradi G, Tóth GK, Fajka-Boja R, Monostori E, Sármay G (2006) Optimization of the cellular import of functionally active SH2-domain-interacting phosphopeptides. *Cell Mol Life Sci* 63: 2682–2693.
- Sármay G, Angyal A, Kertész A, Maus M, Medgyesi D (2006) The multiple function of Grb2 associated binder (Gab) adaptor/scaffolding protein in immune cell signaling. *Immunol Lett* 104: 76–82.

## GYÖRGY PARAGH (2009)

### Novel insights into transcriptional changes during keratinocyte differentiation and ultraviolet radiation response

Supervisor: Norbert Wikonkál

The epidermis is the major interface between the human body and the environment. It protects against dehydration, chemical agents, infection and physical environmental effects. The barrier function is mutually susceptible to alterations in the normal keratinocyte differentiation process and direct barrier disruption by chemical agents or physical effects such as ultraviolet (UV) light capable of both inflicting direct and indirect barrier damage. Given the paramount importance of keratinocyte differentiation in epidermal homeostasis it might not be surprising that therapeutic agents capable of altering keratinocyte differentiation via transcriptional modification such as retinoids and active vitamin D proved to be invaluable tools in the treatment of dermatologic diseases. During our studies we aimed to uncover new aspects of the transcriptional regulation of the keratinocytes. We established a short term confluence induced normal human keratinocyte differentiation model, and validated the ability of GeneChip gene expression analysis to test differentiation effects of sphingolipid derivatives. Analysis of expression data of more than 50 known keratinocyte differentiation genes found some of the test compounds to be potent inducers of keratinocyte differentiation. Moreover we identified previously not described keratinocyte differentiation genes involved in redox regulation and skin immune homeostasis, and verified their differentiation related expression also by long term (10 day) Ca<sup>2+</sup> induced keratinocyte differentiation. When studying transcriptional regulation of the lamellar ichthyosis 2 gene, the sphingolipid transporter ABCA12, we established a potential role for nuclear receptor ligands in the therapy of this debilitating genodermatosis. Aiming to uncover novel aspects of UV mediated transcriptional changes in keratinocytes we identified the biphasic response of hypoxia factor 1-alpha to UV light, and thus a link between epidermal effects and the hypoxia system. Taken together, during our work focusing on transcriptional changes in keratinocyte differentiation and homeostasis we uncovered new genes regulated during the process, a new regulatory pathway (HIF-1alpha) involved in the maintenance of epidermal homeostasis. Furthermore we found an important new target (ABCA12) for previously identified homeostatic regulatory circuits (nuclear receptors) in keratinocytes and identified several potential novel therapeutic agents (salicylsphingolipid derivatives), which modify keratinocyte differentiation.

- Paragh G, Schling P, Ugocsai P, Kel AE, Liebis G, Heimerl S, Moehle C, Schiemann Y, Wegmann M, Farwick M, Wikonkál NM, Mandl J, Langmann T, Schmitz G (2008) Novel sphingolipid derivatives promote keratinocyte differentiation. *Exp Dermatol* 12: 1004–1016.
- Wunderlich L\*, Paragh G\*, Wikonkal NM, Banhegyi G, Karpáti S, Mandl J (2008) UVB induces a biphasic response of HIF-1J in cultured human keratinocytes. *Exp Dermatol* 17: 335–342 (\*Authors contributed equally).
- Jiang YJ, Lu B, Kim P, Paragh G, Schmitz G, Elias PM, Feingold KR (2008) PPAR and LXR activators regulate ABCA12 expression in human keratinocytes. *J Invest Dermatol* 128: 104–109.

## FERENC PINTÉR (2009)

### Targeted therapy of lung adenocarcinomas with Epidermal Growth Factor Receptor (EGFR) inhibitors

Supervisor: István Peták

Following successful BR 21 phase III examination, erlotinib—as the only EGFR inhibitor—became accessible as secondary and tertiary treatment of the EGFR positive, non-small-cell lung cancer. Based on the clinical experiences, the treatment in our country was restricted to the adenocarcinomas. The exact definition of EGFR positively, as it appears in the register too, and the most appropriate examination to test for the predictability of the efficiency of the EGFR TKI treatment are remained undetermined. Our primary objective was to assess the precise EGFR status of the lung-adenocarcinomas and to define the relationship between one another and the efficiency of the EGFR TKI therapy by analysing retrospectively 9 samples of patients responding well to the EGFR TKI and by investigating prospectively 127 specimen of patients prior to the treatment. It was important to answer the question as to whether the overproduction of the EGFR protein necessarily goes hand in hand with the activating mutation of the EGFR or with the gene copy elevation and the TKI therapy-sensitivity. The presence of sensitising mutation of the EGFR TK subdomain was analysed by sequencing, gene copy number was assessed by FISH, and the EGFR protein expression was determined by immunohistochemistry. Based on the retrospective analysis of samples of 9 patients (3xCR, 5xPR, 1xSD) responding well to the EGFR TKI treatment: 8/9 sample were EGFR mutation+, 6/6 were FISH+ and only 5/9 were IHC+. Based on the prospective investigation of 127 patients: the ratio of TK mutations was 13.5% (17/126), the FISH+ was 40% (39/97), the IHC+ was 59% (68/116). None of the groups covered entirely the other. 27% of the mutant samples and 34% of the FISH+ were IHC negative. In case of those who have never smoked, the proportion of mutations was considerably larger (42%, 13/31) compared to the smokers (smokers + ex-smokers: 4%, 2/51) ( $P < 0.0001$ ). Following the detection of the EGFR mutation, all the 10 patients who were treated with gefitinib or erlotinib gave therapy responses (5xCR, 5xPR). Four of them were FISH negative and three were IHC negative. Three out of the 18 patients who carried EGFR mutations and responded well to the EGFR TKI treatment have smoked before. Our results prove that each lung-adenocarcinoma which contains the EGFR activating mutation has to be treated with EGFR inhibitor regardless of other biomarkers and the smoking anamnesis.



- Pinter F, Papay J, Almasi A, Sapi Z, Szabo E, Kanya M, Tamasi A, Jori B, Varkondi E, Moldvay J, Szondy K, Keri G, Dominici M, Conte P, Eckhardt S, Kopper L, Schwab R, Petak I (2008) Epidermal growth factor receptor (EGFR) high genecopy number and activating mutations in lung adenocarcinomas are not consistently accompanied by positivity for EGFR protein by standard immunohistochemistry. *J Mol Diagn* 10: 160–168.
- Schwab R, Pinter F, Moldavy J, Papay J, Strausz J, Kopper L, Keri G, Pap A, Petak I, Oreskovich K, Mangel L (2005) Modern treatment of lung cancer: case 1. Amplification and mutation of the epidermal growth factor receptor in metastatic lung cancer with remission from gefitinib. *J Clin Oncol* 23: 7736–7738.
- Varkondi E, Pinter F, Robert K, Schwab R, Breza N, Orfi L, Keri G, Petak I (2008) Biochemical assay-based selectivity profiling of clinically relevant kinase inhibitors on mutant forms of EGF receptor. *J Recept Signal Transduct Res* 28: 295–306.

## GYÖNGYI RÁBAI (2010)

### The alternative pathway and lipid modulators of fibrinolysis in thrombi

Supervisor: Kraszimir Kolev

**Background:** Thrombolysis is conventionally regarded as dissolution of the fibrin matrix of thrombi by plasmin, but the clots formed in vivo contain additional constituents (free fatty acids, leukocytes, platelets) that modulate their solubilization. **Objective:** We examined the presence of free fatty acids in thrombi and their effects on the distinct stages of fibrinolysis (plasminogen activation, plasmin activity). To express in quantitative terms the impact of neutrophils on the lytic processes in obliterating vascular thrombi based on the local presence of elastase-specific fibrin degradation products (NE-FDP) in relation to the leukocyte, platelet and fibrin content. **Methods and Results:** Presence of free fatty acids was demonstrated in surgically removed human thrombi by fluorescent probe. Free fatty acids present in the thrombi reversibly inhibit the amidolytic activity of plasmin on a synthetic substrate, but only partially inhibit its fibrinolytic activity. The plasminogen activation induced by tissue-type plasminogen activator (t-PA) is completely blocked by oleic acid in the fluid phase, but this process is accelerated on a fibrin matrix. Sections of thrombi from 28 patients subjected to thrombectomy were immunostained for fibrin, NE-FDP and platelet antigens, as well as stained for DNA. The fluorescent microscopic images were digitalized and decomposed according to the separate color channels. The integrated intensity values for all thrombus constituents were statistically evaluated. Association between the NE-FDP and leukocyte content of thrombi is evidenced by a significant Pearson correlation coefficient of 0.71 ( $p=0.00002$ ). Cluster analysis identifies two classes of thrombi according to NE-FDP, leukocyte and platelet content and also two according to NE-FDP, leukocyte and fibrin content. When NE-FDP, fibrin and platelet content is normalized to the leukocyte count in the same thrombus, clusters with platelet-dependent thrombolytic resistance (inversely related NE-FDP and platelet content) as well as advanced cell-dependent thrombolysis (inversely related NE-FDP and fibrin content) can be identified. **Conclusion:** Free fatty acids stimulate the plasminogen activation on fibrin surface and they inhibit the plasminogen activation and plasmin activity in the fluid phase. We showed the morphological signs of quantitatively significant cell-

dependent fibrinolytic activity, which proves the dynamic interrelation between the thrombi invaded leukocytes and the thrombi's fibrin and platelet content.

- Rábai Gy, Váradi B, Longstaff C, Sótónyi P, Kristóf V, Tímár F, Machovich R, Kolev K (2007) *Fibrinolysis in a lipid environment: modulation through release of free fatty acid. J Thromb Haemost* 5: 1265–1273.
- Rábai Gy, Szilágyi N, Sótónyi P, Kovalszky I, Machovich R, Kolev K (2010) *Contribution of neutrophil elastase to the lysis of obliterating thrombi in the context of their platelet and fibrin content. Thromb Res* 126: e94–101.
- Váradi B, Kolev K, Tenekedjiev K, Meszaros Gy, Kovalszky I, Longstaff C, Machovich R (2004) *Phospholipid barrier to fibrinolysis: role for the anionic polar head charge and the gel phase crystalline structure. J Biol Chem* 279: 39863–39871.

## KLÁRA ROSTA (2009)

### The role of $\text{Na}^+/\text{K}^+$ -ATPase in the pathomechanism of diabetes mellitus

Supervisor: Ágota Vér

Long-term complications of diabetes mellitus (DM) lead to structural and functional damage in the heart and kidney. Both tissues require the presence of  $\text{Na}^+/\text{K}^+$ -ATPase (NKA) which facilitates secondary active transport mechanisms and serves as the initiator of a number of signaling pathways. The aim of our studies was to examine the changes in the function of NKA in DM and the effect of therapeutic agents used in everyday clinical practice. NKA expression decreases by 30–55% in diabetic (D) heart muscle. Upon chronic insulin administration, this effect is abolished while the activity of the antioxidant system (TSC%) decreases and cardiac muscle hypertrophy is observable. Acute insulin administration recruits NKA and Glut4 from the intracellular membranes to the plasma membrane. This translocation can be inhibited by phosphoinositide (PI) 3-kinase inhibitor. The insulin dependent translocation of NKA and Glut4 is decreased in DM, probably due to the large decrease in protein expression. NKA expression increases by 50% in D kidney proximal tubular cells. In contrast, the density of NKA molecules remains constant in the plasma membrane. According to our study the  $\alpha 1$  subunit of NKA accumulates under the plasma membrane in intracellular vesicles; these pumps are Ser23 phosphorylated and due to their localization fail to exhibit effective ion transport. Long-term angiotensin (ANGII) administration increases the amount of NKA localized in intracellular vesicles, along with Ser23 phosphorylation. The antihypertensive AT1R blocker, losartan, and the antihyperglycaemic metformin normalized the pathologic localization of NKA. According to our studies, changes in the subcellular distribution of NKA may contribute to tissue-specific malfunctions in the heart- and kidney-related complications of DM. The pathological subcellular distribution not only alters ion transport, but may also influence tissuespecific abnormal remodeling in DM.

- Fekete A\*, Rosta K\*, Wagner L, Prokai A, Degrell P, Ruzicska E, Toth M, Vegh E, Ronai K, Rusai K, Somogyi A, Tulassay T, Szabo AJ, Ver A (2008)  *$\text{Na}^+/\text{K}^+$  ATPase is modulated by angiotensin II in diabetic rat kidney—another reason for diabetic nephropathy? J Physiol* 586: 5337–5648 (\*Authors contributed equally to the work).
- Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy G (2007) *Antioxidant measurements. Physiol Meas* 28: R41–55.

- Somogyi A, Ruzicska E, Blazovics A, Ver A, Rosta K, Toth M (2005) *Insulin treatment decreases the antioxidant defense mechanism in experimental diabetes. Med Sci Monit 11: BR206–211.*

## GÁBOR SOBEL (2009)

### Expression profile of tight junction proteins in premalignant cervical lesions and cervical cancer

*Supervisor: Anna Kádár*

Although several events contribute to cancer development, progression and metastasis, it is accepted that the loss of cell-to-cell adhesion is one of the important mechanisms in carcinogenesis. Claudins (CLDNs), of which 24 types have been identified in humans, are integral transmembrane proteins of the tight junctions (TJ) that are critical for maintaining cell adhesion, polarity and play role in signal transduction. Cells and tissues are characterized by individual CLDN patterns; the composition and levels of expression change during differentiation and tumor formation. Alterations in the expression of individual CLDNs have been detected in several carcinomas and shown to be related with progression and invasion. Using a panel of polyclonal (CLDN-1,-3,-7) and monoclonal (-2,-4) antibodies, CLDN pattern was studied by immunohistochemistry and evaluated by morphometry in 105 cervical samples including 20 normal, 27 cervical intraepithelial neoplasia (CIN I-III), 15 *in situ* carcinoma (CIS) and 33 invasive squamous cell cervical carcinoma specimens. Statistically significant increase in the expression of CLDN-1,-2,-4 and -7 was detected in CIN/CIS lesions and invasive cancers compared with normal cervical epithelia. Reduced reactivity of CLDNs-1 and -2 was observed in invasive cervical cancer as compared to CIN/CIS lesions. Another TJ protein, occludin showed the same alteration as CLDN-2. The altered expressions of CLDNs and occludin displayed in our study seem to be closely associated with premalignant lesions and carcinomas in the uterine cervix and are most likely related to progression and invasion. Because of the significantly increased expression of CLDN-1 in abnormal epithelia, this transmembrane TJ protein may serve as a good marker for detection of premalignant lesions or early invasive cancers of cervical squamous epithelia.

- Sobel G, Páska Cs, Szabó I, Kiss A, Kádár A, Schaff Zs (2005) *Increased expression of claudins in cervical squamous intraepithelial neoplasia and invasive carcinoma. Human Pathol 36: 162–169.*
- Sobel G, Szabó I, Páska Cs, Kiss A, Kovalszky I, Schaff Zs (2005) *Changes of cell adhesion and extracellular matrix (ECM) components in cervical intraepithelial neoplasias. Pathol Oncol Res 11: 26–31.*
- Páska Cs, Bögi K, Szilák L, Tőkés A-M, Szabó E, Sziller I, Rigó J, Sobel G, Szabó I, Kaposi-Novák P, Kiss A, Schaff Zs (2004) *Effect of formalin, acetone and RNAlater fixatives on tissue preservation and different size amplicons by real-time PCR from paraffin-embedded tissue. Diagn Mol Pathol 13: 234–240.*

## FLÓRA MÁRIA SZERI (2009)

### **Drosophila MRP, a high turnover model of the long human Multidrug Resistance-associated Proteins**

*Supervisors: András Váradi, Balázs Sarkadi*

The human Multidrug Resistance-associated Proteins, MRP1, 2, 3 and 6, have only one fruit fly orthologue, Drosophila MRP (DMRP). We have expressed this protein in Sf9 cells, and characterised it in comparative functional assays. We demonstrated that DMRP is a useful model protein of human MRPs, harbouring combined substrate specificity and inhibitory profile to its human orthologues. However, DMRP possessed outstanding turnover for the established human MRP substrates, LTC<sub>4</sub>, E217βD-glucuronide, calcein, fluo3 and CDCF. Moreover, DMRP had a high-level NEM-GS and probenecid inducible vanadate-sensitive basal ATPase activity. As a surprise, this activity was inhibited by the transported substrates. As an explanation we hypothesised the presence of an endogenous substrate/allosteric activator in Sf9 cells. Taking advantage of the remarkable activity of DMRP, we determined the transition-state thermodynamic parameters of the ATPase cycle. Our analysis revealed two distinct rate-limiting steps for basal and for modulated ATPase activities, resulting in the same change of activation free energy. Since the modest ATPase activity of human MRPs did not allow such investigations we are the first to present data concerning thermodynamics of an MRP-type transporter. Our data suggested similarities of the steady-state thermodynamics of the catalytic cycle of MDR and MRP-type proteins. In addition, we provided data for the expression of endogenous DMRP and identified two potential pesticide substrates.

- Szeri F, Iliás A, Pomozi V, Robinow S, Bakos E, Váradi A (2009) *The high turnover Drosophila multidrug resistance-associated protein shares the biochemical features of its human orthologues. Biochim Biophys Acta-Biomembr 788: 402–409.*
- Tarnay JN, Szeri F, Iliás A, Annilo T, Sung C, Le Saux O, Váradi A, Dean M, Boyd CD, Robinow S (2004) *The dMRP/CG6214 gene of Drosophila is evolutionarily and functionally related to the human multidrug resistance-associated protein family. Insect Mol Biol 13: 539–548.*
- Bodó A, Bakos É, Szeri F, Váradi A, Sarkadi B (2003) *Differential modulation of the human liver conjugate transporters MRP2 and MRP3 by bile acids and organic anions. J Biol Chem 278: 23529–23537.*

## DÁVID VARGA-SZABÓ (2009)

### **Study of calcium transport processes in blood platelets using knock out mouse models. Regulation and role of “store-operated calcium entry” (SOCE)**

*Supervisor: Ágnes Enyedi*

Platelet activation and aggregation are essential to limit posttraumatic blood loss at sites of vascular injury but also contributes to arterial thrombosis, leading to myocardial infarction and stroke. Agonist-induced elevation of [Ca<sup>2+</sup>]<sub>i</sub> is a central step in platelet activation, but the underlying mechanisms are not fully understood. A major pathway for

Ca<sup>2+</sup>-entry in non-excitabile cells, such as platelets, involves receptor-mediated release of intracellular Ca<sup>2+</sup> stores, followed by activation of store-operated calcium (SOC) channels in the plasma membrane. In my Ph.D. work I investigated the molecular background and the physiological relevance of store-operated calcium entry (SOCE) in platelets. I have identified stromal interaction molecule 1 (STIM1) and Orai1 to be the key components of SOCE in these cells, where STIM1 is the calcium sensor in the endoplasmic reticulum—the major calcium store—that upon store release signals to Orai1—the major SOC channel in platelets—and open it to allow Ca<sup>2+</sup>-entry. Furthermore, I could exclude canonical transient receptor potential channel 1 (TRPC1) to have a major impact on this process. Using *in vivo* thrombosis models I could show that SOCE is of huge importance for stable thrombus formation under high shear flow conditions, such as found in diseased vessels, but lack of the process does not significantly increase bleeding risk. These latter findings establish platelet SOCE and the proteins involved in it as promising targets in the prevention and treatment of ischemic cardio- and cerebrovascular events.

- Varga-Szabo D, Braun A, Kleinschnitz C, Bender M, Pleines I, Pham M, Renné T, Stoll G, Nieswandt B (2008) The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction. *J Exp Med* 205: 1583–1591.
- Varga-Szabo D, Authi K, Braun A, Bender M, Ambily A, Gudermann T, Dietrich A, Nieswandt B (2008) Store-operated Ca<sup>2+</sup> entry in platelets occurs independently of transient receptor potential (TRP) C1. *Pflugers Arch* 457: 377–387.
- Grosse J, Braun A, Varga-Szabo D, Beyersdorf N, Schneider B, Zeitlmann L, Hanke P, Schropp P, Mühlstedt S, Zorn C, Huber M, Schmittwolf C, Jagla W, Yu P, Kerkau T, Schulze H, Nehls M, Nieswandt B (2007) An EF hand mutation in Stim1 causes premature platelet activation and bleeding in mice. *J Clin Invest* 117: 3540–3550.

## EDIT VÁRKONDI (2009)

### Development of *in vitro* biological and biomechemical assay platform for rational drug design

Supervisor: István Peták

According to the conception of signaltransduction therapy, distinct signalling molecules play roles in some important aspects of cancer and other diseases' development and are promising targets for modern drug design. One of the relevant validated target molecules is EGFR small mutations of that has been linked to the sensitivity of nonsmall cell lung cancer (NSCLC) patients to some anilinoquinazoline inhibitors (e.g. gefitinib or erlotinib). The major focus of our research program is to develop ATPanalogues that inhibit EGFR tyrosine kinase with consideration of these observations. For this purpose we established a simple, rapid and cost-effective ELISA-based enzymatic bioassays to characterise the efficacy of inhibitory compounds, and a *Baculovirus* expression vector system to produce wild type and four mutant recombinant proteins of EGFR in our laboratory for the screening. On the basis of docking simulation of 6 well-known compounds, this recombinant protein-based assay seems to be suitable testing model for selecting effective compounds. We invetigated 96 compounds in our biochemical assay system, one of them inhibited the resistance mutant form, and anti-proliferation effect of 143 compounds on A431 cells in Methylene blue and MTT test set up earlier in our laboratory.

Screening data will be used in the refinement of the original pharmacophore model and in the optimization of virtual lead molecules. To extend our cell-based assay system we also established a cancer model system involving 4 lung cancer cell lines with different status of EGFR. In efficacy characterization of 6 well-known compounds, we confirmed first the difference in sensitivity of different forms of EGFR to gefitinib, erlotinib and other two clinical and two preclinical compounds in biochemical assay. Our results may provide scientific reality for an optimized therapeutic application of various EGFR inhibitors and may provide basis to find new template structures for the development of 2nd generation drugs for patients with mutant EGFR, principally with resistance to the first generation EGFR-targeted therapy.

- Varkondi E, Pinter F, Robert K, Schwab R, Breza N, Orfi L, Keri G, Petak I (2008) *Biochemical assay-based selectivity profiling of clinically relevant kinase inhibitors on mutant forms of EGF receptor*. *J Recept Signal Transduct Res* 28: 295–306.
- Varkondi E, Schafer E, Bokonyi G, Gyokeres T, Orfi L, Petak I, Pap A, Szokoloczi O, Keri G, Schwab R (2005) *Comparison of ELISA-based tyrosine kinase assays for screening EGFR inhibitors*. *J Recept Signal Transduct Res* 25: 45–56.
- Breza N, Pato J, Orfi L, Hegymegi-Barakonyi B, Banhegyi P, Varkondi E, Borbely G, Petak I, Keri G (2008) *Synthesis and characterization of novel quinazoline type inhibitors for mutant and wild-type EGFR and RICK kinases*. *J Recept Signal Transduct Res* 28: 361–373.

---

## PROGRAM 7/3.

---

### EMBRYOLOGY, THEORETICAL, EXPERIMENTAL AND CLINICAL DEVELOPMENTAL BIOLOGY

#### **Coordinator:**

**Imre OLÁH M.D., D.Sc.**

Department of Human Morphology  
and Developmental Biology

58 Tűzoltó st, Budapest H-1094

Tel.: +36 1 218 2101; fax: +36 1 215 3064

E-mail: [olah@ana2.sote.hu](mailto:olah@ana2.sote.hu)

#### **Program overview**

The program deals with the progressive changes of the vertebrate organisms from the primordial germ cells through the fertilization up to the organogenesis. During this progressive development primary tissues (epithelial, supporting, muscle and nervous tissues) are formed from different stem cells or progenitors, what is timely and spatially strictly regulated at both genetic and molecular levels. The Program focuses on the early formation of lymphoid organs including the epithelio-mesenchymal interactions (thymus and bursa of Fabricii), formation of hemopoietic sites, cardiac anlage, accessory dendritic cells of the immune system and the effect of different environmental agents on the ontogeny. The ontogeny of the visual system and its relationship with the circadian rhythms (pineal body) is a significant and progressive topic of the Program. The endocytosis by the monocyte-macrophage system is also a rapidly expanding area of the Program. Finally, characterization



of the accessory cells of the MALT in avian species and human completes the series of scientific topics. The methods used by the Program incorporate a wide range of different techniques like light- and electron microscopy, immunocytochemistry, immunofluorescence combined with confocal microscopy, monoclonal antibody production, tissue culture, embryo manipulation (ablation and transplantation of embryonic organ rudiments, chimaerism, parabiosis) and Western blotting.

***Titles of research projects***

- Role of caveolae and caveolin isoforms in various function of the cells
- In vitro* organotypic retinal cultures to study cone differentiation in mammals
- Developmental biology of the hemopoietic organs
- Development of lympho-myeloid organs and supporting tissue
- Photosensitive molecules and photoreceptors in the vertebrate retina and pineal gland
- Embryonal and postembryonal development of the pineal organ and epithalamus

***Supervisors***

- Anna L. Kiss
- Ákos Lukáts
- Nándor Nagy
- Imre Oláh
- Ágoston Szél
- Béla Vígh

***Ph. D. students***

- Ágnes Ida Berta ft
- Ildikó Bódi ft
- Viktória Doma ft
- Gergely Halász ft
- Dávid Molnár ft
- Erzsébet Lackó ft

***Supervisors***

- Ágoston Szél
- Imre Oláh
- Ákos Lukáts
- Ágoston Szél
- Nándor Nagy
- Imre Oláh

***Ph. D. candidates***

- Éva Bíró ft
- Zoltán Hajdú ft

***Supervisors***

- Imre Oláh
- Imre Oláh

***Ph. D. graduates***

- Erzsébet Botos ft
- Zsolt Fejér na

***Supervisors***

- Anna L. Kiss
- Béla Vígh

ft, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009***

### **ERZSÉBET BOTOS (2009)**

#### **Regulation and intracellular route of the caveolin-mediated endocytosis in rat peritoneal macrophages and HepG2 cells**

*Supervisor: Anna L. Kiss*

Caveolae are detergent-insoluble membrane microdomains seen as omega-, or flask-shaped membrane invaginations with the electron microscope ( $d=50\text{--}100\text{ nm}$ ). The major protein components of the cytoplasmic coat are caveolins. Literature data suggest that caveolae can have multiple functions. (1) Since caveolin-1 can bind various signal transduction molecules, caveolae are thought to function as signalling organelles. (2) Originally caveolae were described as stable components of the plasma membrane, recently increasing number of evidence suggested that caveolae are directly involved in the internalisation of membrane components and extracellular ligands. In our work we were interested in the caveolin-mediated endocytosis in macrophages and HepG2 cells using morphological and biochemical methods. We studied the possible regulation of caveolar internalization, the intracellular route of caveolin-1 and the possible organelles along this alternative endocytotic pathway. We found that phosphatase inhibitors and albumin stimulate the internalization of caveolae. In macrophages we immunoprecipitated a 29kDa molecular weight protein, which was tyrosine phosphorylated after OA treatment and assume this protein to be a macrophage-specific isoform of caveolin 2, possibly involved in regulation of internalization. In long term albumin internalization experiments the intracellular pathway of caveolin-1 was followed. Our morphological and biochemical data indicated that caveolin-1 that originates from caveolae pinching off from the plasma membrane appears later in numerous CD63 (LIMP-1) positive late endosomes/multivesicular bodies, suggesting that upon albumin incubation caveolin-1 enters the degradative pathway. Based on this finding we assume that clathrin-coated and caveolin mediated endocytoses are not pathways independent from each other but can be joined at the level of late endosomes. Many grape-like multicaveolar structures observed in our internalization experiments and morphologically identifiable as caveosomes were found to be continuous with the plasma membrane by a thin neck. With the aid of the extracellular marker, Ru-red we proved that the majority of these structures were indeed deep membrane invaginations with multiple caveolae. Finally, morphological and biochemical data support the idea that cells use de novo caveolin synthesis to substitute for caveolin eliminated from the plasma membrane by previous caveola internalization.

- Botos E, Turi Á, Müllner N, Kovalszky I, Tátrai P, Kiss AL (2007) *Regulatory role of kinases and phosphatases in caveolae internalisation in HepG2 cells. Micron 38: 313–320.*
- Botos E, Klumperman J, Oorschot V, Igyártó B, Magyar A, Oláh M, Kiss AL (2008) *Caveolin-1 is transported to multivesicular bodies after albumin-induced endocytosis of caveolae in HepG2 cells. J Cell Mol Med 12: 1632–1639.*
- Kiss AL, Botos E, Túri Á, Müllner N (2004) *Ocadaic acid treatment causes tyrosine phosphorylation of caveolin-2 and induces internalization of caveolae in rat peritoneal macrophages. Micron 38: 707–715.*

**ZSOLT FEJÉR (2009)****Comparative fine structure and immunocytochemical analysis of the corpus pineale responsible for the regulation of daily and seasonal rhythm in different vertebrates***Supervisor: Béla Vigh*

According to their morphology and development, the pinealocytes as neural cells, bear a lot of resemblance to retinal rods and cones. The dendritic processes of pinealocytes thicken to inner segments that reach the pineal lumen. In the inner segments of all investigated species we found a primary cilium (9x2+0), as well as structures resembling to outer segments of rods. The efferent pole of pinealocyte continues in an axon-like process. Some of these processes make ribbon-type synapses with pineal neurons, while others end as a neuro-haemal bulb. These bulbs have contacts with the outer liquor space on the surface of the pineal gland, so their products (e. g. melatonin) reach directly it, or indirectly the lumen of blood vessels crossing this space. The afferent autonomic fibers of the pineal gland arrive with the meningeal connective tissue. Our important observation is that these nerve fibers never enter the pineal gland, but remain in the meninges. These fibers end on muscle cells of blood vessels as vasomotor terminals. Using immunohistochemistry we mapped the phylogenetic expression of pinopsin and other retinal photopigments. According to our results, the pinopsin could be detected in the pineals of all investigated vertebrates with the exception of Cyclosomata and Mammalia, but not in the parapineal organs or in the retina of lower vertebrates. On the other hand, the retinal photopigment rhodopsin expresses in the pinealocytes of all vertebrates including Cyclostomata. Apart from rhodopsin, OS-2, RET-2 and COS-1 antibodies could detect other photoreceptor molecules in the pinealocytes of different species. We found Ca-accumulation and acervulus formation in avian pineal gland with the pyroan-timonate method. The source of this Ca accumulation could be the cellular activity coupled to photoreception as well as the hampered Ca elimination due to the folded nature of the pineal gland.

- Fejér Zs, Röhlich P, Szél Á, Dávid C, Zádori A, Manzano MJ, Vigh B (2001) *Comparative ultrastructure and cytochemistry of the avian pineal organ. Micr Res Techn* 53: 12–24.
- Fejér Zs, Haldar C, Ghosh M, Frank LCs, Szepessy Zs, Szél Á, Manzano e Silva MJ, Vigh B (2001) *Pineal organ-like organization of the retina in megachiropteran bats. Acta Biol Hung* 52: 17–27.
- Vigh B, Röhlich P, Görcs T, Manzano e Silva MJ, Szél Á, Fejér Zs, Vigh-Teichmann I (1998) *The pineal organ as a folded retina: immunocytochemical localization of opsins. Biol Cell* 90: 653–659.

**PROGRAM 7/4.****BASIS OF HUMAN MOLECULAR GENETICS AND GENE DIAGNOSTICS*****Coordinator:*****András FALUS M.Sc.,****member of the Hungarian Academy of Sciences**

Department of Genetics, Cell- and Immunobiology

4 Nagyváradi sq, Budapest H-1089

Tel.: +36 1 210 2930/ext. 6241

Fax: +36 1 303 6968

E-mail: [faland@dgci.sote.hu](mailto:faland@dgci.sote.hu)**Program overview**

To provide an overview on various fields of human medical and molecular genetics, genomics, including theory and methodology.

***Titles of research projects***

Studies on the pathomechanism of rheumatological diseases

Regulation of histidine decarboxylase (HDC) gene expression in physiological and pathological processes

Study on the inflammation agents and regulatory molecules of the acute-phase response

Molecular immunological characterization of BAL monocytes

Molecular genetic analysis of adhesion protein family

Diagnostic methods of gene analysis in clinical paediatrics

The examination of the influences of genetic and immunological factors in rheumatological conditions

Molecular biological methods in examining patients with muscular dystrophies

Chemotaxis: its biological and clinical significance

Role of histamine in antigen presentation and cell differentiation

Cell cycle-specific expression changes of Waf1/p53/PCNA/ciclins/cdk-s in cell cultures studied by Northern, Western and rt-PCR techniques

The introduction of systems biology in the research of the diagnosis, prevention and therapy of neurological and psychiatric disorders

Genetic and immunological factors in rheumatological conditions

The active immunologic relationship between foetus and mother means fetal antigen presentation, maternal antigen recognition and immunologic response

Studies on the genomic background of haematological malignancies

Forensic aspects of the DNA investigation

Study on the pathomechanism of asthma with molecular genetic methods

***Supervisors***

Edit Buzás

Zsuzsanna Darvas

András Falus

András Falus

András Falus

György Fekete

Pál Géher

Veronika Karcagi

László Kőhidai

Valéria László

Zoltán Marcsek

Mária Judit Molnár

György Nagy,  
Pál Géher

Erna Pap

Éva Pállinger

Péter Sótónyi

Csaba Szalai

Evaluation of medical biology data	Csaba Szalai
ALL genomics	Csaba Szalai
Analysis of cardiac functions in histamine-free transgenic murine model	Sára Tóth
Single nucleotide polymorphisms in the development of para-dontal disease and missing tooth-germs	Gábor Varga
Isolation and characterization of dental postnatal stem cells	Gábor Varga
The human MRP1, ABC-transporter examination of the nucleotide-binding protein domains and study of the catalytic cycle	András Váradi
The significance of genetic polymorphism studies in systemic mastocytosis	Judit Várkonyi

***Ph.D. students***

Borbála Aradi	ft
Zsuzsanna Baka	pt
Sándor Békási	ft
Edit Cságoly	ft
Angéla Dajnoki	ft
Barbara Érsek	ft
Anna Katalin Gilicze	ft (a)
András Gézsi	ft
Bence György	ft
Éva Hadadi	ft
Orsolya Ildikó Lautner-Csorba	ft
Réka Lepesi-Benkő	ft
Petra Misják	ft
Zsuzsanna Pál	ft (a)
Gergely Temesi	ft
Viktor Virág	ft

***Ph.D. candidates***

Ágnes Semsei (Félné)	pt
Orsolya Hegyesi	a
Kristóf Kádár	a
László Losonczi	ft
Viktor Molnár	ft
Krisztián Németh	a
Petra Mária Scharek	a
Barbara Patocs (Zentainé)	pt

***Ph.D. graduates***

Dániel Erdélyi	ft
Annamária Glász-Bóna	pt
Zsuzsanna Horváth	ft
Ilona Milánkovics	pt
Henriett Pikó	pt
Péter Pócza	ft

***Supervisors***

Edit Buzás
György Nagy
Zsuzsa Darvas
Csaba Szalai
György Fekete
György Nagy
Sára Tóth
Csaba Szalai
Edit Buzás
Csaba Szalai
Csaba Szalai
Sarolta Kárpáti
Edit Buzás
Mária Judit Molnár
Csaba Szalai
Csaba Szalai

***Supervisors***

Csaba Szalai
Gábor Varga
Gábor Varga
György Fekete
András Falus
Sarolta Kárpáti
András Falus
György Fekete

***Supervisors***

Csaba Szalai
Sarolta Kárpáti
Edit Buzás
Veronika Karcagi
Veronika Karcagi
András Falus

Mária Szente-Pásztói

ft

Edit Buzás

Gergely Tölgyesi

ft

Csaba Szalai

a, absolutorium; ft, full-time, pt, part-time; i, individual

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

**DÁNIEL ERDÉLYI (2010)**

### **Role of ABC-transporter gene polymorphisms in childhood acute lymphoblastic leukaemia**

*Supervisor: Csaba Szalai*

**Objectives:** The fast development of genomics and bioinformatics provide hope for future's personalized chemotherapy. Our ambition was to establish a paediatric oncology/haematology DNA-bank and a related databank, and to start testing certain already identified functional gene polymorphisms to assess and characterize their clinical relevance.

**Methods:** With the participation of all Hungarian paediatric oncology centres, we collected samples in a retrospective manner from children diagnosed with acute lymphoblastic leukaemia (ALL) between 1990 and 2003. As for control groups, samples and data were gathered from blood donors, osteosarcoma and testicular tumour patients at collaborating institutes. So far, five polymorphisms of two drug transporter genes were analyzed on the ALL data available at the given stage of data collection. **Results:** A DNA bank was set up containing 623 ALL patients. Within this, a 445-member study group was determined, whose toxicity data were also collected. In our studies, the ABCB1 3435TT genotype group was characterized by increased infectious complications. Regarding acute toxic encephalopathy, we observed a multiplicative synergistic interaction between the above mentioned genotype and the carriage of the ABCG2 421A allele. Individual allele frequencies did not differ between our ALL and control blood donor population. **Conclusion:** In order to gain practical utilization of these results, the above mentioned polymorphisms should be further tested in chemotherapy protocols using only one of the transporters' substrates, with concurrent pharmacokinetic studies. Those harbouring ABCB1 3435TT together with ABCG2 421AA/CA genotypes need special attention because of their high risk for severe toxicities.

- Erdélyi DJ, Kámory E, Zalka A, Félné-Semsei Á, Csókay B, Andrikovics H, Tordai A, Borgulya G, Magyarosy G, Galántai I, Fekete Gy, Falus A, Szalai Cs, Kovács GT (2006) The role of ABC-transporter gene polymorphisms in chemotherapy induced immunosuppression, a retrospective study in childhood acute lymphoblastic leukaemia. *Cell Immunol* 244: 121–124.
- Erdélyi DJ, Kámory E, Csókay B, Andrikovics H, Tordai A, Kiss C, Félné-Semsei Á, Janszky I, Zalka A, Fekete G, Falus A, Kovács GT, Szalai C (2008) Synergistic interaction of ABCB1 and ABCG2 polymorphisms predicts the prevalence of toxic encephalopathy during anticancer chemotherapy. *Pharmacogenomics J* 8: 321–327.



- Semsei Á, Erdélyi DJ, Ungvári U, Kámory E, Csókay B, Andrikovics H, Tordai A, Cságoly E, Falus A, Kovács GT, Szalai Cs (2008) Association of some rare haplotypes and genotype combinations in the MDR1 gene with childhood acute lymphoblastic leukaemia. *Leukemia Res* 32: 1214–1220.

## ANNAMÁRIA GLÁSZ-BÓNA (2010)

### Mutation analysis of KRT5 and KRT14 genes with epidermolysis bullosa simplex

Supervisor: Sarolta Kárpáti

Epidermolysis bullosa simplex (EBS) belongs to the most common subtype of hereditary bullous disorders characterized by lysis of the basal keratinocytes due to mild mechanical trauma. In the basal forms of EBS, the blisters heal without scarring. The most frequently occurring types of the basal form of EBS are the localized EBS (EBSloc), EBS Dowling-Meara (EBS-DM) and other generalized EBS (EBS, gen-nonDM). EBS with mottled pigmentation (EBS-MP) is a rare variant basal form of EBS. It is characterized by mild intraepidermal blistering associated with progressive reticular hyperpigmentation on the trunk and extremities. EBS patients are known to carry defects in either the keratin genes KRT5 or KRT14 expressed mostly in the basal layer of the epidermis. Methods for mutation analysis in the disease have been used routinely. The presence of a partially and a completely inactive KRT14 pseudogene in the human genome. Contrary to KRT14, KRT5 has no identified pseudogene. To examine KRT14 mutations in Hungarian families with EBS we developed a simple allele-specific method to study the KRT14 gene excluding pseudogene sequence contamination. In conclusion, this method made the molecular analysis of EBS DNA in the routine laboratory easier, faster, more precise, more economic, more robust and more cost-effective. Using this approach we were able to identify 6 novel and 4 previously described mutations as well as 8 polymorphisms. 3 novel (p.I183V, p.E190D, p.R331G) missense mutations were verified in the KRT5 and 3 novel (p.L136Q, p.E411K, p.I412N) in the KRT14. Out of the EBS families carrying new mutations 2 had the Dowling-Meara and 4 localised phenotype. A limited number of EBS-MP cases—to date twenty unrelated families—published from all over the world including thirteen reports from Europe. We present herein the first reported case from Hungary. In summary, we could validate a new and easy way of KRT14 mutation analysis strategy in EBS which efficiently excludes the KRT14 pseudogene. Furthermore, the described new mutations provide further evidence of phenotype-genotype correlations in EBS, should be helpful for genetic counselling and may pave the way for gene therapy.

- Glasz-Bona A, Medvecz M, Sajo R, Lepesi-Benkő R, Tulassay Zs, Katona M, Hatvani Zs, Blazsek A, Karpáti S (2009) Easy method for keratin 14 gene amplification to exclude pseudogene sequences: new keratin 5 and 14 mutations in epidermolysis bullosa simplex. *J Invest Dermatol* 129: 229–231.
- Glasz-Bona A, Medvecz M, Viragh Zs, Hatvani Zs, Blazsek A, Karpáti S (2010) Epidermolysis bullosa simplex with mottled pigmentation—mutation analysis proved the diagnosis in a four-generation pedigree. *Eur J Dermatol* 20: 698–700.
- Csikós M, Becker K, Rácz E, Bóna A, Benkő R, Czippán A, Katona M, Bruckner-Tuderman L, Kárpáti S, Horváth A (2004) Hereditær epidermolysis bullosa molekuláris genetikai vizsgálata. *Bőrgyógy Venerol Szemle* 80: 195–202.

**ZSUZSANNA HORVÁTH (2010)****The modulating effect of histamine on hematopoiesis***Supervisor: Edit Buzás*

Hematopoiesis is a complex system, which consists of various mature and immature cell populations, regulated by a network of intercellular connections and soluble factors. All hematopoietic cells originate from a common precursor, the hematopoietic stem cell, characterized by the ability to self-regenerate, proliferate and differentiate. By these functions, they can establish all hematopoietic cell lineages, and react to hematopoietic stress by complete bone marrow regeneration. In the present study we analyzed the role of histamine in the functions of hematopoietic stem and progenitor cells. First, we demonstrated the expression of HDC enzyme and histamine in the various stem cell subtypes. These cell populations showed a differential HDC and histamine production, correlating with their cycling status. We showed that there were defects in both the numbers and repopulative functions of stem cells in genetically histamine-free (HDC<sup>-/-</sup>) mice. Although no marked alterations were noted in the overall steady-state hematopoiesis, regeneration after hematopoietic stress was delayed in histamine-free mice. To discover the underlying mechanisms of impaired stem cell functions, we investigated the modulating effect of histamine in the signalling pathways of IL-3, one of the most important stem cell regulating factors. Our results showed that the lack of histamine negatively affects IL-3 signalling, through impaired activation of STAT5. Correlating with this, we observed a lag in G1-S-phase progression in proliferating cells of HDC<sup>-/-</sup> mice, suggesting that histamine affects cell cycle entry and consequent differentiation processes. We also demonstrated that while histamine affects bone marrow cells by directly regulating their proliferation, in the spleen it exerts its immunomodulatory effect on other indirect regulatory mechanisms as well, thereby affecting extramedullary hematopoiesis. Finally, we proved that absence of histamine results in increased apoptosis of hematopoietic cells. Summarizing, these studies provide evidence that histamine has a fundamental role in regulation of hematopoiesis. This effect is probably exerted through interacting with proliferation processes, such as cell cycle regulation, and with signalling pathways of growth factors. Our results may contribute to a better understanding of the physiology of hematopoietic stem cells and their sophisticated regulation by a complex cytokine network. This knowledge could be potentially used in treating pathological bone marrow processes.

- Horvath Z, Pallinger E, Horvath G, Jelinek I, Falus A, Buzas EI (2006) Histamine H1 and H2 receptors but not H4 receptors are upregulated during bone marrow regeneration. *Cell Immunol* 244: 110–115.
- Horvath Z, Pallinger E, Horvath G, Jelinek I, Veszely G, Furesz J, Falus A, Buzas EI (2010) Extramedullary hematopoiesis is dysregulated in histamine-free histidine decarboxylase knockout (HDC<sup>-/-</sup>) mice. *Inflamm Res* 59(6): 429–436.
- Jelinek I, Laszlo V, Buzas E, Pallinger E, Hangya B, Horvath Z, Falus A (2007) Increased antigen presentation and T(h)1 polarization in genetically histamine-free mice. *Int Immunol* 19: 51–58.

**ILONA MILÁNKOVICS (2010)****Molecular and clinical analysis of patients with classical galactosaemia, galactokinase deficiency and biotinidase deficiency***Supervisor: Veronika Karcagi*

Classical galactosaemia, a disorder of carbohydrate metabolism, is caused by a defect of the galactose-1-phosphate-uridylyltransferase enzyme. Affected infants present in the neonatal period with feeding problems, failure to thrive, vomiting, diarrhea, jaundice, bleeding, liver and renal failure, physical and mental retardation, cataract, neonatal sepsis, which can lead to death. Galactokinase deficiency is an inborn error in the first step of galactose metabolism. Its major clinical manifestations are hypergalactosemia and galactosuria, which cause cataract. Biotinidase deficiency is a complex inherited disorder. Untreated patients develop various clinical symptoms: neurological deficits, skin rash, alopecia, seizures, ataxia, organic aciduria, hearing loss, metabolic ketoacidosis and occasionally developmental delay. Early recognition of these three inborn errors and early initiation of the treatment result in rapid clinical improvement, for that reason in Hungary, the national programme for screening was launched. Our aim was to apply mutation and clinical analysis for classical galactosaemia, galactokinase deficiency and biotinidase deficiency in the Western Hungarian setting, both retrospectively and prospectively. 13 different mutations were identified in 46 patients with classical galactosaemia including two novel missense mutations (p.S297P; p.E146D). The two most frequent mutations were the p.Q188R and the p.K285N with an allele frequency of 41.2% and 27.1%, respectively. We examined the sequence of the human galactokinase gene from 6 patients exhibiting galactokinase deficiency and identified two germline mutations including p.L263P novel mutation. Twenty-four different mutations were identified in 60 patients with biotinidase deficiency. Among these, eighteen were missense mutations (out of these, two were double missense mutations in the same allele), one was a nonsense mutation, two were a one-base deletions, one was a seven-base deletion coupled with a three-base insertion, one was an intronic nucleotide change at the splice site of exon 3, and one was a point mutation creating a cryptic 3' splice acceptor site. We identified five novel mutations (p.E46X; p.T152P; p.R157C; p.N195S; c.406delC) in the biotinidase gene. Similar to the worldwide incidence, the five most frequent mutations were: p.D444H, p.Q456H, c.98:d7i3, p.A171T:p.D444H and p.R538C. These mutations accounted for 76.7% of all abnormal alleles. A relatively high proportion of mutations (14.2%) proved to be unique to the Hungarian population. The incidence of the biotinidase deficiency in Western Hungary was more than three times the worldwide incidence. In autosomal recessive disorders the clinical manifestations do not always correlate closely with the genotypes. The symptoms depend on other genetic and environmental factors. The aim of our population genetical study was to analyse a healthy Hungarian population for the frequencies of the most frequent galactose-1-phosphate uridylyltransferase and biotinidase variant alleles. The allele frequencies of GALT mutations in the Hungarian population correlate well with other healthy Caucasian populations. While, the allele frequencies of the most biotinidase variant alleles in the Hungarian population are usually higher than in other healthy Caucasian populations.

- Milánkovics I, Kámory E, Csókay B, Fodor F, Somogyi Cs, Schuler Á (2007) *Mutations causing biotinidase deficiency in children ascertained by newborn screening in Western Hungary. Mol Genet Metab* 90: 345–348.
- Milánkovics I, Schuler Á, Kámory E, Csókay B, Fodor F, Somogyi Cs, Németh K, Fekete Gy (2010) *Molecular and clinical analysis of patients with classical galactosaemia from Western Hungary. Wien Klin Wochenschr* 122: 95–102.
- Milánkovics I, Schuler Á, Németh K, Somogyi Cs, Fekete Gy (2009) *A Los Angeles és Duarte galaktóz-1-foszfát-uridiltranszferáz variánsok allélgyakorisága a magyar populációban. Orv Hetil* 150: 1299–1303.

## HENRIETT PIKÓ (2009)

### Molecular genetics analyses of the muscular dystrophies in the Hungarian affected families

*Supervisor: Veronika Karcagi*

Duchenne/Becker muscular dystrophy is a severe, recessive, X-linked neuromuscular disease with an incidence of 1/3500 (Duchenne type) and 1/30000 (Becker type) in newborn boys. The gene responsible for the Duchenne/Becker muscular dystrophy phenotype is located at Xp21 and its 427 kD protein product is called dystrophin. Deletions, point mutations and rarely duplications can occur almost anywhere in the DMD gene, which makes the molecular diagnosis difficult. Multiple polymerase chain reactions detect 95% of deletions in affected males, but are not suitable for carrier detection in female relatives. Southern blot analysis with six different cDNA probes covers the whole 14 kb dystrophin transcript and allows the detection of female carriers by comparing the intensity of the signals corresponding to the different exons. This method is time consuming compared to the newly introduced multiple ligation-dependent probe amplification method. Multiple ligation-dependent probe amplification is a method suitable for relative quantification of several DNA sequences in one reaction. The author report results on 149 DMD/BMD affected male patients where the dystrophin gene analyses were carried using multiplex PCR, Southern blot with cDNA probe hybridisation and MLPA technique and confirmed deletion or duplication on 93 cases. The carrier status was analysed simultaneously by cDNA hybridisation and MLPA technique in 93 females and the carrier state was confirmed in 42 cases. In this carrier population the author additionally detected two cases with duplication, two cases with one copy of the whole dystrophin gene and three manifest carrier females. On the basis of these results the MLPA technique, which has been newly introduced in Hungary, proved to be a sensitive and quick method for the detection of carrier state in the DMD/BMD disease. Moreover, the exact deletion or duplication border can be detected and as a result, prediction on the phenotype can be given. This will provide the right therapeutic intervention for the affected patients in the future.

- Pikó H, Vancsó V, Nagy B, Bán Z, Herczegfalvi Á, Karcagi V (2009) *Dystrophin gene analysis in Hungarian Duchenne/Becker muscular dystrophy families—Detection of carrier status in symptomatic and asymptomatic female relatives. Neuromusc Disord* 19: 108–112.
- Müller JS, Pikó H, Schoser BGH, Schlotter-Weigel B, Reilich P, Gürster S, Born S, Karcagi V, Pongratz D, Lochmüller H, Walter MC (2006) *Novel splice site mutation in the caveo-*

*lin-3 gene leading to autosomal recessive limb girdle muscular dystrophy. Neuromusc Disord* 16: 432–436.

- *Pikó H, Nagy B, Balog J, Bán Z, Hercegfalvi Á, Karcagi V (2007) Hordoóság szűrés Duchenne–Becker izomdystrophiával érintett családokban. Orv Hetil* 148: 2403–2409.

## PÉTER PÓCZA (2010)

### Oncogenomic investigation in the field of colon cancer carcinogenesis and melanoma-stroma interaction

*Supervisor: András Falus*

In the past decades, colorectal cancer became one of the main foci of cancer research. One of the reasons of this is that colorectal cancer is one of the most serious problems of the public health (the third most common cancers and the second common cause of cancer death in developed countries). On the other hand, the relative ease with which pathological specimens can be obtained by either surgery or endoscopy from different stages of tumor progression has facilitated the application of genomic technologies. In our study, we have investigated the gene expression profiles of lymph-node metastasis negative and distant metastasis positive colon cancer tissues compared to the adjacent non-cancerous mucosa from surgical resections, in order to improve our understanding of the genetic mechanism of carcinogenesis in human colorectal cancer and to identify new potential tumor markers useful for clinical practice. Two-colour whole human genome oligonucleotide microarray was carried out and significantly deregulated genes were analyzed. Our results were validated on extended pieces of specimens of human colorectal cancer and on colon cancer cell lines. In the course of pathway analysis we found that the decreased level of IGF-1 was associated with the decreased level of MDR1/P-gp and regulated via the MAPK-cascade. After the validation on tissue samples we provided evidence that increased IGF-1 resulted in the increased expression of MDR1/P-gp via the MAPK signalling pathway in three human colon cancer cell lines. We focused on the tumor-stroma interaction as well and we investigated the effects of elastin-derived peptides on the invasion potential of melanoma cells. We provide evidence that VGVAPG and VAPG elastin-derived peptides bind directly to three cell surface receptors: galectin-3, integrin  $\alpha v \beta 3$  and elastin-binding protein. We found that EDPs increase the migration, the adhesion, the expression of the elastin-degrading MMP-2 and MMP-3 enzymes and the expression of the lymphangiogenic VEGF-C of melanoma cells via the galectin-3 receptor. Our data gives new insights into the genetic mechanisms underlying neoplastic transformation of colorectal cells and into the interaction of elastin protein and melanoma cells.

- *Pocza P, Suli-Vargha H, Darvas Zs, Falus A (2008) Locally generated VGVAPG and VAPG elastin-derived peptides amplify melanoma invasion via the galectin-3 receptor. Int J Cancer* 122: 1972–1980.
- *Pos Z, Wiener Z, Pocza P, Racz M, Toth S, Darvas Zs, Molnar V, Hegyesi H, Falus A (2008) Histamine coordinately suppresses fibulin-5 and insulin-like growth factor 2 receptor expression in melanoma. Cancer Res* 68: 1997–2005.
- *Boer K, Hellinger E, Hellinger A, Pocza P, Pos Z, Demeter P, Baranyai Zs, Dede K, Darvas Zs, Falus A (2008) Decreased expression of histamine H1 and H4 receptors suggests dis-*



turbance of local regulation in human colorectal tumours by histamine. *Eur J Cell Biol.* 87: 227–236.

## MÁRIA SZENTE-PÁSZTÓI (2010)

### The investigation of the role of regulator and effector factors in the pathomechanism of autoimmune diseases

*Supervisor: Edit Buzás*

There is an increasing awareness of the importance of posttranslational autoantigen modifications and glycobiology in the course of investigating the pathomechanism of rheumatoid arthritis (RA). Thus, for the first time we systematically investigated the expression of cartilage degrading glycosidases ( $\beta$ -D-hexosaminidase,  $\beta$ -D-glucuronidase, hyaluronidase, sperm adhesion molecule 1 and klotho) within the joints. Next, we explored the glycosaminoglycan (GAG) reactivity of natural autoantibodies found in high amount in the circulation. Since citrullinated antigen-reactive antibodies are widely used diagnostic and prognostic tools in RA, we investigated if there was a differential T cell recognition of differently citrullinated variants of a disease relevant, shared epitope-containing aggrecan peptide (P135). Finally, we set out to explore the contribution of T cells to nitrogen monoxide (NO) production in RA. Our main results are as follows: (1) We characterized the expression of glycosidases in the synovial membrane, in synovial fibroblasts and also in synovial fibroblast-derived microvesicles. (2) We have shown for the first time that in contrast to proteases widely investigated in RA pathomechanism, glycosidases, expressed by the synovial membrane and synovial fibroblasts, are under negative regulation by some locally expressed cytokines. (3) We have shown that anti-GAG antibodies were readily detectable in adult controls, and were significantly elevated in patients with RA. (4) We provided evidence that in RA the level of anti-chondroitin sulphate C IgM antibody shows inverse correlation both with the Disease Activity Score (DAS) 28 scores and C-reactive protein (CRP) levels. (5) We found that the P135 peptide induced a significant IL-17 response in patients with RA but not in controls. (6) We found that subgroups of controls and patients with RA were “citrullination sensitive” showing an enhanced Th17 response to citrullinated variants of P135. 7. We found that T cells from RA patients produced significantly more NO than healthy donor T cells in association with an increased cytoplasmic  $\text{Ca}^{2+}$  concentration, that was further increased by *in vitro* treatment with TNF.

- Pasztoi M, Nagy G, Geher P, Lakatos T, Toth K, Wellinger K, Pocza P, Gyorgy B, Holub MC, Kittel A, Paloczky K, Mazan M, Nyirkos P, Falus A, Buzas EI (2009) Gene expression and activity of cartilage-degrading glycosidases in human rheumatoid arthritis and osteoarthritis synovial fibroblasts. *Arthritis Res Ther* 11: R68.
- Pap E, Pállinger E, Pásztoi M, Falus A (2009) Highlights of a new type of intercellular communication: microvesicle-based information transfer. *Inflamm Res* 58: 1–8.
- György B, Tóthfalusi L, Nagy G, Pásztoi M, Géher P, Lőrinc Z, Polgár A, Rojkovich B, Ujfalussy I, Poór G, Pócza P, Wiener Z, Misják P, Koncz A, Falus A, Buzás EI (2008) Natural autoantibodies reactive with glycosaminoglycans in rheumatoid arthritis. *Arthritis Res Ther* 10: R110.



**GERGELY TÖLGYESI (2009)****Genomic investigation of the pathomechanism of allergic asthma***Supervisor: Csaba Szalai*

Asthma is a complex polygenic disorder, and its prevalence is on the rise. Fortunately, in parallel with this tendency, the more and more expanding knowledge of genomics and the rapidly developing technologies offer powerful tools to be able to gain deeper insight into the genetic background of the disease. In our studies, we used gene expression microarray and single nucleotide polymorphism (SNP)-based methods to investigate the pathogenesis of allergic asthma. We carried out gene and microRNA expression profile analysis in the lung of mice undergoing allergen-induced experimental asthma at different time points during the experimental protocol. The study showed extensive changes in gene expression in the lungs in response to allergen at all time points. We applied GO and Gene Set Enrichment Analyses in order to gain a comprehensive insight into the biological processes and functions of the asthmatic response. Using these methods, we were able to relate gene expression changes to cellular processes and integrate our results into multiple levels of information available in public databases. We identified the high upregulation of miR-155, a microRNA that plays an important role in the development of immune response, in the lungs of allergic mice. Among the top downregulated transcripts, an antioxidant enzyme, paraoxonase-1 (PON1) was identified. In human asthmatic patients we found that serum PON1 activity was reduced at asthma exacerbations, but increased in parallel with improving asthma symptoms. PON1 gene polymorphisms, which affect the expression and the activity of the coded enzyme, did not influence the susceptibility to the disease. Our observations suggest that an altered PON1 activity might be involved in the pathogenesis of asthma, and PON1 might be a potential new therapeutic target as well as a diagnostic tool for following up the effect of therapy. In our gene-environment interaction studies, we showed that *C. pneumoniae*-specific IgG positivity is associated with asthma, when children carrying the TNF $\alpha$  -308A allele are considered. Furthermore, children infected with *C. pneumoniae* in the past (IgG positivity) carrying the TNF $\alpha$  -308A allele have considerably higher risk of developing asthma than children with similar infection status carrying normal genotypes.

- Tolgyesi G, Keszei M, Ungvari I, Nagy A, Falus A, Szalai C (2006) Involvement of TNF- $\alpha$  -308A promoter polymorphism in the development of asthma in children infected with *Chlamydophila pneumoniae*. *Pediatr Res* 60: 543–548.
- Ungvari I, Tolgyesi G, Semsei AF, Nagy A, Radosits K, Keszei M, Kozma GT, Falus A, Szalai C (2007) CCR5 Delta 32 mutation, *Mycoplasma pneumoniae* infection, and asthma. *J Allergy Clin Immunol* 119: 1545–1547.
- Szalai C, Ungvari I, Pelyhe L, Tolgyesi G, Falus A (2008) Asthma from a pharmacogenomic point of view. *Br J Pharmacol* 153: 1602–1614.

**PROGRAM 7/5.****IMMUNOLOGY*****Coordinator:*****Péter GERGELY M.D., Ph.D., D.Sc.**

Central Laboratory of Immunology

41 Mária St, Budapest H-1085

Tel./fax: +36 1 266 0926/ext. 5737

E-mail: [gergely@immun.sote.hu](mailto:gergely@immun.sote.hu)**Program overview**

The recognition of immunology as an independent scientific discipline is recent, therefore, in many universities immunological research is carried out in various (e.g. biochemical etc) departments. Qualified immunologists are greatly demanded in many areas, including clinical science. This project, completing the curriculum of undergraduate training, yields a perspective to qualify in several areas of immunology. The purpose of this Ph.D. Program is to train independent, reliable and competent research scientists. The Program emphasizes the importance of studying basic immunology and laboratory methods, both being prerequisites of any work in experimental and clinical immunology.

***Titles of research projects***

Expression of protooncogenes and tumor suppressor genes in gestational trophoblastic tumors and normal pregnancy

Study of the associations with the major histocompatibility complex (MHC) with diseases

Genetic, biomolecular and clinical interactions in multifactorial diseases

Regulation of immune reactions and role of cytokines in the pathomechanism of autoimmune diseases

Acute phase proteins in clinical diagnostics

The effects of immunological and environmental factors in various inflammatory and/or malignant cellular proliferations

Effects of bacterial products on the progression of HIV infection

Genetical, molecular and clinical interactions in multifactorial diseases

Infection and autoimmunity in inflammatory diseases

***Supervisors***

Vilmos Fülöp,

László Kopper

György Füst

Zoltán Prohászka

Péter Gergely

László Kalabay

Beatrix Kotlán

Károly Nagy

Zoltán Prohászka

Gyula Poór

***Ph.D. students***

Zsófia Bánlaki

ft (a)

Judit Czúcz

ft (a)

Rita Margit Ivanics

pt

Gyula Schäffer

ft

Krisztián Vörös

pt

***Supervisors***

György Füst

György Füst

Vilmos Fülöp

Zoltán Prohászka

László Kalabay

***Ph. D. candidates***

István Aladzsity	ft
Veronika Makó	ft

***Supervisors***

Zoltán Prohászka
Zoltán Prohászka

***Ph. D. graduates***

Tamás Constantin	na
Tímea Gombos	ft
Gábor Hutás	na
Attila Zoltán Bokor	pt

***Supervisors***

Péter Gergely
Zoltán Prohászka
Péter Gergely
Vilmos Fülöp

ft, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2010*****TAMÁS CONSTANTIN (2010)****Prognostic factors of juvenile dermatomyositis**

*Supervisor: Péter Gergely*

Juvenile dermatomyositis (DM) belongs to the heterogeneous group of idiopathic inflammatory myopathies (IIMs). The main characteristics of the disease are the weakness of the proximal muscles, as mediated by an autoimmune process and typical cutaneous symptoms. The disease may affect other organs, as well. As these disorders are rare it is very important to manage the patients in specialized centres. My goals were partly analysing the clinical aspects of juvenile DM (focusing on clinical characteristics, course, survival, functional outcome and quality of life), partly finding genetic risk factors in a Hungarian myositis population. The knowledge of these prognostic factors may be used in the clinical practice. To the proper assessment of the clinical characteristics, a national registry of patients with juvenile IIMs was elaborated in Hungary. Analysis was performed using data of 54 patients diagnosed between 1976 and 2004. Concerning the evaluation of clinical signs and symptoms their frequency was similar to the data of the relevant literature. It was an important aim to investigate the characteristics of clinical course and prognosis. In view of the disease course more than half of the patients have monophasic disease, while one third of them are suffering from polycyclic disease and the others have chronic illness. Chronic disease course was more frequent among JDM patients, than in DM patients. The risk of the relapse was found to be the highest during the first year after the remission. As relapses may occur after a prolonged disease-free interval, patients should be followed up for at least 2 years. There was no correlation between relapse free survival and initial therapeutic regimen. Since the initiation of glucocorticoid treatment 60 years ago the prognosis of JDM has become better and better. Nowadays the mortality rate is less than 3%. In this study, none of the juvenile patients died, while among IIM patients CAM has the worst prognosis. My work indicates that patients with juvenile and adult DM have similar or higher survival rates as it has been

previously reported worldwide. I have not found significant difference between groups of juvenile and adult DM in point of survival. The improvement of the survival can be attributed to three main changes: firstly, the widespread use of new diagnostic tools and new serologic testing lead to early diagnosis and to the recognition of milder forms of the disease; secondly the early use of more appropriate and more aggressive immunosuppressive and supportive therapy and lastly, the regular follow-up in departments specialized on the disease. Despite favourable survival probability, further investigations were needed to assess functional outcome. As it is well known, in adult cases CAM has the worst prognosis. One of my goals was to find out if there were CAM cases in juvenile patients. However, based on our IIM registry and the data of the Hungarian National Tumor Registry, no cases were identified. Routine cancer screening is not recommended in JDM patients, however, JDM patients treated with aggressive therapeutic regimens, e. g., cyclophosphamide or biologic treatment need particular attention in the future. One of my objectives was to longitudinally analyse disease activity, functional outcome and quality of life and to assess disease damage in regularly followed-up patients. Before the glucocorticoid era, half of the patients had disease damage. Now it is a real challenge to cure patients to achieve perfect functional outcome. Long-term outcome of the disease is poorly investigated in the scientific literature. Considering both medium- and long-term course functional outcome and quality of life are affected in a certain proportion of the patients. This was the first study to investigate quality of life in JDM patients. At the time of diagnosis the majority of the patients had limitations to some extent in performing activities of everyday life, and the quality of life was significantly different from healthy children. After the treatment period a significant increase in functional outcome was found; however, the patients' life quality was affected on the long run as well. In the heterogeneous group of IIMs different subtypes can be identified with different phenotypic features (different signs and symptoms, and autoantibodies as well) based on the genetic background. Investigations of the immunogenetic characteristics may help with predicting the prognosis. Genetic analysis of the TNF- $\alpha$  G(-308)A polymorphism revealed that our patients have similar characteristics to the Caucasian myositis population. TNF- $\alpha$ -308A allele was a genetic factor for JDM, DM, PM and OM. There were no associations found between TNF- $\alpha$ -308A allele and clinical symptoms, except for Raynaud-phenomenon and sicca symptoms in DM patients. Anti-Jo-1 antibody was associated with the TNF- $\alpha$ -308A allele in PM patients. No association was found between TNF- $\alpha$ -308A allele and treatment, course, calcinosis, and functional outcome. The TNF- $\alpha$ -308 locus was linked with HLA-DRB1, HLA-DQA1 and DQB1 in DM and PM patients. The most frequent haplotype was the ancestral 8.1 (HLA-DRB1\*03-DQA1\*05-DQB1\*02-TNF- $\alpha$ -308A).

- Constantin T, Ponyi A, Orbán I, Molnár K, Dérfalvy B, Dicső F, Kálovics T, Müller J, Garami M, Balogh Z, Szalai Z, Fekete G, Dankó K (2006) National registry of patients with juvenile or adult idiopathic inflammatory myopathies in Hungary—clinical characteristics and disease course of 54 patients with juvenile IIM. *Autoimmunity* 39: 223–232.
- Constantin T, Ponyi A, Garami M, Gergely L, Fekete G, Dankó K (2003) A juvenilis dermatomyositis klinikai jellemzői. *Orv Hetil* 144: 1245–1250.
- Dankó K, Ponyi A, Constantin T, Borgulya G, Szegedi G (2004) Long-term prognosis and survival of patients with idiopathic inflammatory myopathies. *Medicine (Baltimore)* 83: 35–42.

**TÍMEA GOMBOS (2010)****New prognostic markers and the role of inflammation in the pathomechanism of chronic heart failure***Supervisor: Zoltán Prohászka*

Activation of the neurohormonal system, chronic inflammation and endothelial dysfunction are playing role in the pathomechanism of chronic heart failure. The parameters describing the three connected pathways are potential prognostic markers. Methodological developments provide reliable measurement of the plasma levels of the vasoactive adrenomedullin (ADM) and endothelin-1 (ET-1). Heat shock proteins with 70kDa are ancient intracellular molecules, but during stress response they can be found in the circulation (sHsp70). Von Willebrand factor (VWF) is a marker of the endothelial dysfunction. Its level increases in chronic heart failure patients, but there is no data about the activity of its cleaving protease, ADAMTS13. The imbalance between the two proteins may predict thromboembolic complications. We aimed to perform a prospective clinical cohort with chronic heart failure patients to find prognostic mortality and morbidity markers, and to study the relation of these markers with clinical and laboratory parameters. According to our results high MR-proADM levels and high CT-proET-1 levels had similar predictive value to NT-proBNP in mortality and morbidity analysis. The two studied vasoactive peptides showed a strong positive correlation with the markers of inflammation, independently of the severity of the disease. The supposed mutual regulation may play a crucial role in the progression of heart failure. The sHsp70 level was higher in patients with severer disease, and showed strong positive correlation with the markers of the failure of cell integrity. Carrying the G allele of HspA1B (1267) is connected with high sHsp70 levels in patients with severer disease. In chronic heart failure the ADAMTS13 protease activity was significantly decreased, while the VWF antigen level was increased. The low ADAMTS13/VWF ratio is an independent predictor of mortality and showed correlation with thromboembolic complications in chronic heart failure patients.

- Gombos T, Föhrécz Z, Pozsonyi Z, Jánoskúti L, Prohászka Z (2008) Interaction of serum 70-kDa heat shock protein levels and HspA1B (+1267) gene polymorphism with disease severity in patients with chronic heart failure. *Cell Stress Chaperones* 13: 199–206.
- Gombos T, Föhrécz Z, Pozsonyi Z, Walentin S, Papassotiriou J, Kunde J, Morgenthaler NG, Jánoskúti L, Prohászka Z (2009) Adrenomedullin and endothelin-1 are related to inflammation in chronic heart failure *Inflamm Res* 58: 298–305.
- Gombos T, Makó V, Cervenak L, Papassotiriou J, Kunde J, Hársfalvi J, Föhrécz Z, Pozsonyi Z, Borgulya G, Jánoskúti L, Prohászka Z (2009) Levels of von Willebrand factor antigen and von Willebrand factor cleaving protease (ADAMTS13) activity predict clinical events in chronic heart failure. *Thromb Haemost* 102: 573–580.

## GÁBOR HUTÁS (2010)

### The mechanism of action of anti-CD44 monoclonal antibody treatment in experimental arthritis

*Supervisor: Péter Gergely*

Granulocytes play a central role in the effector phase of chronic autoimmune arthritis. Migrating inflammatory cells entering the joint cavity induce events leading to progressive, irreversible tissue damage. Therefore focused anti-granulocyte treatment may be a therapy of choice in treating inflammatory conditions in the future. The improvement of fluorescent imaging techniques makes it possible for the first time to directly observe the recruitment of inflammatory cells in the arthritic joints. We give a short description of both *in vitro* and *in vivo* techniques used in the investigation of granulocyte-endothelial interactions under inflammatory conditions. CD44, the leukocyte adhesion receptor for hyaluronan, has been considered a therapeutic target on the basis of the robust anti-inflammatory effect of CD44-specific antibodies in animal models of immune-mediated diseases. However, CD44 deficiency does not provide substantial protection against inflammation. Using intravital video microscopy in a murine model of rheumatoid arthritis, we show that CD44 deficiency and anti-CD44 antibody treatment exert disparate effects on leukocyte recruitment in inflamed joints. Leukocyte rolling, which is increased in CD44-deficient mice, is promptly abrogated in anti-CD44-treated wild-type mice. CD44-specific antibodies also trigger platelet deposition on granulocytes and subsequent depletion of this leukocyte subset in the circulation. These *in vivo* effects require CD44 cross-linking and are reproducible with an antibody against Gr-1, a molecule that, like CD44, is highly expressed on granulocytes. Anticoagulant pretreatment, which prevents platelet deposition, mitigates both granulocyte depletion and the suppressive effect of CD44-specific antibody on joint swelling. Our observations suggest that cross-linking of prominent cell surface molecules, such as CD44 or Gr-1, can initiate a rapid self-elimination program in granulocytes through engagement of the coagulation system. We conclude that the robust anti-inflammatory effect of CD44-specific antibodies in arthritis is primarily the result of their ability to trigger granulocyte depletion. Our study also implicates that different antibody treatments against certain cell surface markers may cause unwanted side effects by interfering with the coagulation system.

- Hutás G, Bajnok E, Gal I, Finnegan A, Glant TT, Mikecz K (2008) CD44-specific antibody treatment and CD44 deficiency exert distinct effects on leukocyte recruitment in experimental arthritis. *Blood* 112: 4999–5006.
- Farkas B, Boldizsar F, Tarjányi O, Laszlo A, Lin SM, Hutás G, Tryniszewska B, Mangold A, Nagyri G, Rosenzweig HL, Finnegan A, Mikecz K, Glant TT (2009) BALB/c mice genetically susceptible to proteoglycan-induced arthritis and spondylitis show colony-dependent differences in disease penetrance. *Arthritis Res Ther* 11: R21.



**ATTILA BOKOR (2010)****Semi-invasive diagnosis of endometriosis***Supervisor: Vilmos Fülöp*

Endometriosis is a common, benign, oestrogen-dependent, gynaecological disorder associated with pelvic pain and infertility. While endometriosis has been described for more than one hundred years, our current knowledge of its pathogenesis remains unclear. The aim of our study was to test the hypothesis that multiple sensory small diameter nerve fibers are present in a higher density in endometrium from patients with endometriosis when compared to women with a normal pelvis. The assumed difference enables the development of a semi-invasive diagnostic test for minimal-mild endometriosis. In our second study we have tested the hypothesis that menstruation is associated with a higher concentration of endometrial cells in peritoneal fluid (PF). To prove that endometriosis is associated with an active immunologic process with increased white and red blood cell concentration in PF when compared to nonmenstrual phases of the cycle. The density of small nerve fibres is significantly higher in endometrium from patients with minimal-mild endometriosis when compared to women with a normal pelvis. The combined analysis of neural markers PGP9.5, VIP, SP could predict the presence of minimal-mild endometriosis with 95% sensitivity, 100% specificity, and 97.5 % accuracy. The results of our second study demonstrate for the first time that menstruation in women is associated with an increased PF concentration of leucocytes, erythrocytes and hemoglobin when compared to nonmenstrual phases of the cycle, supporting the concept of retrograde menstruation. An increased PF concentration of PF endometrial cells was not observed during menstruation when compared to nonmenstrual phases of the cycle.

- Bokor A, Kyama CM, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A, De Moor B, Fülöp V, D'Hooghe T (2009) Density of small diameter sensory nerve fibres in endometrium: a semi-invasive diagnostic test for minimal to mild endometriosis. *Hum Reprod* 24: 827–834.
- Bokor A, Debrock S, Drijkoningen M, Goossens W, Fülöp V, D'Hooghe T (2009) Quantity and quality of retrograde menstruation: a case control study. *Reprod Biol Endocrinol* 7: 123.

---

# SCHOOL OF PH.D. STUDIES

---

## 8. PATHOLOGICAL SCIENCES

---

***Chairman:*****László KOPPER M.D., Ph.D., D.Sc.**1st Department of Pathology and Experimental  
Cancer Research

26 Üllői st, Budapest H-1085

Tel./fax: +36 1 317 0891

E-mail: [kopper@korb1.sote.hu](mailto:kopper@korb1.sote.hu)**General overview**

The Doctoral School of Pathology includes six postgraduate teaching Programs as follows: Oncology, Pathomorphology, Microbiology, Transplantation studies, Health Sciences, Public Health. Consequently, the training covers a rather broad area of medical sciences involving both the etiopathogenesis, diagnostic and therapeutic activities of the most common human diseases and health education. The training concentrates on individual research work guided by the tutors who makes proposal for the topic of the research, provides the facilities, warrant the technical/intellectual up-to-dateness and the progress of study. At present 39 Ph.D. students with diploma in medicine, pharmacy, or biology are holders of fellowship, in addition 46 medical doctors as corresponding Ph.D. students are preparing their dissertation. The Ph.D. degree has been awarded to 42 students trained in the frame of Doctorate School of Pathology in the last years.

***PROGRAM 8/1.***

---

**ONCOLOGY*****Coordinator:*****László KOPPER M.D., Ph.D., D.Sc.**1st Department of Pathology and Experimental  
Cancer Research

26 Üllői st, Budapest H-1085

Tel./fax: +36 1 317 0891

E-mail: [kopper@korb1.sote.hu](mailto:kopper@korb1.sote.hu)**Program overview**

The Program invites those who intended to learn and study tumor biology as well as experimental and clinical oncology. The Ph.D. students are also trained in updated techniques in cell biology, pathology, biochemistry and recombinant gene technology. Following courses are organized by the program: Experimental Oncology, Clinical Oncology, Molecular Oncology.

***Titles of research projects***

Markers of efficacy in therapy of against urological cancer  
 Epigenetic gene inactivation in breast carcinoma and in head and neck tumors  
 Predictive and prognosticatory factors in moderate tumours  
 Pregnancy breast cancer  
 Pediatric oncology  
 System biological modelling of chemoresistance  
*In vitro* examination of pediatric malignancies  
 Experimental modelling, characterization and chemotherapeutic response in osteosarcoma  
 Brachytherapy of brain tumors  
 Regulation of cell proliferation and cell death  
 Role of extracellular matrix elements in the regulation of liver behavior  
 Role of proteoglycans in carcinogenesis  
 Gene defects related to malignancy  
 Clinical and experimental aspects of oncotherapeutic markers in colorectal cancer  
 Epidermal growth factor receptor (EGFR) in giant cell bone tumors in the progression of giant cell bone tumors  
 Upregulation of collagen XVII in malignant transformation and tumor progression  
 Defects of direct cell-cell communication in malignant melanoma due to failures in connexin junctions  
 Studying bioelectromagnetic effects in tumor models  
 Tumor immunology—tumor infiltrating immunocells in human tumors and immunological parameters in sentinel lymph nodes  
 The effect of ionizing radiation on the immune system and its role in the modulation of antitumor immune response  
 Molecular genetics in genesis and progression of lymphomas  
 Signalling pathways directed by receptors and metabolism in cell death and their pharmacological characterization  
 Role of liver stem cells in hepatic disorders and tumors  
 Tumor induced angiogenesis  
 Morphological study on the biliary tract and its vascular network during regeneration and carcinogenesis  
 Death receptor signalling as target for tumor therapy  
 Host and tumor factors in metastatization (mainly in melanomas)  
 The examination of individual beam sensitivity in radiation therapy patients, the identification of genes is responsible for the beam sensitivity  
 The increase of the sensitivity of tumours for radiotherapy with gene therapy procedures  
 The examination of the late genetic effects of the ionising radiation  
 Chromosomal instability in giant cell bone tumors

***Supervisors***

István Bodrogi  
 Orsolya Csuka  
 Magdolna Dank  
 Magdolna Dank  
 Miklós Garami  
 Balázs Gyórfy  
 Péter Hauser  
 András Jeney  
 Jenő Julow  
 László Kopper  
 Ilona Kovalszky  
 Ilona Kovalszky  
 Ilona Kovalszky  
 Judit Kralovánszky  
 Tibor Krenács  
 Tibor Krenács  
 Tibor Krenács  
 Tibor Krenács  
 Andrea Ladányi  
 Katalin Lumniczky  
 András Matolcsy  
 Rudolf Mihalik  
 Péter Nagy  
 Sándor Paku  
 Sándor Paku  
 István Peták  
 Erzsébet Rásó  
 Géza Sáfrány  
 Géza Sáfrány  
 Géza Sáfrány  
 Zoltán Sági

Repair of the function of proapoptotic regulators to increase the effect of chemotherapy	Anna Sebestyén
Clinical progression and ECM components in oral precancerosis and squamous cell cc	Zsuzsanna Suba
Induction of apoptosis	Béla Szende
Role of microRNA in the pathogenesis of non-Hodgkin lymphomas	Ágota Szepesi
Gene expression maps to predict individual behavior and response of tumors	József Tímár
Metastatization and angiogenesis	József Tímár
Modelling and regulation of the movement of human tumor cells	József Tóvári

**Ph. D. students**

Houman Ahmadbehbahani	it
Péter Balla	pt
Tamás Barbai	ft
Mansour Bassel	it
Edina Bugyik	ft
Katalin Dobos (Dömötörné)	ft
Siamak Eftekhari	it
Alexandra Fullár	ft
Éva Gagyí	ft
Tamás Márton Garay	ft
Eleonóra Imrédi	ft
Navid Kabiri	it
Katalin Kiss	ft
András Lőrincz	ft
Ágnes Márk	ft
Nóra Meggyesházi	pt
Gyöngyi Munkácsy	ft
Orsolya Németh	pt (a)
Zoltán Órfi	crc
Gergő Papp	ft
Zsófia Péntzváltó	ft
Tímea Pócza	ft
Hajnalka Rajnai	ft
Krisztián Somlai	pt
Tamás Sticz	ft
Balázs Szabó	pt (a)
Gyöngyvér Szentmártoni	pt
Dániel Takács	ft (a)
Bálint Tegze	ft
József Virág	ft (a)
Ivett Zsákovics	ft

**Ph. D. candidates**

Kornélia Baghy	ft
Helga Barti-Juhász	ft

**Supervisors**

Ilona Kovalszky
Tibor Krenács
Erzsébet Rásó
István Bodrogi
Sándor Paku
Ilona Kovalszky
Zsuzsanna Suba
Ilona Kovalszky
András Matolcsy
József Tímár
József Tímár
Ilona Kovalszky
Ilona Kovalszky
István Peták
Anna Sebestyén
Tibor Krenács
Balázs Gyórfy
Miklós Garami
István Peták
Zoltán Sági
Balázs Gyórfy
Péter Hauser
András Matolcsy
Magdolna Dank
László Kopper
József Tímár
Magdolna Dank
Zsuzsanna Suba
Balázs Gyórfy
Miklós Garami
Tibor Krenács

**Supervisors**

Péter Nagy
István Peták

Katalin Dezső	ft	Péter Nagy
Zsuzsanna Dunai	ft	Rudolf Mihalik
Tibor Fekete	na	Balázs Győrffy
Lajos Viktor Komlósi	ft	Judit Kralovánszky
Linda Moskovszky	ft	Zoltán Sági
Veronika Papp	ft	Sándor Paku
Bálint Péterfia	ft	Ilona Kovalszky
Eszter Turányi	na	Péter Nagy

***Ph.D. graduates***

Zsófia Balogh	ft
Bíborka Bereczky	pt

Krisztina Biró	na
Zsófia Egyed	pt
Melinda Hajdu	ft
Péter Hollósi	ft
Gergely Huszty	pt
István Kenessey	ft

Viktória László	ft
Judit Müller	na
Judit Pápay	na
Andrea Réti	ft
Erzsébet Szabó	ft
Tibor Szarvas	ft
Tünde Szatmári	pt

***Supervisors***

András Matolcsy
József Tímár,
József Tóvári
László Kopper
Zoltán Papp
Anna Sebestyén
Ilona Kovalszky
Géza Sáfrány
József Tímár,
József Tóvári
Sándor Paku
Judit Kralovánszky
József Tímár
Judit Kralovánszky
Zsuzsa Schaff
Ilona Kovalszky
Géza Sáfrány

ft, full-time; pt, part-time; it, international; na, not affiliated; crc, Cooperation Research Centre

***Abstracts of Ph.D. theses successfully defended in 2009 and 2010*****ZSÓFIA BALOGH (2010)****Genomic imbalances in chronic lymphocytic leukemia with increased prolymphocytes and in pseudofollicles**

*Supervisor: András Matolcsy*

Chronic lymphocytic leukaemia (CLL) is a low-grade non-Hodgkin's lymphoma that may transform to a more aggressive form, termed CLL with increased prolymphocytes (CLL/PL). The molecular mechanism of the transformation is not fully known. To further characterize the biological features of this disease, we performed IgVH gene mutational status, fluorescent *in situ* hybridization (FISH) and high-resolution comparative genomic hybridization (HR-CGH) analysis in 17 cases of CLL/PL. All CLL/PL utilized members of VH1, VH3 and VH4 families, with the highest prevalence of the VH1-69 gene. In all but

one cases analyzed, the VH genes were unmutated. The FISH and HR-CGH analyses showed frequent occurrence of trisomy 12, del(11)(q23), del(17)(p13) and genetic imbalances, but recurrent genetic lesion characteristic for CLL/PL was not found. The follow up HR-CGH analysis of two cases showed that increasement of prolymphocytes in the course of CLL or CLL/PL are associated with clonal evolution and selection of the tumor clone. In conclusion, this study suggests that CLL/PL is a relatively homogeneous disease regarding VH gene mutation, but heterogeneous regarding genetic lesions. The heterogeneity and high number of genomic imbalances found in CLL/PL suggest that a genome-wide instability of the neoplastic cells may play a role in the development of the disease. Although CLL is characterized by the accumulation of small B-lymphocytes, prolymphocytes and paraimmunoblasts form pseudofollicles (PF) in the lymph nodes which are also known as proliferation centers. 12 formalin-fixed paraffin-embedded (FFPE) CLL lymph nodes containing PF were analyzed. The FFPE sections were stained with methylene blue and marked by laser. Subsequent FISH analysis was performed relocalizing the previously defined regions. In 7 cases the PF areas contained a significantly higher ratio of genetic alterations than the surrounding interfollicular (IF) areas. The accumulation of the genetic lesions in PF suggests that the genetic progression and transformation of CLL may begin in these proliferation centers.

- Balogh Zs, Reiniger L, Deák L, Bődör Cs, Csomor J, Szepesi Á, Gagyí É, Kopper L, Matolcsy A (2007) *IgVH gene mutation status and genomic imbalances in chronic lymphocytic leukemia with increased prolymphocytes (CLL/PL)*. *Hematol Oncol* 25: 90–95.
- Reiniger L, Bődör Cs, Bognár Á, Balogh Zs, Csomor J, Szepesi Á, Matolcsy A (2006) *Richter's and prolymphocytic transformation of chronic lymphocytic leukemia are associated with high mRNA expression of activation-induced cytidine deaminase and aberrant somatic hypermutation*. *Leukemia* 20: 1089–1095.

## BÍBORKA BERECHKY (2010)

### Analysis of certain aspects of hematogenous and lymphogenous dissemination in preclinical tumor models

*Supervisors: József Tímár*

Key steps of tumor progression are invasion, tumor angiogenesis and metastatization. These processes depend on interactions between tumor cell and its microenvironment. According to literature data, dissemination of tumor cells is affected by the coagulation system, so by anticoagulants either. In our *in vitro* experiments we analyzed the effects of unfractionated heparin (UFH) and its low molecular weight derivative (LMWH) on human melanoma cell proliferation, adhesion, migration and invasion. Their effects were tested *in vivo* on lung and liver colonization in SCID mice. Heparins failed to influence the growth of human melanoma cells, but significantly inhibited lung and liver colony formation. These presented experimental data prove that clinical doses of heparins can specifically inhibit the adhesion, migration, invasion and metastasis of tumor cells. Inflammatory cells and cytokines in tumor microenvironment are involved in the neoplastic process, regulating the survival and metastatization is a highly inflammatory and multifunctional of the tumor cells. Interleukin-1 $\beta$  is a highly inflammatory and multifunctional cytokine, which can induce angiogenesis, but its role in lymph-



angiogenesis remains unclear. In this study we used *in vivo* mouse cornea model and confirmed that IL-1 $\beta$ -induced haemangiogenesis and lymphangiogenesis are on similar level with 100 ng FGF-2-induced one. IL-1 $\beta$  selectively enhanced the migration of human endothelial and lymphatic endothelial cells, but had no effect on proliferation. Anti-VEGFR-3 treatment significantly inhibited the IL-1 $\beta$ -induced lymphangiogenesis. In mouse cornea IL-1 $\beta$  can induce the expression of angiogenic growth factors on mRNA level via NF- $\kappa$ B signal transduction pathway. Macrophage depletion assay showed that the above mentioned growth factors are produced by macrophages. These results demonstrate that IL-1 $\beta$  can induce lymphangiogenesis and this process is mediated via VEGF-C/-D/VEGFR-3 pathway. These findings offer new strategies in lymphovascular targeted antitumor therapy. Also the antiadhesion and antimigratory effects of commonly used heparins open up new perspectives for clinicians and may help in the battle against developing metastases.

- Berezsky B, Gilly R, Rásó E, Vágó Á, Tímár J, Tóvári J (2005) *Selective antimetastatic effect of heparins in preclinical human melanoma models is based on inhibition of migration and microvascular arrest. Clin Exp Met* 22: 69–76.
- Tímár J, Tóvári J, Rásó E, Mészáros L, Berezsky B, Lapis K (2005) *Platelet-mimicry of cancer cells: epiphenomenon with clinical significance. Oncology* 69: 185–201.
- Watari K, Nakao S, Fotovati A, Basaki Y, Hosoi F, Berezsky B, Higuchi R, Miyamoto T, Kuwano M, Ono M (2008) *Role of macrophages in inflammatory lymphangiogenesis: enhanced production of vascular endothelial growth factor C and D through NF- $\kappa$ B activation. Biochem Biophys Res Comm* 377: 826–831.

## KRISZTINA BIRÓ (2009)

### Detection of ototoxic effect in testicular cancer patients with otoacoustic emission

Supervisor: László Kopper

The aim of the research was to detect the acute and long-term ototoxic effect of cisplatin in testicular cancer patients, using OAE (otoacoustic emission), a highly sensitive new objective method, for detecting medication-related hearing loss. Secondary objective was to evaluate the risk factors that contribute to hearing loss. In the study for acute hearing loss ten males with different histological types of testicular germ cell tumor were examined with TOAE (transiently evoked otoacoustic emission), before the 1st and after the 5th day of their 1st cycle of cytostatic therapy. 100 mg/m<sup>2</sup> cisplatin were administered per cycle, (20 mg/m<sup>2</sup> for five days). Ten age-matched healthy volunteers of good hearing and without treatment were also examined with the same method. Wilcoxon and paired t-tests were used for statistical evaluation. In this acute phase study no differences were found either in otological physiological examination or in conventional audiometry, or in tympanometry. There were no statistically significant differences in amplitude either before and after therapy, or between patient and control group. No patient complained of hearing loss or tinnitus. In the long term hearing loss study 223 cured patients were assessed by DPOAE (distorsion product otoacoustic emission) 100 mg/m<sup>2</sup> cisplatin were administered per cycle, in EP, BEP, VeIP, VIP or VPB regimens. The control group consisted of 40 testicular cancer patients without chemotherapy. A detailed medical history of the patient

and his family evaluated audiological risk factors and hearing complaints. Before DPOAE, otoscopic examination and tympanometry tests were used to exclude any conductive component. DPOAE was measured in eight frequencies from 750 to 8000 Hz. Paired t-test, Mann-Whitney test and stepwise discriminant analysis were used for statistical evaluation. Symptomatic ototoxicity was observed in 20% of the patients. In patients receiving  $\leq 300$  mg/m<sup>2</sup> cisplatin, no amplitude changes were detected. Beyond this dose, hearing impairment proved to be dose dependent. Contrary to the literature, not only high frequencies were affected. In patients receiving  $\geq 400$  mg/m<sup>2</sup>, our method could also detect significant hearing impairment at lower frequencies that are important for speech perception. (1000–3000 Hz). The lowest amplitudes were detected in those patients who had symptomatic ototoxicity. The only statistically significant risk factors were the cumulative dose of cisplatin and age; neither smoking nor previous noise exposure proved to be risk factors. As the life expectancy of testicular cancer patients matches in most cases the life expectancy of healthy males, studying long-term side effects is of great importance. OAE is a fast, noninvasive and reliable method in detecting ototoxicity in testicular cancer patients. Cisplatin dose regimens should be reduced to the minimum required for cure, based on a risk-adapted treatment.

- Biro K, Baki M, Buki B, Noszek L, Jokuti L (1997) *Detection of early ototoxic effect in testicular cancer patients treated with cisplatin by transiently evoked otoacoustic emission: a pilot study. Oncology 54: 387–390.*
- Biro K, Noszek L, Prekopp P, Nagyivanyi K, Geczi L, Gaudi I, Bodrogi I (2006) *Characteristics and risk factors of cisplatin induced ototoxicity in testicular cancer patients, detected by distortion product otoacoustic emission. Oncology 70: 177–184.*
- Biró K, Noszek L, Prekopp P, Nagyivanyi K, Geczi L, Gaudi I, Bodrogi I (2006) *Cisplatinnal kezelt heretumoros betegek késői halláskárosodásának vizsgálata disztorziós otoakusztikus emissziós (DOAE) készülékkel. Magyar Onkol 50: 329–335.*

## ZSÓFIA EGYED (2009)

### **New challenges in complex breast cancer diagnostics: the role of pathologic prognostics, the correlation of breast density and hormone replacement therapy**

*Supervisor: Zoltán Papp*

Breast cancer has been the most frequent malignant tumor until recently: in 1999 it caused 2345, in 2001 2301 deaths. The complex imaging and cytology reports of 2028 breast change, verified by postoperative histology—which were diagnosed in the past 10 years at the breast clinic of MaMMA Healthcare Co.—were reviewed according to the following points: (1) Statistic evaluation of individual and combined preoperative diagnostics with a focus on the effect of mammographic density on the results of the evaluation of the different radiomorphologic forms. (2) Can the different tumors of the prognostic factor driven pathology classification be separated by the current preoperative diagnostic methods?—Nottingham Prognostic Index (NPI) and Ki-67 classification, triple negative and basal cancers. (3) The correlation of hormone replacement therapy, breast density increase and cancer diagnostics. Can the increased density be considered as an independent risk factor or it only makes diagnosis more difficult? Are there any possible differences in the tumors of the population of prolonged perimenopausal combined hor-

mone replacement therapy vs. age related untreated ones? The study resulted in the following conclusions: The results of all four diagnostics methods equal the figures known from the literature. The analysis of sum score and mammographic appearance proved significant results which are important for the evaluation of the connection of modern pathologic diagnostics and prognosis. Putting together the radiologic methods into the hands of one doctor and organizing them to a single occasion along with the close cooperation with the cytopathologist is crucial for this achievement. There are specific changes in the sum score and mammographic radiomorphologic distribution of the different prognostic groups according to NPI and the Ki-67 reaction. The triple negative and basal carcinomas which have the worst prognosis showed the most malignant signs in the case of all of our diagnostic tools and have appeared on mammography as indeterminate circumscribed lesions in increased density breast baseline pattern. The diagnostic results of these aggressive tumors are encouraging to look for further correlations between the preoperative diagnostics attributes, mammographic appearance and prognostic factors. The followings could have been concluded when comparing all of our malignant cases with the cancers of women on longer than 5 years of combined hormone replacement therapy as the evaluation of the correlation between peri- and postmenopausal hormone replacement therapy and breast cancer: Hormone replacement therapy causes breast density increase, which decreases mammographic sensitivity, but this deficiency is made even by the other diagnostic methods. In these cases compared to low density breasts the probability of having a mammographically occult tumor is quadrupled. There is no significant difference in low or high density baseline breast parenchyma in the mammographic or histologic evaluation of tumor size, but exists in focality recognition. Increased density can more often be noticed—compared to normal population—in the case of either malignant or benign breast pathology. Within the scope of this study the diagnostics and clinicopathologic factors (radiomorphologic distribution, axillary node involvement, histologic grade, and hormone receptor positivity) of the hormone taking women were not significantly worse than that of untreated ones. These results encourage us to seek for further correlations between modern histologic prognostics and the routine breast diagnostics, especially in the case of specific subgroups of mammographic forms, malignant microcalcifications.

- Egyed Zs, Péntek Z, Ormándi K, Járay B Kulka J, László Zs (2007) Difficulties in the diagnosis of intracystic tumors of the female breast. *Neoplasma* 54: 75–82.
- Egyed Zs, Péntek Z, Járay B, Kulka J, Svastics E, Kas J, László Zs (2008) Radial scar—significant diagnostic challenge. *Pathol Oncol Res* 14: 123–129.
- Egyed Zs, Járay B, Kulka J, Péntek Z (2009) Triple test score for the evaluation of invasive ductal and lobular breast cancer. *Pathol Oncol Res* 15: 159–166.

**MELINDA HAJDU (2009)****The elements and functional significance of Notch-signaling and its potential cross-talk with the TGF $\beta$ -pathway in the survival of human B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia cells***Supervisor: Anna Sebestyén*

The survival of lymphoid cells is regulated by the interplay of signaling pathways comprising an intricate network. The aim of our studies was to decipher the role of the Notch and the transforming growth factor-beta (TGF $\beta$ ) signaling pathways—two members of this signaling network—in the regulation of human B-cell non-Hodgkin lymphoma (B-NHL) cell survival, and to explore potential cross-talk between these two pathways. The pathogenetic importance of the Notch pathways has been well established in acute T-cell leukemia, however, its role in B-cell malignancies remains controversial. We showed that the mRNA expression pattern of Notch-receptors, -ligands and the Deltex regulator molecule is similar in normal B-cells and in circulating chronic lymphocytic leukaemia (CLL) cells. The expression of Hairy/Enhancer of Split-1 (HES-1) was generally higher in normal B-cells than in CLL. The expression level of the genes studied showed no correlation with clinical and prognostic parameters of CLL. Inhibition or -secretase inhibitor-GSI $\gamma$  ligand-induced activation of the Notch pathway (by a  $\gamma$ - and by Dll4 ligand, respectively) did not affect the rate of apoptosis in B-NHL cell lines *in vitro*. TGF $\beta$  generally inhibits proliferation and induces apoptosis in lymphoid tumors, however, tumor cells often lose their sensitivity to TGF $\beta$ . Interaction between the two signaling pathways has already been described in other cells types, therefore we hypothesized that such a cross-talk might exist in B-NHL cells, which may also modify TGF $\beta$ -sensitivity. Based on our results, GSI was able to decrease TGF $\beta$ -induced apoptosis in TGF $\beta$ -sensitive B-NHL cell lines; however, neither GSI, nor Dll4 restored TGF $\beta$ -sensitivity in TGF $\beta$ -resistant cell lines. HES-1 was shown to be a transcriptional target gene of TGF $\beta$  in TGF $\beta$ -sensitive cell lines. We showed for the first time that TGF $\beta$ -induced apoptosis can be independent of Smad4, which was previously believed to be a core molecule of the pathway. TGF $\beta$ -induced, Smad4-independent apoptosis required the inactivation of ERK1/2 and JNK MAP-kinases and the activation of PP2A phosphatase in B-lymphoma cells. These results suggest that Notch-signaling itself is not a central regulator of apoptosis in B-NHL cells, however, it may interact with TGF $\beta$ -signaling and modify its effects. Our data also warns that the clinical application of Notch-inhibitors needs to be preceded by detailed studies assessing their specific, cell-type dependent effects. In addition, the characterization of Smad4-dependent and -independent effects of TGF $\beta$  may lead to the identification of more efficient and specific targets in lymphoma therapy.

- Hajdu M, Sebestyén A, Barna G, Reiniger L, Jánosi J, Sréter L, Várkonyi J, Demeter J, Kopper L (2007) Activity of the notch-signalling pathway in circulating human chronic lymphocytic leukaemia cells. *Scand J Immunol* 65: 271–275.
- Sebestyén A\*, Hajdu M\*, Kis L, Barna G, Kopper L (2007) Smad4-independent, PP2A-dependent apoptotic effect of exogenous Transforming Growth Factor beta1 in lymphoma cells. *Exp Cell Res* 313: 3167–3174 (\*Authors contributed equally to this work).
- Hajdu M, Luttun A, Pelacho B, Burns CT, Chase L, Gutiérrez-Pérez M, Jiang Y, Lenvik T, Vas V, Uher F, Sebestyén A, Verfaillie C (2007) Transcriptional characterization of the notch signaling pathway in rodent multipotent adult progenitor cells. *Pathol Oncol Res* 13: 302–310.

PÉTER HOLLÓSI (2009)

**Implications of lysyl oxidase and lysyl oxidase-like 2 enzyme expression for epithelial and neuroepithelial tumor progression**

*Supervisor: Ilona Kovalszky*

Lysyl oxidases are copper-dependent amine oxidases responsible for covalent crosslinking of collagen and elastin molecules in the extracellular matrix. The role of lysyl oxidases in tumor progression has been shown by multiple studies. Both LOX and LOXL2, two members of the family, have been implicated in both tumor suppression and tumor promotion. The aim of our studies was to investigate roles of LOX and LOXL2 enzyme expression in certain tumor types of epithelial and neuroepithelial origin. For our studies we generated and characterized two polyclonal antibodies specifically recognizing LOX and LOXL2. Our results provide evidence that enzymatically active LOX is produced by astrocytes and shows increased expression in high grade astrocytomas. Elevated LOX activity contributes to increased astrocytic cell migration, facilitating focal adhesion formation by means of H<sub>2</sub>O<sub>2</sub> release. We also show that compared with normal colon tissue, LOXL2 shows increased expression in colon adenocarcinomas, where the positive correlation between LOXL2 expression and tumor grade is statistically significant. Similarly, compared to normal esophagus tissue, esophageal SSC tumors show increased LOXL2 expression. Loss of heterozygosity affecting the *lox12* locus can be detected in approximately one third of these two tumor types. Furthermore, we identified promoter hypermethylation and histone deacetylation as two epigenetic gene silencing mechanisms contributing to decreased LOXL2 expression in normal colon epithelial cells and low grade colon adenocarcinoma cells, compared to high grade colon adenocarcinoma cells. We also show experimental evidence proving that LOXL2 produced by mammary epithelial cells is catalytically active. Overexpression of LOXL2 in normal mammary epithelial cells and in noninvasive mammary adenocarcinoma cells causes loss of epithelial appearance and induces a more mesenchymal cell morphology; and stimulates cell migration but only in transformed mammary epithelial cells, not in normal mammary epithelial cells, possibly due to altered extracellular processing of LOXL2. The low level of LOXL2 expression, typical in non-invasive transformed mammary epithelial cells, may also be explained by epigenetic silencing mechanisms. In summary, our results help to clarify some of the controversy around the biological importance of lysyl oxidases in tumor progression.

- Hollosi P, Yakushiji J, Fong KS, Csiszar K, Fong SFT (2009) Lysyl oxidase-like 2 promotes migration in non-invasive breast cancer cells but not in normal breast epithelial cells. *Int J Cancer* 125: 318–327.
- Laczko R, Szauter KM, Jansen MK, Hollosi P, Molnar J, Muranyi M, Fong KS, Hinek A, Csiszar K (2007) Lysyl oxidase (LOX) promotes FAK/paxillin activation and migration in invasive astrocytes. *Neuropathol Appl Neurobiol* 33: 631–643.
- Fong SF, Dietzsch E, Fong KS, Hollosi P, Asuncion L, He Q, Parker MI, Csiszar K (2007) Lysyl oxidase-like 2 expression is increased in colon and esophageal tumors and associated with less differentiated colon tumors. *Gene Chromosome Canc* 46: 644–655.



**GERGELY HUSZTY (2009)****Aspects of immuno- and suicide gene therapies for cancer - a combination with radiation therapy***Supervisor: Géza Sáfrány*

Gene therapies may serve as adjuvant treatments against tumors. We studied potential immuno-gene and gene-directed enzyme pro-drug (GDEPT) therapy systems in rat models; and examined the presence of transferrin receptor in human pancreatic tumors. Systemic Flt3-ligand (FL) treatment leads to expansion of dendritic cells with antitumoral effect in animal models. We hypothesized, that intratumoral FL gene transfer would have effect on the antitumoral immune response and tumor growth in experimental DSL6A rat pancreatic cancers. The unknown rat FL cDNA was sequenced, and cloned into a plasmid. Transfection of s.c. growing tumors was augmented by cationic liposomes—10% transfection rate was achieved. While control tumors grew continuously, 6 times repeated injections of FL-coding plasmids resulted in shrinkage of tumors in half of the treated animals; total regression and tumor size stabilization could also be achieved in some cases. Most treated tumors regained proliferative activity after cessation of treatment. The therapy was accompanied by considerable increase in the expression of CD80 on splenic dendritic cells in some treated animals and increase in splenic NK cell number in therapy responders. The effect of therapy was limited; combinational strategies aiming to activate dendritic cells may be helpful. We present the first experimental attempt to enhance the radiosensitizing effect of the widely used chemotherapeutic agent gemcitabine by means of GDEPT. Both the cytotoxic and radiosensitizing effect of gemcitabine could be significantly improved by adenovirus mediated overexpression of the dCK enzyme in murine C6 and human U373 glioma cell lines. dCK overexpression in pre-transduced C6 gliomas significantly improved the survival rate of tumor bearing rats in response to chemoradiotherapy by enhancing the radiosensitizing effect of gemcitabine. After further *in vivo* studies in a therapeutic setting (local transfection), the dCK/gemcitabine GDEPT system might be a candidate of adjuvant gene-therapeutical protocols against tumors, where gemcitabine and radiation is already in clinical use—such as pancreatic cancer and gliomas. We found that malignant human ductal and neuroendocrine pancreatic tumor cells express considerable amount of transferrin receptor in most cases (90%), while healthy pancreatic tissue and benign tumors do not show expression by immunohistochemistry. This observation may have implication in the diagnosis and (vector-) targeting of these malignancies.

- Ryschich E, Huszty G, Wentzensen N, Schmidt E, Knaebel HP, Märten A, Büchler MW, Schmidt J (2007) Effect of Flt3-ligand-gene transfer in experimental pancreatic cancer. *Int J Colorectal Dis* 22: 215–223.
- Szatmári T, Huszty G, Désaknai S, Spasokoukotskaja T, Sasvári-Székely M, Staub M, Esik O, Sáfrány G, Lumniczky K (2008) Adenoviral vector transduction of the human deoxycytidine kinase gene enhances the cytotoxic and radiosensitizing effect of gemcitabine on experimental gliomas. *Cancer Gene Ther* 15: 154–164.
- Ryschich E, Huszty G, Knaebel HP, Hartel M, Büchler MW, Schmidt J (2004) Transferrin receptor is marker of malignant phenotype in human pancreatic cancer and in neuroendocrine carcinoma of the pancreas. *Eur J Cancer* 40: 1418–1422.



**ISTVÁN KENESSEY (2010)****The role of the motogenic signal in human melanoma cells**

*Supervisors: József Tímár, József Tóvári*

The components of the extracellular matrix (ECM) are more than just adhesion sites for migrating tumor cells: following enzymatic degradation of the ECM, the release of sequestered growth factors increases, thus become available for tumor cells. In a number of cancers dysfunction of the epidermal growth factor receptor (EGFR) or the hepatocyte growth factor receptor (c-Met) contribute to the malignant transformation that directly regulates cell proliferation, survival and motility. Furthermore, the intracellular calcium level plays an important role in the regulation of the tyrosine kinase pathway. In our preclinical experiments, by administrating heparin-derived oligosaccharides we influenced the interaction between human melanoma cells and ECM. The *in vitro* cell migration was inhibited by heparin-fragments. Moreover, two of the effective oligosaccharides reduced the number of lung colonies formed in SCID mice. In human melanoma cells an important element of  $\text{Ca}^{2+}$  homeostasis, the purinergic  $\text{Ca}^{2+}$  channel P2X7 proved to be an anti-apoptotic protein. EGFR and c-Met showed constitutive activity in human melanoma cells, and their inhibition *in vitro* caused decreased proliferation, migration and elevated apoptosis. Administration of a selective c-Met-TKI, significantly decreased primary tumor growth *in vivo* as well as the capacity for liver colony formation in SCID mice. Selective EGFR-TKI had less inhibitory effect on metastasis formation, and had no effect on the primary tumor. Our results suggest the necessity of a rational dual-specific drug design for the purpose in the therapy of malignant melanoma.

- Kenessey I, Simon E, Futosi K, Bereczky B, Kiss A, Erdődi F, Gallagher JT, Tímár J, Tóvári J (2009) Antimigratory and antimetastatic effect of heparin-derived 4-18 unit oligosaccharides in a preclinical human melanoma metastasis model. *Thromb Haemost* 102: 1265–1273.
- Deli T\*, Varga N\*, Ádám A\*, Kenessey I\*, Rásó E, Puskás LG, Tóvári J, Fodor J, Fehér M, Szigeti GP, Csernoch L, Tímár J (2007) Functional genomics of calcium channels in human melanoma cells. *Int J Cancer* 121: 55–65 (\*Equally contributed to this work).
- Harisi R, Kenessey I, Oláh JN, Tímár F, Babó I, Pogány G, Paku S, Jeney A (2009) Differential inhibition of single and cluster type tumor cell migration. *Anticancer Res* 29(8): 2981–2985.

**VIKTÓRIA LÁSZLÓ (2009)****The effect of primary mitogens on stem cell mediated regeneration of the rat liver**

*Supervisor: Sándor Paku*

The AAF/PH model is widely used to induce intrahepatic progenitor/oval cell proliferation in the rat liver. We used this model to study the impact of primary mitogens triiodothyronine (T3) and lead-nitrate on oval cell mediated liver regeneration. The administration of the mitogens caused the increase of the relative liver weight in 48 hours, due to accelerated proliferation and differentiation of the oval cells. 2 days after the mitogen treatment

(7 days after the PH) small polygonal basophilic hepatocytes appeared. The oval cell origin of these cells has been proven by two different methods. We labeled the DNA of the cells in S phase either with BrdU, or with a retroviral vector, which contained the gene of the  $\beta$ -galactosidase enzyme. The BrdU and the  $\beta$ -galactosidase could be detected in the newly formed small hepatocytes. The mechanism of the accelerated differentiation is the same as it has been described earlier in this model, and as the spontaneous differentiation of the oval cells that occurs on the 11th day after the PH in the untreated rats. The expression of the oval cell specific OV-6 and alpha-fetoprotein (AFP) is lost, while differentiating oval cells start to express hepatocyte specific markers like HNF-4, CYP450, cx 32,  $\alpha 1$  integrin. CD 26 bile canaliculi are formed between the newly differentiated small hepatocytes. The basal membrane, surrounding the oval cells is fragmented during the differentiation, it probably plays a regulative role. The expression of hepatocyte specific mRNAs e.g. albumin, tyrosine aminotransferase (TAT) and tryptophan 2, 3-dioxygenase (TO2) can be detected by real-time PCR in microdissected small hepatocytes. Liver function tests improved concurrently with the accelerated differentiation, bilirubin concentration decreased, while the prothrombine level increased. We conclude that administration of the primary hepatocyte mitogen T3 and lead-nitrate accelerate the differentiation of hepatic progenitor cells into hepatocytes *in vivo*. Since activation of the hepatic progenitor cell compartment is often described in the diseased human liver, the support of the differentiation process may have a therapeutic potential.

- Laszlo V, Dezso K, Baghy K, Papp V, Kovalszky I, Safrany G, Thorgeirsson SS, Nagy P, Paku S (2008) Triiodothyronine accelerates differentiation of rat liver progenitor cells into hepatocytes. *Histochem Cell Biol* 130: 1005–1014.
- Dezso K, Jelnes P, László V, Baghy K, Bodor C, Paku S, Tygstrup N, Bisgaard HC, Nagy P (2007) Thy-1 is expressed in hepatic myofibroblasts and not oval cells in stem cell-mediated liver regeneration. *Am J Pathol* 171: 1529–1537.
- Szabó E, Lódi C, Korpos E, Batmunkh E, Rottenberger Z, Deák F, Kiss I, Tóké AM, Lotz G, László V, Kiss A, Schaff Z, Nagy P (2007) Expression of matrilin-2 in oval cells during rat liver regeneration. *Matrix Biol* 26: 554–560.

## JUDIT MÜLLER (2009)

### Efficiency and pharmacogenetic aspects of treatment of pediatric malignancies

*Supervisor: Judit Kralovánszky*

In the last three decades the biggest leap forward in cancer treatment was in the improvement of therapy results of the pediatric malignancies both, in abroad and in Hungary. The aim of our work was on one hand to summarize the Hungarian data, to assess the survival rates and to compare them with the international results in four pediatric malignancies: LCH, NHL, EWS and ALL. The survival rates of Hungarian children with LCH were parallel, with ALL were similar, only lower with some %, and with NHL and EWS were lower, but approached the international results. In NHL the prevalence of death during primary chemotherapy was reduced from 10% to 3% presumably due to the better supportive treatment and earlier use of broad spectrum antibiotics and antimycotics. In NHL patients with residual tumor or relapse survival rates can be raised with high-dose chemotherapy followed by stem cell transplantation or with immuno-

therapy. We drew attention to the importance of early diagnosis and careful supportation after the myeloablative treatment in children suffering from EWS. We emphasized the importance to make efforts to reduce early deaths and lethal complications after achieved remission. In the treatment of pediatric tumors prevention and reduction of severe complications are important factors. During our molecular biological studies we performed a comprehensive evaluation of the marker value of MTHFR gene SNP's for HD-MTX toxicities in childhood OSC and ALL. Our genetic studies proved that genotyping of MTHFR gene before HD-MTX treatment in children suffering from OSC can help us to select patients with higher risk for toxic complications. Patients with 677 TT or 1298 AC+CC genotypes have higher risk for liver adverse effects, and lower risk for hematopoietic complications. In patients with ALL, evaluating MTHFR 677CT in a recessive setting showed higher liver toxicity probability in homozygous girls and in evaluation independently from sex. In boys with 1298CC genotype risk of hematotoxicity is raised. Further studies with high number of involved patients are needed for obvious verification of the role of MTHFR polymorphisms in risk of MTX caused toxicities.

- Müller J, Garami M, Hauser P, Schuler D, Csóka M, Kovács G, Rényi I, Kovács G, Marosi A, Galántai I, Békési A, Kajtár P, Kiss CS, Nagy K, Bartyik K, Masáth P, Kriván G (2006) Hungarian Pediatric Oncology Network: Hungarian experience with Langerhans cell histiocytosis in childhood. *Pediatr Hematol Oncol* 23: 135–142.
- Müller J, Csóka M, Jakab Zs, Ponyi A, Erlaky H, Garami M, Kovács G (2008) Hungarian Pediatric Oncology Network: treatment of pediatric non-Hodgkin's lymphoma in Hungary: 15 years experience with NHL-BFM 90 and 95 protocols. *Pediatr Blood Cancer* 50: 633–635.
- Müller J, Kralovánszky J, Adleff V, Pap É, Németh K, Komlósi V, Kovács G (2008) Toxic Encephalopathy and delayed MTX clearance after high-dose methotrexate therapy in a child homozygous for the MTHFR C677T polymorphism. *Anticancer Res* 28: 3051–3054.

## JUDIT PÁPAY (2009)

### Factors in progression of lung cancer

Supervisor: József Tímár

Lung cancer is the leading cause of tumor mortality worldwide. Most of the cases are in advanced stage at the time of diagnosis. The prognosis of patients is poor. Investigations which are far from each other in time and research methods, focused on the characteristic features of lung cancers, that may have a role in the connections between tumor cells and their environment, tumor cells and their metastatic potential and profiles with predictive value in the chemotherapeutic plan. *In vitro* and *in vivo* investigations of the human small cell lung cancer (SCLC-H69) cell line and its subclones demonstrated that the NCAM- $\alpha$ -2,8 linked polysialic acid reduces the cell to cell adhesion and increases the metastatic ability. The synthesis and intracellular transport of polysialic acid are associated with the Golgi complex: it is a *de novo* process in SCLC cells. The results of the investigations of protein expression profile of primary and brain metastatic non-small cell lung cancer groups concluded that the  $\beta$ -catenin is the only marker which has correlation with the patients' survival. The effect of platinum-based neoadjuvant chemotherapies on certain biomarkers, including apoptosis, cell proliferation, and DNA repair

mechanisms, was also investigated. The low patients' number could not allow us to compile statistics, but the results suggest that platinum-based chemotherapy has no influence on low activity level apoptosis, and it induces a selection of tumor cells with increased proliferating activity. Therapy-induced change in the expression—in our cases the loss of expression—of tissue marker ERCC1 (excision repair cross-complementation group 1), which plays a role in DNA repair, may have a predictive value in therapeutic response. The study comparing methods to detect different levels of EGFR expression provides evidence that tumor tissue groups positive to different EGFR diagnostics only partly overlap. Most importantly, lung cancers which do not overexpress EGFR protein, therefore present negative EGFR immunohistochemical reaction, can carry activating mutations and the patients can have excellent response to EGFR TKI therapy.

- Pápay J, Sápi Z, Egri G, Gyulai M, Szende B, Losonczy G, Tímár J, Moldvay J (2009) *Platinum-based chemotherapy in lung cancer affects the expression of certain biomarkers including ERCC1. Pathol Oncol Res 15: 445–450.*
- Papay J, Krenacs T, Moldvay J, Stelkovics E, Furak J, Molnar B, Kopper L (2007) *Immunophenotypic profiling of nonsmall cell lung cancer progression using the tissue microarray approach. Appl Immunohistochem Mol Morphol 15: 19–30.*
- Scheidegger EP, Lackie PM, Papay J, Roth J (1990) *In vitro and in vivo growth of clonal sublines of human small cell lung carcinoma is modulated by polysialic acid of the neural cell adhesion molecule. Lab Invest 70: 95–106.*

## ANDREA RÉTI (2010)

### Application of non-steroidal anti-inflammatory drugs to enhance 5-fluorouracil efficacy on experimental systems

*Supervisor: Judit Kralovánszky*

The elevated cyclooxygenase-2 (COX-2) expression has been shown to affect the carcinogenesis and tumor progression processes, including cell proliferation, motility and angiogenesis. COX-2 is overexpressed in approximately 80% of sporadic colorectal carcinomas and COX-2 enzyme is the best defined target of non-steroidal anti-inflammatory drugs (NSAIDs). In the chemotherapy of colorectal carcinomas 5-fluorouracil (5-FU) has been the most important of the basic drugs for more than 40 years. In order to improve the effectiveness of 5-FU therapy different biological modifiers i.e. inhibitors of its catabolism or activators of anabolism have been studied recently. The rate-limiting enzyme of 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD) since more than 80% of the administered 5-FU is catabolised by DPD. Tumoral DPD has become of clinical interest because elevated intratumoral DPD can decrease the tumor response to 5-FU therapy. The main purpose of our experiments was to investigate the effect of COX inhibitors on the efficacy of 5-FU on high and low COX-2 expressing HCA-7 and HT-29 human colon adenocarcinoma cell lines, respectively and also on xenografts derived from HT-29 cells. The cytotoxic and antitumor effects of 5-FU in the presence of low doses of indomethacin (non-selective COX-2 inhibitor) and that of NS-398 (highly selective COX-2 inhibitor) on the HT-29 and HCA-7 cells and also on the HT-29 xenograft were investigated. In addition our intention was to understand the mechanism(s) by which NSAIDs could enhance the cytotoxic effect of 5-FU. Our data indicated that, the

elevated COX-2 expressions of the HCA-7, the collagen-induced HT-29-C cells and of the HT-29 xenograft were associated with reduced 5-FU sensitivity. Based on the fact that at the same time the DPD activity was also increased it might be conceivable that a possible explanation for the decrease of 5-FU sensitivity is the co-existence of high COX-2 and DPD activity. Indomethacin or NS-398 enhanced in a simultaneous and significant manner the sensitivity and cytotoxic effect of 5-FU on high COX-2 expressing cells and xenografts through the modulation of DPD-decrease of its mRNA expression and/or enzyme activity. Based on our results it could be presumable that 5-FU efficacy is limited by the COX-2 associated high DPD expression and activity in patients with colorectal cancers as well, therefore further clinical studies are warranted to decide if NSAIDs in the therapeutic protocol might improve the antitumor potency of 5-FU.

- Réti A, Barna G, Pap E, Adleff V, L Komlósi V, Jeney A, Kralóvanszky J, Budai B (2009) *Enhancement of 5-fluorouracil efficacy on high COX-2 expressing HCA-7 cells by low dose indomethacin and NS-398 but not on low COX-2 expressing HT-29 cells. Pathol Oncol Res* 15: 335–344.
- Réti A, Pap E, Zalatnai A, Jeney A, Kralóvanszky J, Budai B (2009) *Co-inhibition of cyclooxygenase-2 and dihydropyrimidine dehydrogenase by non-steroidal anti-inflammatory drugs in tumor cells and xenografts. Anticancer Res* 29: 3095–3101.
- Réti A, Pap E, Adleff V, Jeney A, Kralóvanszky J, Budai B (2010) *Enhanced 5-fluorouracil cytotoxicity in high cyclooxygenase-2 expressing colorectal cancer cells and xenografts induced by non-steroidal anti-inflammatory drugs via downregulation of dihydropyrimidine dehydrogenase. Cancer Chemother Pharmacol* 66(2): 219–227.

## ERZSÉBET SZABÓ (2009)

### Expression of matrilin-2 during liver regeneration in an experimental model and in hepatocellular carcinoma

Supervisor: Zsuzsa Schaff

Hepatocellular carcinoma (HCC) is among the most common causes of cancer deaths worldwide. The malignant transformation of the hepatocytes is a longterm multistep process, in which the genetic alterations and molecular changes in the extracellular matrix (ECM) play role together in carcinogenesis and tumor progression. The recently described matrilin protein family is part of the ECM, their pathophysiological role as well as distribution in the liver have not yet been studied. Matrilin-2 displays a broad tissue distribution and has been detected in basement membranes of other tissues. Considering its role in cell growth and tissue remodeling we aimed to study the expression of matrilin-2 during liver regeneration, in normal human liver, HCC and surrounding cirrhotic liver. Liver regeneration was induced in rats by partial hepatectomy (PH) and 2-acetylaminofluorene (AAF)/partial hepatectomy (PH) in experimental models. A total of 35 cases of surgically resected HCCs, 35 corresponding surrounding liver tissues and 10 normal liver samples were used for the study of human tissues. The expression of matrilin-2 was analyzed both at mRNA and protein levels. Matrilin-2 was detected in normal rat liver and partially hepatectomized liver in the portal area, but could not be demonstrated in the acini. In the AAF/PH model the oval cells but not the hepatocytes produced matrilin-2 mRNA. Increase in protein level in the AAF/PH regenerating liver



model was demonstrated by Western blotting. The protein was present in the basement membrane zone around the tubules formed by oval cells. In normal human liver matrilin-2 expression was detected in normal liver, portal blood vessels, while sinusoids were negative. Cirrhotic surrounding tissues of HCCs showed intensive matrilin-2 staining along the sinusoids. Tumorous neovasculature was found to be strongly positive in HCCs. No differences, however, were detected by morphometry based on the grade of HCC. Taken together, our data show that among mesenchymal cells hepatic oval cells produce matrilin-2, a novel ECM protein which appears during capillarization in liver cirrhosis and hepatocarcinogenesis. Based on our findings, it is suggested that matrilin-2 is one of the important components of ECM during stem cell-driven liver regeneration and is also synthesised during the remodeling of the cirrhotic ECM and tumor stroma. Matrilin-2 as putative adaptor protein might help other molecules for proper ECM assembly during the reorganization of matrix.

- Szabó E, Lódi C, Korpos E, Batmunkh E, Rottenberger Z, Deák F, Kiss I, Tóké AM, Lotz G, László V, Kiss A, Schaff Z, Nagy P (2007) Expression of matrilin-2 in oval cells during rat liver regeneration. *Matrix Biol* 26: 554–560.
- Szabó E, Korpos É, Batmunkh E, Lotz G, Holczbauer Á, Kovalszky I, Deák F, Kiss I, Schaff Zs, Kiss A (2008) Expression of matrilin-2 in liver cirrhosis and hepatocellular carcinoma. *Pathol Oncol Res* 14: 15–22.
- Szabo E, Lotz G, Paska C, Kiss A, Schaff Z (2003) Viral hepatitis: new data on hepatitis C infection. *Pathol Oncol Res* 9: 215–221.
- Szabó E, Páska C, Kaposi Novák P, Schaff Z, Kiss A (2004) Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis. *Pathol Oncol Res* 10: 5–11.

## TIBOR SZARVAS (2009)

### The diagnostic value of microsatellite LOH analysis and the prognostic relevance of angiogenic gene expression in urinary bladder cancer

*Supervisor: Ilona Kovalszky*

The present work tries to answer some current diagnostic and prognostic questions of urinary bladder cancer using new molecular methods. In the diagnostic applications, we focused on detection of DNA alterations in human urine samples. We installed the UroVysion FISH method and microsatellite deletion analysis and defined their specificity and sensitivity in our laboratory conditions. The analysis of cell-free DNA of urine supernatant provided higher sensitivity as urine sediment. By performing a genome wide screening by a set of 400 microsatellite markers (mapped on all chromosomes) we were able to reduce the minimal number of microsatellite primers to 4 with a sensitivity of 100%. Furthermore we identified chromosomal regions deleted only in muscle-invasive or in high grade tumors. The analysis of these regions would have an importance in cases with unclear results of histological examination. Based on their molecular characteristics, future behavior of tumors become more and more predictive. The prognostic value of angiogenesis was confirmed by both morphologic and molecular biologic evidence. We identified tissue mRNA expressions and serum protein concentrations of five angiogenic factors critically involved in normal and pathological angiogenesis. To explore these markers clinical relevance, their expression data was compared with a long



follow-up period. We found a characteristic “angiogenic switch” in gene expression pattern with strong down-regulation of Ang-1 and concurrent up-regulation of Ang-2 and VEGF expression in bladder tumor stage pTa, a superficial non-invasive tumor stage. This shift is probably a main driving force of vascular destabilization and initiation of angiogenesis in bladder cancer. Remarkably, this switch is less pronounced in later stages of bladder cancer. We identified Ang-2 and VEGF as an independent predictor of disease recurrence and demonstrated the predictive potential of Tie2 expression for bladder cancer metastasis and disease-specific survival. Serum protein levels of the same factors were analyzed by sandwich ELISA. These experiments identified high serum concentrations of extracellular domain of Tie2 as a significant risk factor for bladder cancer metastasis. Based on these we concluded that Tie2 plays an important role in preventing bladder cancer metastasis. The detailed results above show that the expressions of angiogenic factors do have a significant impact on patients’ survival in bladder cancer. This indicates the need for new antiangiogenic therapy modalities in this disease. The advances in this field lay claim to markers to measuring the biological effects of targeted agents and to identifying patients most likely to benefit from treatment.

- Szarvas T, Kovalszky I, Bedi K, Szendrői A, Majoros A, Riesz P, Füle T, László V, Kiss A, Romics I (2007) Deletion analysis of tumor and urinary DNA to detect bladder cancer: urine supernatant versus urine sediment. *Oncol Rep* 18: 405–409.
- Szarvas T, Jäger T, Tötsch M, vom Dorp F, Kempkensteffen C, Kovalszky I, Romics I, Ergün S, Rübber H (2008) Angiogenetic switch of angiopoietins-Tie2-system and its prognostic value in bladder cancer. *Clin Canc Res* 14: 8253–8262.
- Szarvas T, Jäger T, Droste F, Becker M, Kovalszky I, Romics I, Ergün S, Rübber H (2009) Serum levels of angiogenic factors and their prognostic relevance in bladder cancer. *Pathol Oncol Res* 15: 193–201.

## TÜNDE SZATMÁRI (2009)

### Improving the radiosensitivity of brain tumors by gene-directed enzyme prodrug therapy

Supervisor: Géza Sáfrány

The efficacy of radiotherapy in glioma patients is limited by the radioresistance of the tumor cells. Therefore there is an urgent need for new therapeutic approaches in the treatment of these tumors and for tumor models to elaborate the effects of the new treatment modalities. In the first part of this work a few important biological characteristics of the murine GL261 glioma model were presented. We determined that the GL261 murine glioma cells harbor the main growth characteristics of most glioma models. GL261 cells can be efficiently transduced with adenovirus vectors. The genes introduced in the cells by adenoviral vectors have an increased expression for several days. GL261 cells have an elevated basal MHC I expression compared to that of the healthy brain tissue, and also express low levels of B7-1 and B7-2 RNA. The only cytokine that modified MHC I and MHC II expression was IFN- $\gamma$ . GL261 is a moderately immunogenic tumor model: prevaccination of mice with irradiated GL261 cells 7 days before intracranial tumor transplantation protects the animals from tumor outgrowth. In the second part of the work we studied the efficacy of a radiosensitizing genetherapy on the GL261 cells

and in two other well-characterized models: the rat C6 and human U373 models. The objective was to increase the cytotoxic and radiosensitizing effects of the prodrug gemcitabine by using a gene directed enzyme prodrug therapy approach. The glioma cell lines were transduced with an adenoviral vector encoding the deoxycytidine kinase (dCK) enzyme. Intracranial tumors were established either in C57Bl/6 mice or in Wistar rats using either wild-type or dCK over-expressing GL261 or C6 tumor cells. Both *in vitro* growing cells and tumor-bearing animals were treated with gemcitabine, irradiation, or with their combination. dCK overexpression enhanced the toxic and radiosensitizing effect of gemcitabine in all three cell lines, but the enhancement rate varied. *In vivo* experiments showed a moderate radiosensitizing effect of dCK overexpression both in the GL261 and C6 models. The combination of dCK overexpression, gemcitabine treatment and irradiation improved the survival rate of C6 bearing rats significantly. In conclusion, the GL261 brain tumor model might be efficiently used to study the antitumor effects of various therapeutic modalities but the moderate immunogenicity of the cells should be considered. Overexpression of the dCK gene can improve the cytotoxic and radiosensitizing effect of gemcitabine both *in vitro* and *in vivo* in a tumor-specific manner.

- Szatmari T, Lumniczky K, Desaknai S, Trajcevski S, Hidvegi EJ, Hamada H, Safrany G (2006) Detailed characterization of the mouse glioma 261 tumor model for experimental glioblastoma therapy. *Cancer Sci* 97: 546–553.
- Szatmari T, Huszty G, Desaknai S, Spasokoukotskaja T, Sasvari-Szekely M, Staub M, Esik O, Safrany G, Lumniczky K (2008) Adenoviral vector transduction of the human deoxycytidine kinase gene enhances the cytotoxic and radiosensitizing effect of gemcitabine on experimental gliomas. *Cancer Gene Ther* 15: 154–164.

## ***PROGRAM 8/2.***

### **ALTERATIONS OF CELLS, FIBRES AND EXTRACELLULAR MATRIX. DIAGNOSTIC PATHOMORPHOLOGICAL STUDIES IN THE COURSE OF HEART AND VASCULAR DISEASES AND IN CERTAIN TUMOURS. EXPERIMENTAL AND DIAGNOSTIC PATHOMORPHOLOGICAL STUDIES**

#### ***Coordinator:***

**Zsuzsa SCHAFF M.D.**

**member of the Hungarian Academy of Sciences**

2nd Department of Pathology

93 Üllői st, Budapest H-1091

Tel.: +36 1 215 0815; fax: +36 1 215 6921

E-mail: [schaff.zsuzsa@med.semmelweis-univ.hu](mailto:schaff.zsuzsa@med.semmelweis-univ.hu)

#### **Program overview**

Leading causes of morbidity and mortality in Hungary are the cardiovascular, gastrointestinal, hepatic, pancreatic diseases and malignant tumors. The Program offers a multi faceted analysis of the above diseases, completed with the development of liver diseases including liver, breast and pancreatic tumors. The research studies apply various patho-

histological approaches with the extension of clinical retrospective and prospective studies. The project is dealing mostly with human materials, though experimental models are also induced. Several modern molecular pathological methods have been introduced in the well equipped laboratories. Recent studies focuses on the significance of microRNAs in chronic inflammations and human tumors and alterations of cell adhesion molecules during carcinogenesis.

***Titles of research projects***

Prognostic factors and locoregional staging  
Molecular genetic analysis of adhesion proteins  
Pathology of vascular network  
Exogenous restrictive pulmonary diseases  
Adhesion proteins and cell-connecting structures in viral hepatitis  
Claudins in breast cancer  
Prognostic factors in breast cancer  
Effect of hepatotrop viruses and other hepatotoxins on the metabolism of liver cells and related disorders  
Factors in the development of liver diseases  
Role of extracellular matrix in chronic liver diseases  
Diagnostic and pathophysiological significance of alterations of cell surface and extracellular matrix molecules ingastrointestinal tumors

***Supervisors***

Gábor Cserni  
András Falus  
Anna Kádár  
Tibor Kerényi  
András Kiss  
Janina Kulka  
Janina Kulka  
Gábor Lotz

Zsuzsa Schaff  
Zsuzsa Schaff  
Péter Tátrai

***Ph. D. students***

Zsuzsanna Baranyák	pt
Mónika Nóra Erős	pt
Mónika Gyugos	ft
Anna Korompay	ft
Borbála Székely	ft
Péter Törzsök	ft

***Supervisors***

Janina Kulka  
Janina Kulka  
Zsuzsa Schaff  
Zsuzsa Schaff  
Janina Kulka  
András Kiss

***Ph. D. candidates***

Attila Patonai	ft
Áron Somorác	ft
Attila Marcell Szász	ft
Eszter Judit Székely	na

***Supervisors***

Zsuzsa Schaff  
András Kiss  
Janina Kulka  
József Tímár

***Ph. D. graduates***

Batmunkh Enkhjargal	it
Rita Bori	na
Katalin Borka	na
Hajnalka Gyórfy	na
Judit Halász	na
Anikó Keller Pintér	ft
Csaba Lódi	ft
Júlia Irma Németh	na
Zsuzsanna Németh	ft

***Supervisors***

András Kiss  
Gábor Cserni  
Zsuzsa Schaff  
András Kiss  
Zsuzsa Schaff  
Janina Kulka  
András Kiss  
Zsuzsa Schaff  
András Kiss

ft, full-time; pt, part-time; it, international; na, not affiliated

## ***Abstracts of Ph.D. theses successfully defended in 2009 and 2010***

### **BATMUNKH ENKHJARGAL (2009)**

#### **Expression of agrin in primary liver tumors**

*Supervisor: András Kiss*

Heparan sulphate proteoglycans mediate cell adhesion and control the activities of numerous growth and motility factors. They play a critical role in carcinogenesis and tumour progression. Agrin is a large, multidomain heparan sulphate proteoglycan which is associated with basement membranes of several tissues. Recently, the tissue expression of agrin was described in several organs, including the liver, under physiological conditions. So far, little is known about its role in malignancies, especially in primary liver tumours. Our goal, therefore, was to investigate mRNA and protein expression of agrin in primary liver tumors (HCC, CC, HCA and FNH) to analyze its involvement in tumorigenesis. The mRNA expression was measured on 52 surgically resected, RNA later fixed samples by real-time PCR method. Agrin immunostaining was carried out on 114 formalin fixed, paraffin-embedded surgical biopsy specimens (35 CC, 35 HCC, 14 HCA, 20 FNH, 10 normal liver). Western blot analysis on representative samples was also performed. Immunohistochemistry showed mild positivity around the bile ducts and the blood vessels within the portal area in normal liver, however, no expression within the hepatic lobules was found. HCC presented intense expression along the neovascular basement membrane. CC samples showed increased mRNA expression of agrin in comparison to both normal liver and HCC. Intense protein expression of agrin was observed in the tumor-specific basement membrane in well differentiated areas of CCs, which staining was fragmented, decreased or even disappeared in less differentiated fields and sites of infiltration. Western blot analysis confirmed the presence of increased agrin expression. In HCA, agrin expression was variable within the tumors. In the some fields agrin showed strong expression along the sinusoids, while elsewhere it was negative. In FNH intensive agrin expression was detected in the basement membrane of the proliferated bile ducts, while no agrin expression was observed along the sinusoids. Agrin may play an important role in neoangiogenesis in human HCCs and HCAs, as part of the newly formed vasculature. In CCs, agrin is also thought to play an important role in tumour progression.

- Batmunkh E, Tátrai P, Szabó E, Lódi C, Holczbauer A, Páska C, Kupcsulik P, Kiss A, Schaff Z, Kovalszky I (2007) Comparison of the expression of agrin, a basement membrane heparan sulfate proteoglycan, in cholangiocarcinoma and hepatocellular carcinoma. *Human Pathol* 38: 1508–1515.
- Tátrai P, Dudás J, Batmunkh E, Máthé M, Zalatnai A, Schaff Z, Ramadori G, Kovalszky I (2006) Agrin, a novel basement membrane component in human and rat liver, accumulates in cirrhosis and hepatocellular carcinoma. *Lab Invest* 86: 1149–1160.
- Szijártó A\*, Batmunkh E\*, Hahn O, Mihály Z, Kreiss A, Kiss A, Lotz G, Schaff Z, Váli L, Blázovics A, Geró D, Szabó C, Kupcsulik P (2007) Effect of PJ-34 Parp-inhibitor on rat liver microcirculation and antioxidant status. *J Surg Res* 142: 72–80 (\*Equally contributed to this work).

**RITA BORI (2010)****Examination of conventional staging and prognostic factors in colorectal cancer***Supervisor: Gábor Cserni*

Colorectal cancer (CRC) is a common disease and represents a major health issue worldwide, including Hungary. The natural course of malignant diseases is influenced by a number of so called prognostic factors, and these have a bearing on their treatment, too. The aim of the presented studies was to evaluate some relevant aspects of classical prognostic factors, especially nodal status and the depth of invasion in CRC. Metastatic involvement of the regional lymph nodes is one of the main prognosticators of CRCs. Our data suggest that the histological evaluation of fewer than 6 nodes is associated with a measurable risk of false negative nodal staging, whereas the examination of a minimum of 16 lymph nodes can result in an adequate staging in most cases. Not only the number of lymph nodes assessed, but also some of their qualitative features may be important in recognizing metastatic nodes. Qualitative features which could help in selecting among regional lymph nodes were also looked for. By assessing the fat along the resected bowel length in a segmental manner, it was found that most lymph nodes can be found in the close proximity of the tumor, and the number of nodes that can be recovered decrease with the distance from the tumor. Metastatic nodes are also more likely to be found beneath the tumor or very close to it. The validity of the sentinel node theory was also assessed in CRCs. The status of vital blue dye labelled sentinel nodes was compared to the status of the remainder of the lymph nodes. The false-negative rate of sentinel nodes was high, suggesting that the identification of sentinel nodes in CRCs is less than optimal, which may limit the use of lymphatic mapping in this disease. The size of the largest metastasis and its correlation with the involvement of other regional nodes was also investigated. The larger the largest metastasis is, the greater the frequency of massive metastatic load to the regional lymph nodes. These data indirectly support a stepwise lymphogenic metastatic process and the sentinel node theory in CRC. The depth of invasion is also a major prognostic factor in CRC. Its correlation with other prognosticators (nodal involvement, massive nodal involvement, venous invasion, distant metastases) was proven in this study. The pT3 tumors can be divided into two prognostically distinct subgroups on the basis of the depth of invasion.

- Cserni G, Tarján M, Bori R (2001) Distance of lymph nodes from the tumor, an important feature in colorectal cancer specimens. *Arch Pathol Lab Med* 125: 246–249.
- Bori R, Vinh-Hung V, Vajda K, Svebis M, Cserni G (2007) The impact of the largest metastasis size on nodal tumor burden in colorectal carcinomas: implications for the sentinel lymph node theory in cancers of the large intestine. *J Surg Oncol* 95: 629–634.
- Bori R, Sejben I, Svébis M, Vajda K, Markó L, Pajkos G, Cserni G (2009) Heterogeneity of pT3 colorectal carcinomas according to the depth of invasion. *Pathol Oncol Res* 15: 527–532.

## KATALIN BORKA (2009)

### Claudin expression in different pancreatic cancers and its significance in differential diagnostics

Supervisor: Zsuzsa Schaff

Claudins (CLDNs) are essential proteins of tight junctions. Changes in their expression pattern have been demonstrated in a number of tumors. CLDNs-3 and -4 are receptors of the *Clostridium perfringens* enterotoxin, cytolytic effects of the toxin are well known. The aim of our studies was to compare the different CLDN expression patterns in normal pancreas cells, pancreatic endocrine tumors, adenocarcinomas, mucinous cystic tumors and acinar cell carcinomas. Expressions of CLDN-1, -2, -3, -4 and -7 proteins were examined using immunohistochemical as well as RT-PCR techniques and the following observations were taken:

(1) In addition to the well-known CLDN-1 and -4 expression CLDN-2, -3 and -7 proteins were demonstrated in ductal cells, CLDN-3 and -7 proteins showed expression in acinar cells. Expression of CLDNs-3 and -7 was manifest in endocrine cells.

(2) CLDN-3 and -7 proteins showed high expression in endocrine tumors, CLDN-1, -2, and -4 proteins in exocrine tumors. This is the first description of CLDN protein expression in endocrine tumors.

(3) The level of CLDN-1, -4 and -7 protein expressions in borderline cystic tumors is in-between that of benign and malignant tumors. This supports the sequential development theory regarding mucinous cystic tumors.

(4) This is a first review on childhood acinar cell carcinoma causing Cushing syndrome. According to our results the following conclusions are made:

(1) The presence of CLDN-3 refers to endocrine differentiation. The adenocarcinomas and cystic mucinous tumors of exocrine origin denoted CLDN-1, -2, -4 and -7 positivity, whereas acinar cell carcinomas expressed only CLDN-1 and -2 positivity. Considering the CLDN expression observed in normal pancreas cells, it can be established that CLDN-1, -2 and -4 proteins are definitely markers of ductal differentiation, CLDN-1 protein of acinar and CLDN-3 of endocrine differentiation.

(2) The increased CLDN-4 expression in adenocarcinomas and mucinous cystic tumors, as well as the high CLDN-3 expression in endocrine tumors may open up new prospects in the targeted therapy of these tumours.

(3) The claudin expression pattern of pancreas tumors may be employed in the differential diagnosis of these tumors and may be of help in deciding dignity.

- Borka K, Kaliszky P, Szabó E, Lotz G, Kupcsulik P, Schaff Z, Kiss A (2007) Claudin expression in pancreatic endocrine tumors as compared with ductal adenocarcinomas. *Virchows Arch* 450: 549–557.
- Illyés G, Luczay A, Benyó G, Kálmán A, Borka K, Köves K, Rácz K, Tulassay T, Schaff Z (2007) Cushing's syndrome in a child with pancreatic acinar cell carcinoma. *Endocr Pathol* 18: 95–102.



**HAJNALKA GYÓRFFY (2009)****Claudins and prognostic factors in certain gastrointestinal diseases***Supervisor: András Kiss*

Gastrointestinal tumours are highly ranked regarding tumoral mortality worldwide. The development and progression of gastrointestinal (GI) diseases go hand in hand with the changes of tight junctions (TJ). Claudins (CLDN) are the main TJ proteins, showing different expression by the different tissues, with the expressed CLDN profile being representative.

(1) We explored the changes of CLDN expression in Barrett's esophagus and related adenocarcinoma. CLDN2 and -3 expression in Barrett's esophagus was higher than in normal foveolar epithelium. Adenocarcinoma showed higher CLDN2 and -3 expression compared with normal and Barrett's epithelia. The similar CLDN expression profile of Barrett's esophagus and adenocarcinoma supports their sequential development.

(2) Gastric intestinal metaplasia showed higher expression of CLDN2, -3 and -4 as compared with normal antral foveolar mucosa. Tumours of small and large bowels exhibited higher CLDN2 expression when compared with normal epithelia. Colorectal adenoma and adenocarcinoma could not be differentiated according to their CLDN profile. Intestinal metaplasias of Barrett's esophagus and stomach show similar CLDN profile to small bowel epithelium.

(3) Studies on duodenal mucosa in celiac disease in childhood demonstrated CLDN2 and -3 expression to be higher than in normal mucosa. The expression was significantly higher in the distal part of the duodenum samples. This and the serious histological findings suggesting that, the distal duodenum is more adequate for biopsy testing.

(4) Beside the epithelial cells, mesenchymal tumours express intercellular junctional proteins. Expression of claudins in gastrointestinal stromal tumours (GIST) and other mesenchymal neoplasia was also studied. The CLDN profile was found to be representative to the individual tumour. GIST, angiosarcoma, haemangioma, leiomyosarcoma and leiomyoma showed expression of various CLDNs. CLDN2 was detected in all entities. CLDN1, however, was found positive in leiomyosarcoma only. Leiomyoma, on the other hand, expressed only CLDN2. GISTs and leiomyosarcomas showed CLDN2, 3, 4, 5, and 7 expression. The angiogenic tumors revealed CLDN2 and 5 expression. The similar CLDN profile observable in GIST and leiomyosarcoma is suggestive of an histogenetic relationship. Smooth muscle and vessel tumours of different dignity could also be separated from each other based on CLDN profile.

- Gyórfy H\*, Holczbauer Á\*, Nagy P, Szabó Zs, Kupcsulik P, Páska Cs, Papp J, Schaff Zs, Kiss A (2005) *Claudin expression in Barrett's esophagus and adenocarcinoma. Virchows Arch* 447: 961–968 (\*Equally contributed to this work).
- Nagy Szakál D, Gyórfy H, Tőkés AM, Arató A, Dezsőfi A, Veres G (2008) *Claudin 2, 3 és 4 expressziója coeliakiás gyermekek proximális és disztális duodenumában. Gyermekgyógyászat* 59: 272–276.
- Miettinen M, Kopczynski J, Markhlouf HR, Sarlomo-Rikala M, Gyórfy H, Burke A, Sobin L, Lasota J (2003) *Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the duodenum: a clinicopathologic, immunohistochemical, and molecular genetic study of 167 cases. Am J Surg Pathol* 27: 625–641.

## JUDIT HALÁSZ (2010)

### New differentiation and differential diagnostic markers in human hepatoblastomas

Supervisor: Zsuzsa Schaff

Hepatoblastoma (HB) is the most frequent malignant primary liver tumor in infancy and early childhood, with unknown etiology and mechanism of development. Claudins (CLDNs) are integral proteins of the cell junction structures, the tight junction and changes in the expression pattern of these proteins can be kept track of during the carcinogenetic process. Dlk-1 is a protein expressed in the early organogenetic, fetal phase of the liver. Our aim was to study the expression of CLDN-1, -2, -3, -4, -7 and dlk-1 in the epithelial (fetal and embryonal) components of human hepatoblastomas (HBs), using immunohistochemical and RT-PCR techniques. **Results and conclusions:** (1) We were the first to describe the expression of CLDNs in human HBs. In the epithelial HB components CLDN-1 and -2 were found to show significant expression, while CLDN-3, -4 and -7 showed only slight positivity. (2) CLDN-1 and -2 were markedly expressed in the fetal components as compared with the embryonal components, in which case negative expression was found (disregarding sporadical positivity). Similar results were obtained by Real Time RT-PCR analyses. These findings refer to the fact that CLDN-1 and -2 proteins appear to be the markers of differentiation in HBs. (3) Higher tissue differentiation is accompanied by enhanced CLDN-1, -2 expression and lower proliferative ability in HBs. (4) The nuclear accumulation of  $\beta$ -catenin was demonstrated in almost all our studied HB cases (14/13), the distribution of which did not show correlation with histological subtype.  $\beta$ -catenin gene point mutation was proved in certain cases in both the fetal and embryonal components. These findings refer to the fact that damage to the Wnt/ $\beta$ -catenin signalling plays role in the pathogenesis of HBs regardless of histological subtype. (5) All our HB cases expressed the dlk-1 protein. Distribution of the expression did not correlate with the histological subtype. All the studied typical hepatocellular carcinoma (HCC) cases as well as all the other tumor cases showed dlk-1 negativity. Based on the above, we proved that the dlk-1 protein is suitable for differentiation between HB and first of all HCC. The expression of this protein can therefore be of value in the differential diagnostics of childhood malignant hepatocellular neoplasms.

- Halász J, Holczbauer A, Páska C, Kovács M, Benyó G, Verebély T, Schaff Z, Kiss A (2006) *Claudin-1 and claudin-2 differentiate fetal and embryonal components in human hepatoblastoma. Hum Pathol 5: 555–561.*
- Dezső K, Halász J, Bisgaard HC, Paku S, Turányi E, Schaff Z, Nagy P (2008) *Delta-like protein (DLK) is a novel immunohistochemical marker for human hepatoblastomas. Virchows Arch 4: 443–448.*
- Jakab Cs, Halász J, Kiss A, Szász A. M, Schaff Zs, Rusvai M, Kulka J (2008) *Külső pozitív kontrollok alkalmazása claudin-expressziós immunhisztokémiai vizsgálatokban. Magyar Állatorv Lapja 130: 433–438.*

**ANIKÓ KELLER-PINTÉR (2010)****Investigation of molecular mechanisms playing role in the progression of breast cancers—the complex role of syndecan-4 in MCF-7 cells***Supervisor: Janina Kulka*

Type I transmembrane proteoglycan syndecan-4 has several roles in the cells. It contributes to the formation of acidic sugar surface of the cells, participates in the signal transduction and coordinates the intracellular distribution of certain proteins. Our aim was to investigate the multiple biological functions of syndecan-4 in MCF-7 breast adenocarcinoma cells. We generated different mutant syndecan-4 constructs, which were expressed and analyzed by biochemical and cytochemical methods. According to our results, syndecan-4 regulates the activity of the small GTP-ase Rac1, the phosphorylation of Ser179 syndecan-4 reduced the Rac1 activity restricting the interaction between the Rac1 activator guanine nucleotide exchange factor Tiam-1 and Rac1. Syndecan-4 regulates the cell-matrix and cell-cell adhesions; therefore, it can induce the migration of quiescent cells or enhance the epithelial polarization of MCF-7 cells depending on the phosphorylation state of Ser179. The phosphorylation of Ser179 and the shedding of the ectodomain fluctuate during the cell cycle, both elevated during G2/M phases and decreased after the mitosis. Prerequisite of the ectodomain shedding is the phosphorylation of Ser179. The phosphorylation of Ser179 influences the cytokinesis: the phospho-resistant Ser179Ala mutation increases the number of multinucleated cells, whilst the phospho-mimetic Ser179Glu mutation leads to delay of the abscission. During the endocytosis, syndecan-4 can reach the perinuclear region through the cis- and trans-Golgi networks by retrograde transport, and can enter the nucleus. The phosphorylation of Ser179 induces the formation of nuclear localization signal, the unphosphorylated form cannot accumulate in the nucleus. Our results raised the possibility of the participation of phospho-Ser179 syndecan-4 in the formation and progression of tumors, and therefore it can have a potential prognostic value beside the routinely used prognostic markers (e. g. HER-2) in breast carcinomas.

- Keller-Pinter A, Bottka S, Timar J, Kulka J, Katona R, Dux L, Deak F, Szilak L (2010) Syndecan-4 promotes cytokinesis in a phosphorylation-dependent manner. *Cell Mol Life Sci* 67: 1881–1894.
- Letoha T, Keller-Pintér A, Kusz E, Kolozsi C, Bozsó Z, Tóth G, Vizler C, Oláh Z, Szilák L (2010) Cell-penetrating peptide exploited syndecans. *Biochim Biophys Acta Biomembr* 1798: 2258–2265.
- Kulka J, Tőkés AM, Kaposi-Novák P, Udvarhelyi N, Keller A, Lotz G, Schaff Z (2006) Detection of HER-2/neu gene amplification in breast carcinomas using quantitative real-time PCR—a comparison with immunohistochemical and FISH results. *Path Oncol Res* 12: 197–204.

**CSABA LÓDI (2010)****Examination of the expression pattern of different claudins in human biliary tract cancers, hepatocellular carcinomas and rat liver regeneration model***Supervisor: András Kiss*

The connection of the adjacent epithelial and endothelial cells occurs via different intercellular structures. Tight junctions, the most apical part of the intercellular junctional complex recognizable as a network of anastomosing strands, are crucial structures controlling paracellular permeability and maintaining cell polarity. The claudin superfamily proteins consisting of at least 24 members play a central role in modulating tight junction functions. Beside the fact, that several human diseases, such as psoriasis and collagen colitis are believed to be caused by the down-regulation of claudins, these proteins may provide unique functions during embryogenesis, differentiation and carcinogenesis, too. Knowing that cell polarity and permeability, regulation of solutes, ions and macromolecules are especially important in the liver function suggests an important role of these molecules in tissue remodelling and liver regeneration. Based on the above, the aim of our study was to analyse the expression and pattern of distribution of claudins in normal human liver and biliary epithelium; in biliary cancers and hepatocellular carcinomas; and during experimental liver regeneration. The surgically removed normal extrahepatic and intrahepatic biliary epithelium usually showed weak but distinct claudin-4 expression. We clearly demonstrated a strong expression of claudin-4 in biliary cancers at both protein and mRNA levels. Strong claudin-4 expression in biliary cancers arising from different localisations irrespectively of the grade of differentiation suggests that claudin-4 protein could well become a useful marker of extra- and intrahepatic biliary cancers. None of the hepatocellular carcinomas showed positive immunostaining for claudin-4. Our study reported an increased expression of the studied claudins on proliferating oval cells during experimental liver regeneration and proved the difference between oval cells, hepatocytes and cholangiocytes in claudin expression pattern emphasizing the importance and dynamic nature of these proteins in liver regeneration.

- Lódi C, Szabó E, Holczbauer A, Batmunkh E, Szíjártó A, Kupcsulik P, Kovalszky I, Paku S, Illyés G, Kiss A, Schaff Z (2006) Claudin-4 differentiates biliary tract cancers from hepatocellular carcinomas. *Mod Pathol* 19: 460–469.
- Lódi C, Szabó E, Kiss A, Nagy P, Paku S, Schaff Z (in prep) Hepatic oval cells are characterized by expression of claudins in the rat.

JÚLIA IRMA NÉMETH (2010)

**Claudin expression of endometrial and thyroid carcinomas: may we speak of a common marker of the papillary tumors of these organs?**

*Supervisor: Zsuzsa Schaff*

The role and altered expression of claudins, the main tight junction proteins, are investigated in the tumorigenesis, clinical behavior and progression of many epithelial tumors. According to the dualistic model, there are supposedly two major pathomechanisms in the background of development of endometrial carcinoma; leading to the estrogen-related, endometrioid carcinoma of better prognosis (type I), as well as to the nonestrogen-related, serous papillary carcinoma of aggressive behavior (type II). In our study we searched for answers to whether the expression pattern of claudins reflects the supposed different pathogenetic background of endometrial cancers. Further, whether different claudin expression is characteristic to serous papillary carcinoma of clinically aggressive behavior. Studies on our endometrial samples gave results that raised the question whether the increased claudin-1 expression is also characteristic to the papillary tumors of other organs. The prognostically good papillary thyroid cancers compose the large majority of thyroid follicular epithelial cell-derived tumors. These thyroid tumors have different molecular pathways like the endometrial carcinomas. According to our results the following conclusions could be drawn: (1) The proved, different claudin-1, -2 protein expressions in our endometrial samples support the dualistic model theory in endometrial cancers. Claudins-1, 2 can sharply separate the endometrioid and serous papillary endometrial cancers, which can be useful in the differential diagnosis of endometrial carcinomas. (2) Similarly to serous papillary carcinomas of the endometrium, we detected strong claudin-1 protein expression in the papillary thyroid carcinomas, giving first description in their regional lymph node metastases. (3) On the contrary, weak or no expression of claudin-1 was detected in follicular thyroid cancers, follicular adenomas, and in the peritumoral non-malignant thyroid tissue, supportive of the different molecular pathways of thyroid follicular epithelial cell-derived tumors. Claudin-1 is a useful marker in the differential diagnosis of these carcinomas. (4) Based on our results, it could be stated that claudin-1 could be a useful common marker of endometrial and thyroid papillary tumors. (5) In addition, it could be established that the prognostic value of claudin-1 is different in the serous papillary cancers of the endometrium and papillary thyroid cancers.

- Németh J, Németh Z, Tátrai P, Péter I, Somorácz Á, Szász AM, Kiss A, Schaff Z (2010) *High expression of claudin-1 protein in papillary thyroid tumor and its regional lymph node metastasis. Pathol Oncol Res* 16: 19–27.
- Sobel G\*, Németh J\*, Kiss A, Lotz G, Szabó I, Udvarhelyi N, Schaff Z, Páska C (2006) *Claudin 1 differentiates endometrioid and serous papillary endometrial adenocarcinoma. Gynecol Oncol* 103: 591–598 (\*Equally contributed to this work).

## ZSUZSANNA NÉMETH (2010)

### Tight junction and adherens junction proteins in biliary tract cancers

*Supervisor: András Kiss*

In my dissertation I studied claudin-1, -2, -3, -4, -7, -8, -10, occludin, ZO-1 and E-cadherin protein expressions in biliary tract cancers. Claudins, occludin and ZO-1 are tight junction proteins, while E-cadherin is localized in adherens junctional complex. Based on the literary data, claudins show differing expression in the normal and neoplastic tissues, thus studies on expression of these proteins could help differential diagnosis and—in accordance with the literature—could be usable in therapy as well. I investigated 62 bile duct tumors and 57 normal tissue samples in tissue microarray panels. Following immunohistochemical reactions, slides were analyzed using semiquantitative methods. Further analyses were carried out with statistical softwares.

In the other part of my research I measured the quantity of claudin-1 and -4 proteins in lysates of cell lines and tissue samples by means of ELISA methods, with the aim to develop methods for the detection of these two proteins, possibly suitable in diagnostic procedures as well. Further techniques used were fluorescence immunohistochemistry and -cytochemistry, real-time RT PCR and Western-blot analyses.

In my dissertation I demonstrated for the first time the differences of claudin-1, -2, -3, -4, -7, -8, and -10 protein expressions in several part of the biliary tract in normal and cancerous epithelia, moreover I also made observations on the expressions of occludin, ZO-1 and E-cadherin proteins. Furthermore, I demonstrated two ELISA methods for the detection of claudin-1, and -4 proteins. Based on my results, claudins show groupspecific protein expression in the different regions of normal and tumorous biliary tract, as opposed to the other studied proteins. Further, based on my results, the different parts of the normal biliary tract show notable differences in claudin-2, -3, and -4 protein expressions, whereas biliary tract cancers show significant differences in the expression claudins-2 and -4. The cancer samples may be divided into groups based on this evaluation.

- Németh Z, Szász AM, Tátrai P, Németh J, Győrffy H, Somorácz A, Szíjártó A, Kupcsulik P, Kiss A, Schaff Z (2009) Claudin-1, -2, -3, -4, -7, -8, and -10 protein expression in biliary tract cancers. *J Histochem Cytochem* 57: 113–121.
- Németh Z, Szász AM, Somorácz A, Tátrai P, Németh J, Győrffy H, Szíjártó A, Kupcsulik P, Kiss A, Schaff Z (2009) Zonula Occludens-1, Occludin, and E-cadherin protein expression in biliary tract cancers. *Pathol Oncol Res* 15: 533–539.



**PROGRAM 8/3.****STUDY OF THE IMMUNOBIOLOGICAL EFFECTS OF MICROORGANISMS AND OF THEIR COMPONENTS AT MOLECULAR AND CELLULAR LEVEL AND IN MICROORGANISMS****Coordinators:****Károly NAGY M.D., Ph. D.**

Department of Medical Microbiology

4 Nagyváradi sq, Budapest H-1089

Tel./fax: +36 1 210 2959, +36 1 210 0940

E-mail: [nagykar@net.sote.hu](mailto:nagykar@net.sote.hu)**Program overview**

Infections caused by bacteria, viruses, parasites and fungi have shown significant leading roles in morbidity, mortality and health economy all over the world including Hungary. This Program has offered a variety of studying the causative agents, pathomechanisms, pathogenesis, transmission, epidemiology, control, rapid diagnosis and prevention of most frequent infections in Hungary from molecular to host level. Outstanding parts of the Program have been the *in vitro* and *in vivo* pathomechanism and pathogenesis of natural and nosocomial infections including the pheno- and genotypical features of infectious agents with special regard to the presence and expression of genes responsible for toxin production, cell-surface properties, and resistance to different antimicrobial groups. It has been extended to investigate the structure and function of adenovirus epitopes, the interaction between virus and host cell the origin and spread of hepatitis viruses, the regulation of viral oncogen expression. Most recently the effects of infections on the quality of life as well as on health care economy have been involved in the Program. Molecular mechanisms of the virus-cell interaction of human retroviruses, such as HTLV and the AIDS virus HIV, as well as the analysis of drug resistance of and the effect of antiretrovirals on HIV were added to the program as new fields.

**Titles of research projects**

Differential diagnostics of viral respiratory

Pathogenicity and antibiotic resistance of streptococcus and enterococcus

Molecular biology of the resistance against Gram-negative bacteria

Role of chlamydia in the pathomechanism of chronic diseases (atherosclerosis, infertility)

Host dependent methylation pattern of latent Epstein-Barr viral genomes with automatic fluorescent genomic hybridization

Regulation of expression of latent oncogenes in cells carrying latent Epstein-Barr viral genomes

Molecular study on human retroviruses and their role in immunopathological diseases

Antibiotic resistance in Gram-negative bacteria

Mechanism of resistance against Gram-negative non-fermenting bacteria

**Supervisors**

György Berencsi

Orsolya Dobay

Miklós Füzi

Éva Gönczöl

János Minárovits

János Minárovits

Károly Nagy

Dóra Szabó

Dóra Szabó

Resistant microorganisms in nosocomial infections	Dóra Szabó
Molecular study on hepatitis virus carrying individuals in order to identify origin and spreading of agents	Mária Takács

***Ph. D. students***

Balázs Áron Ivády	pt
Máté Sándor Szász	ft
Katalin Marossy Szinyei	pt
Merséné	
Adrienn Tóthpál	ft

***Supervisors***

Dóra Szabó
Dóra Szabó
Miklós Füzi
Orsolya Dobay

***Ph. D. candidates***

Damjanova Ivelina Borisova	na
Emese Réka Juhász	a
Tamás András Koncz	pt
Ákos Tóth	pt

***Supervisors***

Miklós Füzi
Károly Nagy
László Gulácsi
Miklós Füzi

***Ph. D. graduates***

Valentin Brodsky	pt
Katalin Kristóf	na
Tünde Mag	ft
Fruzsina Petrovay	ft

***Supervisors***

László Gulácsi
Dóra Szabó
Ferenc Rozgonyi
Éva Gönczöl

a, absolutorium; ft, full-time; pt, part-time; na, not affiliated

***Abstracts of Ph. D. theses successfully defended in 2009 and 2010*****VALENTIN BRODSZKY (2009)****Health status and disease burden of patients with psoriatic arthritis in Hungary**

*Supervisor: László Gulácsi*

Psoriatic arthritis is a chronic inflammatory arthritis associated with psoriasis. Psoriatic arthritis is characterized by asymmetrical peripheral joint inflammation and/or axial involvement. From December 2007 to March 2008 a cross-sectional questionnaire survey of 183 consecutive patients with established diagnosis of psoriatic arthritis was conducted in 8 rheumatology outpatient centres in Hungary. Data on clinical status, health related quality of life, health care utilization and disease burden were collected. Cost calculation based on collected data was conducted to assess the cost of illness. Our results revealed that the quality of life of patients with psoriatic arthritis were similar to patients with rheumatoid arthritis and significantly lower than of the general population in every age-groups. Functional status and disease activity were in the strongest correlation with the quality of life of the patients. The average yearly total costs of psoriatic arthritis were 1.4 million HUF/patient/year. Indirect costs due to disability pension had the highest share (49%) in the total costs. The key cost drivers were the functional status and the severity of cutaneous manifestation. The costs of psoriatic arthritis were lower in all cost do-

mains than of rheumatoid arthritis. Comparing to international data (only German results were published) the costs were lower in Hungary than in Germany. Our study offers information on the health status of patients with psoriatic arthritis, the disease progression, health related quality of life and first provide cost-of-illness data for Hungary. These informations are useful for medical decision making, developing guidelines and value based reimbursement.

- Brodszky V, Péntek M, Gulácsi L (2008) *Efficacy of adalimumab, etanercept and infliximab in psoriatic arthritis based on ACR50 response after 24 weeks treatment. Scand J Rheumatol 37: 399–400.*
- Brodszky V, Balint P, Geher P, Hodinka L, Horvath G, Koo E, Pentek M, Polgar A, Sesztak M, Szanto S, Ujjalussy I, Gulacsi L (2009) *Disease burden of psoriatic arthritis compared to rheumatoid arthritis, Hungarian experiment. Rheumatol Int 30: 199–205.*
- Péntek M, Kobelt G, Czirják L, Szekanecz Z, Poór G, Rojkovich B, Polgár A, Genti G, Kiss CG, Brodszky V, Májer I, Gulácsi L (2007) *Costs of rheumatoid arthritis in Hungary. J Rheumatol 34: 1437–1438.*

## KATALIN KRISTÓF (2010)

### Microbiological features of multiresistant bacteria causing nosocomial infections

Supervisor: Dóra Szabó

Diagnostic and therapeutic devices have gone through a great development since Ignác Semmelweis but paradoxically represent the most important factor of recent nosocomial infections. Proper characterization of causative agents is crucial in every nosocomial infection. Microbiologists reveal putative resistance mechanisms and interpret results based on these informations. Being aware of microbes typically occurring on a certain ward and its antibiotic resistance properties they help to establish the strategy of empiric treatment and thus actively participates in infection control of the ward. When a special resistance mechanism appears, by providing substantial examination of microbes and revealing the genetic background of the resistance mechanism we are able to determine putative reasons of its development and epidemiological significance. Results of researches can be built in the routine diagnostic work and infection control. Following this approach we described the molecular epidemiology of infections caused by ESBL-producing *K. pneumoniae* and *K. oxytoca* in a perinatal intensive care unit in a five-year long surveillance. We showed that the epidemiology of outbreaks caused by ESBL-producing strains may vary during years: a strain causing outbreaks may disappear and new strains may replace it causing outbreaks as well. We performed the first national study on *S. haemolyticus* and pointed out the high prevalence of glycopeptide resistance as well as the significance of correct microbiological investigations. We characterized the first VIM-4 metallo- $\beta$ -lactamase producing *K. pneumoniae* and *K. oxytoca* strains in Hungary. We reported the emergence of VIM-4 in a *K. oxytoca* strain. To our knowledge this is the first case when the internationally successful ST11 *K. pneumoniae* clone imported an MBL-coding integron. We also determined the plasmid-mediated quinolone-resistance in ESBL-producing Enterobacteriaceae in Hungary. We established the appearance of qnr gene in Europe referring the widespread dissemination of plasmids

playing role in the transfer of resistance against extended spectrum cephalosporins and quinolones. We determined the antibiotic resistance of *S. aureus* strains collected in our laboratory and compared data to that of Austrian and Macedonian strains. Results of investigations concerning susceptibility and virulence factors suggest that reclassification of MSSA and MRSA strains into two distinct subtypes should be considered. In the lack of international recommendations we determined the antibiotic susceptibility testing methods for *S. maltophilia* strains that can appropriately be performed in routine microbiological laboratories for the drugs available in Hungary.

- Kristóf K, Tóth Á, Damjanova I, Jánvári L, Konkoly-Thege M, Kocsis B, Concan R, Cornaglia G, Nagy K, Szabó D (2010) Identification of *blaVIM-4* gene in the internationally successful *Klebsiella pneumoniae* ST11 clone and in a *Klebsiella oxytoca* strain in Hungary. *J Antimicrob Chemoth* 65: 1303–1305.
- Kristóf K, Szabó D, Marsh JW, Cser V, Janik L, Rozgonyi F, Nobilis A, Nagy K, Paterson DL (2007) Extended-spectrum beta-lactamase-producing *Klebsiella* spp. in a neonatal intensive care unit: risk factors for the infection and the dynamics of the molecular epidemiology. *Eur J Clin Microbiol Infect Dis* 26: 563–570.
- Kristóf K, Kocsis E, Nagy K (2009) Clinical microbiology of early-onset and late-onset neonatal sepsis, particularly among preterm babies. *Acta Microbiol Immunol Hung* 56: 21–51.

## TÜNDE MAG (2010)

### Pathogenetical characterization of *Escherichia coli* strains isolated from human infections

Supervisor: Ferenc Rozgonyi

Pathogenic *Escherichia coli* strains can cause severe illnesses in significant numbers worldwide. They can be categorized in different groups by the harboring virulence factors: EPEC, VTEC, EHEC, ETEC, EIEC, EAaggEC and DAEC, which are responsible for diarrheal diseases, while the UPEC and MAEC for extraintestinal manifestations. The aim of this study was to introduce molecular methods in the National Reference Laboratory of Enteral Pathogen Bacteria to enhance the efficacy of the diagnostics of pathogenic *Escherichia coli*. In the course of my work I have adapted and optimized PCR methods using previously collected *E. coli* strains, so I could detect the genes of intimin (*eae*), enterohaemolysin (*ehlyA*), enteroaggregative heat-stable toxin (*ast1*), ETEC heat-labile enterotoxin (*elt*), ETEC heat-stable enterotoxin (*est*) and the invasion-related plasmid (*Ipa*). Besides the serotyping these assays seem to be useful tools to classify the isolates into different pathogroups. The next step of the development of laboratory diagnostics was to exchange the tube-agglutination for microplate-agglutination. The latter is a simpler, more sensible as well as more economical method than the conventional tube agglutination Widal test. Application of these new molecular methods enabled the characterization of all the VTEC strains isolated from 2000 to 2006 in a retrospective study. Our results reflected expected values in the field of serogroup distribution as well as of related verotoxin subtypes. The most frequent group proved to be the O157 type (58%), while the most severe outbreaks were due to vt2 and vt2c toxintypes producing *E. coli* strains. Surprisingly the two described HUS cases were not caused by O157 but vt2-pro-

ducing O26 strains. Five non-O157 isolates harboured *ast1* gene often described in the literature. We have observed no association between the temporal and geographical origin and the PFGE patterns among the isolates. The results of the serotyping suggest that the verotoxigenic *E. coli* strains are underrepresented in Hungary, however we expect to reveal their etiologic role in more cases after improving the surveillance systems. The other purpose of our investigations was to determine the serotypes of ESBL-producing strains. The results showed that two groups: O25 and O15 appeared in significant by high numbers. Much of the O25 isolates were confirmed by PCR to belong to the world-wide spread O25b-ST131 clone.

- Mag T, Nógrády N, Herpay M, Tóth I, Rozgonyi F (2010) Characterization of verotoxin-producing *Escherichia coli* strains isolated from human patients in Hungary over a 7-year period. *Eur J Clin Microbiol Infect Dis* 29: 249–252.
- Kiss R, Szita G, Herpay M, Csikó Gy, Pásztí J, Mag T, Szita J, Bernáth S (2009) The isolation of verocytotoxin-producing *Escherichia coli* (VTEC) strains from improperly pasteurised cow's milk samples. *Acta Aliment Hung* 40: 23–37.
- Sonnevend Á, Al Dhaheri K, Mag T, Herpay M, Kolodziejek J, Nowotny N, Usmani A, Sheikh FA, Pál T (2006) CTX-M-15-producing multi-drug resistant enteroaggregative *Escherichia coli* in the United Arab Emirates. *Clin Microbiol Infect* 12: 582–585.

## FRUZZINA PETROVAY (2010)

### Molecular diagnostic investigation of human pathogen *Chlamydiae*

Supervisor: Éva Gönczöl

*Chlamydiae* are obligate intracellular bacteria among which three human pathogen species are described for causing widespread infections. In this dissertation I summarized my molecular diagnostic research results on these three *chlamydia* species. *Chlamydia trachomatis* is one of the most prevalent sexually transmitted bacterium worldwide. In Hungary, only limited information is available on the prevalence of *Chlamydia trachomatis* infection, and the distribution of certain urogenital serovars has not been determined yet. The aims of this thesis were to determine the prevalence of *Chlamydia trachomatis* infection in a high-risk population and to develop a genotyping method for characterizing certain serovars. According to the results the prevalence of the infection was 6.6%, which showed a negative correlation with age. In the positive samples seven different serovars could be identified, the most prevalent were serovars D, E and F in correlation with other findings from different countries. *C. psittaci* infection spreading from birds to humans causes respiratory disease, which can be occasionally fatal. In the present work cases of two poultry-processing-plant employees have been discussed, who presumably died due to psittacosis. I have adapted some molecular methods for human samples by which the diagnosis was eventually confirmed. These results point out the relevance of direct detection of *C. psittaci* in the laboratory diagnostics, which could make more effective the management of suspected cases of psittacosis. *C. pneumoniae* usually causes mild respiratory infections and it is responsible for about 10% of community acquired pneumonia cases in developed countries. The pathogen has gained a significant role in the research field of the development of atherosclerosis. The objective of this work was to detect the reactivation of persistent *C. pneumoniae* and cytomegalovirus as

the result of coronary angioplasty intervention. Our results have revealed for the first time a higher prevalence of *C. pneumoniae* and CMV DNA in the peripheral blood samples of patients after the intervention than before. These findings might suggest that certain pathogens present in the atherosclerotic vessel wall, could be reactivated by the mechanical injury, which could be a risk factor for further inflammation process.

- Petrovay F, Balla E, Németh I, Gönczöl E (2009) *Genotyping of Chlamydia trachomatis from the endocervical specimens of high-risk women in Hungary. J Med Microbiol* 58: 760–764.
- Petrovay F, Balla E (2008) *Two fatal cases of psittacosis caused by Chlamydophila psittaci. J Med Microbiol* 57: 1296–1268.
- Petrovay F, Heltai K, Kis Z, Tresó B, Gonczol E, Burian K, Endresz V, Valyi-Nagy I (2007) *Chronic infections and histamine, CRP and IL-6 levels after percutaneous transluminal coronary angioplasty. Inflamm Res* 56: 362–367.

## PROGRAM 8/4.

### PUBLIC HEALTH

#### **Coordinator:**

**Péter SÓTONYI M.D.,**

**member of the Hungarian Academy of Sciences**

Department of Forensic Medicine

93 Üllői st, Budapest H-1091

Tel.: +36 1 215 0538; fax: +36 1 215 6228

E-mail: [sotonyi.peter@med.semmelweis-univ.hu](mailto:sotonyi.peter@med.semmelweis-univ.hu)

#### **Program overview**

The Program includes issues and joint areas of public health, forensic medicine, hygiene and dietetics. The various Subprograms sum up knowledge in the fields of organ damages caused by environmental injuries, endocrinological diseases and virology. A special Program deals with topics of health education and nursing. The various Programs have a common basic and following the branching-off there is an opportunity to select a special subjects.

#### **Titles of research projects**

Importance of medical informatics research in healthcare

Protein structures in oncology

Health history

Carcinogenicity of environmental chemicals, biological markers

Death caused by drugs (opiate and dopaminerg systems in heroin taking)

Role of antioxidants in prevention of certain diseases

Statistical and clinical epidemiology to discover etiology of cancer

Comparative study on the asthmatic children taking part regularly in swimming programs

#### **Supervisors**

Elek Dinya

Judit Fidy

Judit Forrai

Sarolta Gundy

Éva Keller

Andrea Lugasi

Gábor Makara

Györgyi Mezei



Epidemiology of adenoviruses in immunosuppressive conditions.	József Ongrádi
Adenoviral therapy	
Health impacts of climate change. The use of geographical information system in public health	Anna Páldy
Science-, research-, and innovation politics and management, financing systems and their practical applications in Hungary and in European Union	Gábor Pörzse
Toxic injury of the myocardium	Péter Sótónyi
Health and medical relation in Hungarian legal practice	Péter Sótónyi
Effect of globalization on the onset of diseases	Anna Tompa
Health behaviour, health status and health promotion of the vulnerable populations with a special focus on the Roma population	Zoltán Vokó

**Ph. D. students**

Domonkos Bán	pt
Adrienn Bartók	pt
Gábor Grósz	pt
Diána Kaldau	ft
Ildikó Rákóczi	pt
Ágnes Regős	pt
József Révész	pt
Attila Trájer	ft
Katalin Wolff	ft

**Ph. D. candidates**

Zsuzsanna Balogh (Kériné)	ft
Éva Bocskay	pt
Annamária Pakai (Karamánné)	pt
Hedvig Győry (Királyné)	na
Júlia Kis	pt
Erzsébet Mák	pt
Katalin Tátrai (Némethné)	pt
Erzsébet Pálfi	pt
Marcel Marius Pop	pt
Enikő Tátrai	ft
Márta Bálint (Veresné)	pt

**Ph. D. graduates**

Kinga Balla	pt
Sarolta Csatornai	pt
Péter Csépe	na
Erika Erdősi	pt
István Hornyák	pt
Katalin Kardos (Horváthné)	pt
Kálmán Kiss	na
László Papp	pt
Younes Saleh Ali Saleh	na

**Supervisors**

Judit Forrai
Judit Forrai
Anna Tompa
Judit Forrai
Péter Balázs
Endre Morava †
Judit Forrai
Anna Páldy
Péter Sótónyi

**Supervisors**

György Berencsi
Péter Balázs
Péter Balázs
Judit Forrai
Sándor Hollós
István Szabolcs
Mária Barna
Mária Barna
Judit Mészáros
Péter Sótónyi
István Szabolcs

**Supervisors**

Györgyi Mezei
Sándor Hollós
Zoltán Vokó
Kornélia Helembai
Mária Barna
István Szabolcs
Péter Sótónyi
Kornélia Helembai
György Berencsi

Ágnes Simek	na	István Szabolcs
Tímea Tóth	pt	Judit Mészáros
Ibolya Tulkán	pt	Sándor Hollós

ft, full-time; pt, part-time; na, not affiliated

## ***Abstracts of Ph. D. theses successfully defended in 2008, 2009 and 2010***

### **KINGA BALLA (2009)**

#### **Effect of regular physical activity on the fitness and quality of life of asthmatic children**

*Supervisor: Györgyi Mezei*

Up-to-date treatment affords asthmatic children to live an accomplished life. One of the basic requirements is that the patient collaborates with his physician, and lays stress on the long term treatment, which includes physical activity as an integral part. The aim of the examination was to compare the running fitness and quality of life of the young asthmatics who took part in regular therapeutic swimming training program (N=298) with that of the non swimmer asthmatic (N=100), healthy children (N=456) and swimmer subjects with orthopedic disorders (N=236). Running fitness of swimmer asthmatics is significantly better than that of non-swimmer asthmatics and healthy persons. Regarding running fitness, no significant difference was found between swimmer asthmatics and subjects with orthopedic disorders. The trend results underline that there was a tendency between 1985 and 2004, so that the fitness of children worsened. In the swimmer group we found significantly lower overweight and obesity rate, than in the non-swimmer group. Quality of life and psychological status of swimmer asthmatics did not differ from that of non-swimmer asthmatics. Furthermore, quality of life of asthmatics and their caregivers shows strong connections. Asthmatics' high quality of life correlates with the high quality of life of their caregivers, and inversely. With swimming training an excellent fitness level can be developed. This is very important in case of asthmatics, because through the least asthmogenic sport form they can reach good results also in the most asthmogenic, in running. Based on our findings in the swimmer asthmatics group, swimming based fitness development may be also a good alternative for healthy children against the hypokinetic lifestyle. The secular trend of worsened fitness level needs more investigation and, from the public health point of view, needs rush intervention.

- Balla K, Szabó A, Gyene I, Jáky Z, Mezei G (2008) Does long term regular swimming have an influence on quality of life, anxiety and depressive symptoms of adolescents with asthma? *Hung Med J* 4: 551–561.
- Balla K, Gyene I, Szentágothai K, Bak Z, Mezei G (2008) Trends in physical fitness among asthmatic children and healthy boys in Hungary from 1985 to 2004. *Hung Med J* 2: 241–247.
- Belányi K, Gyene I, Bak Z, Mezei G (2007) Asthmás fiatalok összehasonlító futás-állóképesség vizsgálata. *Orv Hetil* 148: 357–361.

**SAROLTA CSATORDAI (2010)****Advancement in the prevention of postnatal depression:  
application of the Leverton Questionnaire***Supervisor: Sándor Hollós*

For screening patients suffering from postnatal depression (PND), we used the Leverton Questionnaire (LQ) with the view of providing social workers with a fast and easily applicable tool for screening pregnant and confined women more efficiently and directing them to specialists if necessary. The validation process proved that PND can be detected with the help of LQ, which is a quick test able to screen pre- and postnatal depression. Our validation has shown that an LQ resulting in 12 points or above increases the probability of antenatal or postnatal depression. LQ can be used only for screening and not for giving diagnoses. LQ has an excellent inner consistence and it corresponds to the validating criteria (Cronbach  $\alpha$ : 0.77). The efficacy of the LQ in determining the severity of depression can be compared to that of the Beck Depression Inventory (BDI). The LQ includes questions concerning anxiety and the somatic symptoms of depression, which show a close correlation with the subscales of BDI. Our test concerning 3842 (N=614) between 1996 and 2006 confined women resulted in 16.1% PND occurrence. The same data was 14.9% in 1996, screened 2229 mothers in puerperium (N=333), while the rate was in 2006, among mothers (N=281), in the 6th week of the postpartum 17.4%. Consequently the tendency was rising. In 2006 introduced team training therapy reduced significantly the occurrence of PND, among those treated (N=177), from 17.5% to 12.7%. The examined team training influenced the major PND risk factors in different degrees. It affected most favourably married women, women who planned their pregnancy, and those who wanted to return to work two years after the postnatal period. The team training was less likely to ameliorate the economic factors (women having financial problems). We developed and used the therapeutic team activity that can be easily applied by social workers. We also proposed the inclusion of the exposition of the Leverton Questionnaire into the training of social workers.

- Csator dai S, Kozinszky A, Pal A (2006) *Forecasting postnatal depression with randomized representational sample. Magazine of Hungarian Gynaecologists* 69(1): 17–22.
- Csator dai S, Krajcsi A, Sefcsik T, Rudisch T, Devosa I, Toth E, Kozinszky Z, Pal A (2007) *Validation of the Leverton Questionnaire for postnatal depression in randomized representational sample. Magazine of Hungarian Gynaecologists* 70(4): 229–235.
- Csator dai S, Kozinszky Z, Devosa I, Toth E, Sefcsik T, Krajcsi A, Pal A (2007) *Obstetric and socio-demographic risk of vulnerability to postnatal depression. Patient Education and Counselling* 67: 84–92.

**PÉTER CSÉPE (2010)****Health surveys and health promotion activities in vulnerable populations especially in the Roma population***Supervisor: Zoltán Vokó*

Vulnerable populations are significantly diverse from the average of the majority population in terms of somatic and mental status, behavior and socio-economic conditions associated with disadvantaged situation. The disadvantaged condition causes negative health consequences and can make difficult for this population to reach health care services. This paper summarizes the methods and findings of several studies of health behavior of vulnerable populations (Roma, homosexual males and sex-workers) in order to improve health education and promotion programs. To categorize certain individuals into certain groups is problematic. Self-identification is not always acceptable and identification by others can cause ethical and legal problems. Consequently the assistance of peers and intermediaries of studied population is needed. Altogether 469 gay and bisexual males and 80 Roma have been participated in the health behavior and need assessment surveys. These surveys were appropriate basis for planning and elaborating on community based disease prevention and health promotion programs. The results of questionnaire surveys should be analyzed with caution. The difficulties can be decreased with appropriate preparation, knowing the specific population well, maintaining strict confidentially or anonymity, and using methods of epidemiology, sociology and anthropology in integrated way. Popular opinion leaders (C\_POL) of target communities (69 gay and bisexual men and 80 Roma people) helped to adapt prevention programs. A health promotion club network was organized for 2000 elderly Roma people with the focus on prevention of cardiovascular diseases and cancer. An oral cancer screening model program was established for 1,146 people living in Roma settlements. Oral lesions were found in 18 or 1.6% of the responders while 12 or 1% have lesions which appeared malignant. All of these programs were effective and sustainable justified by qualitative and quantitative evaluating methods. Basic condition of these prevention programs is the collaboration with the community which can form after a long-relationship. Community-based health promotion projects in vulnerable populations, especially programs involving community opinion leaders, should be a priority.

- Csépe P, Amirkhanian YA, Kelly JA, McAuliffe TL, Mocsonoki L (2001) HIV risk behavior among gay and bisexual men in Budapest, Hungary. *Int J STD AIDS* 13: 192–200.
- Vokó Z, Csépe P, Németh R, Kósa K, Kósa Zs, Széles Gy, Ádány R (2009) How is Roma ethnicity related to health in Hungary? *J Epidemiol Commun Health* 63: 455–460.
- Csépe P, Bánóczy J, Dombi Cs, Forrai J, Gyenes M, Döbrössy L (2007) Modellprogram a roma populációban ajak és szájüregi daganatok szűrővizsgálatára. *Magy Onkol* 51: 95–101.

**ERIKA ERDŐSI (2010)****The personality background of BSc student nurses' assertiveness**

*Supervisor: Kornélia Helembai*

The theme of the paper is the examination of the personality background of assertiveness among nurse students. The research was mainly aimed at what interpersonal characteristics—which are significant in patient management, too—support cooperative effectiveness in the phase of professional training. First we defined the measure of assertiveness, the types of each factor of assertiveness and the particularities of social-interpersonal behaviour and of the management of internal tensions. Then, we assessed the functioning of assertiveness in relation to profession specific requirements, such as empathy, social intelligence and counselling attitude. In the next step, we explored the interrelation of the studied factors with special regard to establishing correlations among assertiveness and the personality traits that define it. The results of the research show that during the acquisition of the profession, characteristic features can be distinguished in the development of assertiveness, and on the basis of it we defined the model of assertiveness characteristic to the period of training. One of the basic dimensions of assertiveness is the successful harmonizing the other-centred attitude (which is built on empathy) and the enforcement of self-interest. The other important feature in the view of the functioning of interpersonal relationships proved to be the psycho-vegetative and emotional harmony, in the sense that the need to establish interpersonal relationships, the quality of behavioural flexibility and the emotional stability have a decisive impact on the effective functioning of interpersonal relationships. Summarising the results of the research, we established the factors that bring about assertive and non-assertive behaviour and on the basis of this we defined the areas that need to be developed. When formulating our conclusions, we put an emphasis on the adequate development of central personality factors, and not only on exploring the flawed attitudes formed by nursing practice but also on covering the whole range of courses relevant to our topic by our professional recommendations. In the possession of the results of the research, it will be possible to compile a new test that examines assertiveness, which suits the professional profile and is able to explore experience contents, too.

- Erdősi E, Papp L, Helembai K (2009) *The personality background of BSc student nurses' assertiveness in Hungary. New Medicine* 1: 2–6.
- Erdősi E, Tulkán I, Papp L, Nagy E, Helembai K (2009) *Az ápolóhallgatók empátia és aszsertivitás szintjének jellemzői. Nővér* 2: 3–12.
- Erdősi E, Papp L, Tulkán I, Helembai K (2009) *A pszichovegetatív és érzelmi kiegyensúlyozottság vizsgálata ápolóhallgatók körében. Nővér* 4: 19–23.

**ISTVÁN HORNYÁK (2008)****An investigation of attitudes and competences to first aid with the purpose of developing school education***Supervisor: Mária Barna*

According to my results, the attitude to First Aid is unacceptable. Although the willingness of pedagogues and lay groups to help is significantly higher than the one of pupil groups, the high proportion of helpfulness to help is wiped out by the nearly same ratio of insufficient knowledge. The groups identifying the consequences of their wrong decision-making as being due to the background fear they felt during giving first aid indicates/shows their unstable knowledge. This influence arose mostly at medical students, which being compared to the groups of lays represented a significant deviation ( $p < 0.001$ ). Although more than half of asked people has learned first aid before, 76% of them is dissatisfied with his present knowledge. The answers to the first aid knowledge questions show that the knowledge of people working in medical is significantly better than the one of other groups. While occasionally, the knowledge of people studying in medical is equal to the level of lay groups. Among those, who have no hygienic qualification, children lag behind adults in knowledge. In each group, those, who have learnt first aid previously, performed better. Each group thought that the necessity of teaching first aid is important. The elementary school age group was marked to be suited to learn first aid in a significantly higher proportion over against grammar school age group. They signed the elementary school group in significantly higher ratio for being suited to study it. A deviation appeared among adults and children about the judgement of the organizational methods of teaching first aid. Adults would like to learn first aid in school lessons, children would do it in a study circle or in other facultative form ( $p < 0.001$ ). In most of the cases, they would entrust a professional to teach the subject. Pedagogue and medical employee groups would undertake the teaching of first aid after a proper preliminary training. According to their pedagogic knowledge, pedagogues would teach the subject by course book, training-school students would use other means of teaching as well. In the acquirement of first aid knowledge every group prefers practical kinds of training. The analysis of books made for public education and suitable for acquiring first aid proved that in the lack of professional guidelines, everybody chooses the categories of the course books personally. In the exposition of certain topics, a lot of obsolete aspects and factual deceptions can be detected. The problem concerns the whole society. Initiation of the conditions required to the improvements is a public object, which need the collaboration of medical, educational civilian organizations.

- Hornyák I, Mészáros J (2007) *Elsősegélynyújtási készség és tudás kérdőíves vizsgálata laikusok körében. Nővér* 20: 14–21.
- Hornyák I (2007) *Gyenge motiváció, alacsony szintű tudás. LAM* 17: 826–827.
- Hornyák I (2008) *A comparative analysis of ten to eighteen-year-old German and Hungarian students attitude and abilities to giving first aid. New Medicine* 3: 112–115.



**KATALIN KARDOS (HORVÁTHNÉ) (2010)****Stakeholder appraisal of policy options for responding to obesity in Europe**

*Supervisor: István Szabolcs*

With obesity reaching epidemic proportions in Europe, it is essential for policy-makers to know how to tackle obesity in an effective way. Being involved in a project with participation of nine European countries I had the opportunity to identify stakeholder's views on different policy options combating obesity using Multi Criteria Mapping, the innovative software based social science research and risk analysis tool.

The project aimed to find answer on several questions: which way are stakeholders supportive of the different types of options; what are the criteria being used by the different stakeholders to influence their judgements; and what are the similarities and differences between stakeholder's views on these possible approaches in nine countries, representing a broad range of dietary and physical activity patterns as well as geographic and demographic types?

During structured interviews, quantitative and qualitative data were gathered from representatives of a broad range of organisations representing relevant stakeholder interest groups. Data were analysed by stakeholder perspectives, clusters of options and issues of criteria also. The data gathered for this study indicate that there is a broad consensus that in order to reverse the rising trend in the incidence of obesity a portfolio of measures integrated into a coherent programme would be needed. When scoring the wide range of different options societal benefits, efficacy, social acceptability and practical feasibility were weighted to be more important than any kind of costs. Educational initiatives as well as the improvement of sports facilities, modifying the food supply and mandatory nutritional labelling were well supported in most countries.

Comparison of findings in nine country resulted in a consensus that the relative costs of implementing a programme of measures were not considered as high as the potential costs of not taking action. Analysing the rich body of information concerning the reasons for differing views of a broad range of stakeholder perspectives in different countries may help decision-makers to find the best way interventions could be introduced and communicated to different stakeholder interest groups resulting in line of least resistance.

- Horvath Z, Gilinger Pankotai M, Szabolcs I (2007) *Stakeholder appraisal of policy options for responding to obesity in Hungary*. *Obesity Reviews* 8(Suppl 2): 75–81.
- Gonzalez-Zapata L, Alvarez-Dardet C, Ortiz-Moncada R, Clemente V, Millstone E, Holdsworth M, Sarri K, Tarlao G, Horvath Z, Lobstein T, Savva S (2009) *Policy options for obesity in Europe: a comparison of public health specialists with other stakeholders*. *Public Health Nutr* 12: 896–908.
- Gonzalez-Zapata L, Alvarez-Dardet C, Millstone E, Clemente-Gómez V, Holdsworth M, Ortiz-Moncada R, Lobstein T, Sarri K, De Marchi B, Horvath KZ (2011) *The potential role of taxes and subsidies on food in the prevention of obesity in Europe*. *J Epidemiol Commun Health* 65(9): 737–739.

**KÁLMÁN KISS (2010)****Comparative study of physique and physical performance in medical university students***Supervisor: Péter Sótonyi*

The aim of the study was to compare the body built, relative body fat content (estimated by the skinfold thicknesses) and physical performance of students studying at three various university faculties with different education profiles. The subjects of this kinanthropometric comparison were the first and second year students of the various medical faculties of Semmelweis University (study group), students of the Faculty of Physical Education and Sport Sciences (active control), and students of the different faculties of Technical University (non-active control group). A total of 2156 students were investigated. Physique and nutritional status were described by anthropometric techniques accepted by the international literature. Physical performance was assessed by the running time in 800 m run. Because the means of the studied variables between the first and second year students did not differ within the identical education profile, the final comparison was carried out by excluding the calendar age differences by genders. The difference between the mean height, body mass and physique characteristics were very moderate. By the small inter-group variability the marked and consistently significant differences between the relative body fat content and physical performance can not be explained in our opinion. There were no significant differences between the kinanthropometric characteristics of the study groups and non-active controls. Nevertheless, the relative body fat content was significantly lower, the lean body mass was remarkably greater, and the running time in 800 m distance was shorter in the physically active control groups in both genders. The joint prevalence of overweight and obese students was similar in the two non-active groups (exceeded the 33%), but no such student was found among the active controls. The performance decreasing effect of greater body fat content was significantly stronger in the active controls. It is out of question, that body composition and physical performance of the medical students were less favourable than the theoretically expected levels. The momentary status and the developing consequences (increased health risks) indicate the necessity of the institutional interference. The first steps of the possible solutions would be the increase of general prestige of physical education and its official appreciation by a credit.

- Kiss K, Mészáros Zs, Mavroudes M, Szmodis BM, Zsidegh M, Ng N, Mészáros J (2009) *Fitness and nutritional status of female medical university students. Acta Phys Hung* 96: 469–474.
- Kiss K, Mavroudes M, Faludi J, Farkas A, Szmodis BM, Uvacsek M (2009) *Medikák testzsírtartalma és állóképessége. Magyar Sporttud Szemle* 10: 3-6.
- Mészáros Zs, Kiss K, Szmodis BM, Zsidegh M, Mavroudes M, Mészáros, J (2009) *Effects of attending elevated level school physical education in 7-to-11-year-old boys. Acta Phys Hung* 96: 349–357.

**LÁSZLÓ PAPP (2010)****Counseling in nursing: examination of student nurses' counseling competence**

*Supervisor: Kornélia Helembai*

The main goal of nursing is to help the patient or client in a way which enhances his/her individuality, and promotes his/her independence from nursing care. This could be achieved by maximizing the patient's personal resources, improving his/her independent problem-solving capacity and assisting the health-related decisions. The tools of this task of nurses are health promotion and counselling, as were declared by the Hungarian Law on Health. An actual question is which skills do nurses need to make counselling, how they acquire and use them during the nursing process. The goal of our study was to examine the theoretical and practical aspects of counselling in nursing and its educational considerations. In the first part of our research, we conducted a concept analysis of counselling in nursing. According to the results, the concept has well-definable attributes, which are interaction, professionalism, orientation, individuality and support. In the second part of our work, we examined the student nurses' relation to counselling with an attitude study. Our goal was to survey the full-time students'—who have no practical nursing experience—, and part-time students'—who already have nursing experience—counselling attitude, and to analyze the possibilities of its development through high-level nursing education. The results showed that all of the students were aware of the importance of client-centeredness, but in the inspected attitude dimensions—acceptance, claiming of independence, problemsolving support, leading of conversation—we measured average levels. The study drew attention to the insecurity of the application of theoretical knowledge in practical nursing situations, which was also confirmed by our study about the nurses' conversation-leading skills, conducted as the third part of our research. The results of this part showed that nurses dominate the nurse-patient interaction, forcing the patient in a passive role. The nurses acted as knowledge-holders, whilst they presented the patients basically as persons with low compliance levels and absence of knowledge. The experiences of our study support the importance of integration of the idea of client-centeredness to the nursing education. The development of counselling programmes based on high-level evidences provides possibilities for this integration, and our results could be conducive to reach this goal.

- Papp L, Erdősi E, Nagy E, Helembai K (2009) *Characteristics of student nurses' attitudes towards counselling: a descriptive study. New Med* 3: 61–64.
- Papp L, Erdősi E, Helembai K (2009) *A tanácsadói attitűd vizsgálata ápoló hallgatók körében. Nővér* 22: 3–10.
- Papp L, Nagy E, Erdősi E, Helembai K (2009) *Az ápoló-beteg interakciók jelentősége a betegellátásban. IME—Az egészségügyi vezetők lapja* 8: 38–41.

**YOUNES SALEH ALI SALEH (2010)****Hepatitis virus markers in the population of the Southern Plain Region of Hungary; perinatal or transplacental transmission of human viruses***Supervisor: György Berencsi*

Nation-wide programmes have been initiated in Hungary for the prevention of perinatal transmission of hepatitis B virus (HBV) in 1995 and mandatory vaccination has been initiated in 2001. The prevalence of hepatitis virus markers have been determined in the Southern Plain Region of Hungary. The presence of oncogenic X-protein (HBxAg), specific antibodies and genotypes of hepatitis B virus were also examined. These data can be used later for the control of efficacy of preventive programmes. Recently conflicting results have been published on the perinatal and transplacental transmission of viruses, therefore a systematic molecular examination of healthy pregnant and amniotic fluids taken at term were carried out. Results confirmed that the preventive measures will reduce the occurrence of HBV prevalence to very low level (<1%) in the age group below 20 years. It has been observed, that the X-antigen and antibodies form probably immunocomplexes in the plasma similar to other hepatitis B viral proteins. One third of the amniotic fluid amples at term were found to contain DNA of herpesvirus types 4, 5, 6, 7 and 8. Low levels of endotoxin and papillomaviruses could be also detected. The comparison of virus content of maternal blood and amniotic fluid suggested protracted transplacental transmission of viruses.

- Younes A Saleh, Csire M, Kapusinszky B, Szomor K, Takács M, Berencsi Gy (2009) *Heterogeneous pathways of maternal-fetal transmission of human viruses. Pathol Oncol Res* 15(3): 451–468.
- Younes Saleh A, Csire M, Pályi B, Mikala G, Vályi-Nagy I, Cseh I, Benczik M, Jeney Cs, Takács T, Simon É, Fülöp V, Berencsi Gy, Visy M (2007) *Endotoxins do not influence transplacental transmission of lymphotropic human herpesviruses and human papillomaviruses into amniotic fluid taken from healthy mothers before parturition in Hungary. Acta Microbiol Immunol Acad Sci Hung* 54: 279–303.
- Pal J, Palinkas L, Nyarady Z, Czompoly T, Marczinovits I, Lustyik G, Saleh Ali Y, Berencsi G, Chen R, Varro R, Par A, Nemeth P (2005) *Sandwich type ELISA and a fluorescent cytometric microbead assay for quantitative determination of hepatitis B virus X antigen level in human sera. J Immunol Methods* 306: 183–192.

**ÁGNES SIMEK (2010)****Status and opportunities of rural health in Hungary***Supervisor: Anna Tompa*

**Introduction:** Rural health did not exist after WWII. Foundation of Hungarian Academic Association of Rural Health was the first step of institutional elaboration of rural health. **Aims:** to have Rural Health in Hungary. To establish educational, research and service system of rural health, to involve it into community care in Hungary. To promote rural health researches, to transfer its proposals toward decision-makers for forming and

improving the services for rural population to ensure equality in medical care for people living and for physicians working in rural area. **Material and methods:** Descriptive and analytic epidemiological surveys and casuistic studies gave arguments on differences in health status, health care especially in accessibility, availability, necessity and information of rural population comparing with urban ones. **Results:** Health service: the basic is the primary care system in the villages. Rural GP-s apply the elements of quality assurance; build the base of community health care for ensuring equality for rural population. **Education-researches:** The conferences on rural health organized for physicians and patients are honoured and scientifically accredited CME events. The results of assessments, studies, surveys, guidelines elaborated by the HAARH, their professional proposals are utilized by MOTESZ, universities, Professional College of Family Medicine, and different ministries. Rural health is the part of under- and postgraduate curricula, and that of CME. Experiences, scientific activities of rural family physicians are reported in several national and international periodicals and congresses. Numerous colleagues are the mentor or lecturer of Departments of Family Medicine in Hungarian Medical Universities. Cooperation with national and international scientific associations (MÁOTE, CSAKOSZ, MOTESZ, EURIPA, IAAMRH and WONCA-Rural,) is widening. So we dare declare: services, the educational, research and institutional system of rural health are elaborated. **Further aims:** to complete the elaboration of independent service, educational and institutional system, to finish running researches, and to set up new ones for the continual improving of rural health. For this to gain young colleagues to provide complex rural health.

- Simek Á (2006) *Health care and the most common physical and mental problems of illegal migrant workers and their families. Family Medicine Primary Care Review* 8: 755–768.
- Simek Á (2008) *A szociális háttér befolyása az egészségügyi ellátás igénybevétele szempontjából. Medicus Universalis* 41: 167–170.
- Simek Á (2010) *A házi orvosok pszichés státusza. Medicus Universalis* 43: 55–60.

## TIMEA TÓTH (2010)

### Value attitude characteristics of young people selecting paramedic careers

*Supervisor: Judit Mészáros*

A few years ago, in 2002, when I selected value research to be the topic of my dissertation, I was not aware of the fact that our current historical processes lend an unprecedented relevance to my task. The permanent and historical nature of value-based conduct and action encompassing multiple eras—the “perpetual” nature from humanization of man sweeping through cultures, religions, ideologies and affecting our time—was clear to me, however, I could only arrive at the present conclusion as part of the scientific process, in exploring and researching the relevant topic and especially after project completion that thinking in terms of values in individual and small community dimensions as well as in those encompassing the whole society or nation has become pressingly relevant, necessary and urgent. Our current task should be to rethink the value specifics of our lives, internal and external worlds, workdays and holidays and environments, the meaning and sometimes the sense of concept. Although words have the same meaning

as in the past, but the substance of their use should be adapted to explosive changes: humanity, power, money, market, competition, love and hate, solidarity and indifference, sense of vocation, individualism, riches or poverty or even “good” and “bad” are all everyday topics the value-centered research, ever-renewed interpretation, correct and targeted interpretation of which are increasingly dominant with regard to our life and destiny. Starting out from and at the same time, arriving at this train of thoughts, we can search for substance and perspectivity of these surveys, which could improve with exact results that the examined students choosing helping professions arrived at the University Faculty with the wanted value system, value priority and social expectations: the asked first-year BsC-students (41–45%) chose the paramedical career because of humanistic motivations in both samples. In 2003 and also in 2007 the concept of altruism was the first in the ranking of value categories (1409–2018 points). In the value profiles of both samples the self-esteem of the students showed a low average (1.6–0.69) on the valuable-worthless axis comparing to the others. And although the dissertation referring to “here and now”, I also wish to quote the following thoughts of Ágnes Heller conveying an eternally valid message to all of us irrespective of era and culture about the importance of human values: “Value can be related to quality. This is the only way to distinguish goals to follow from fatal occurrences.” To sum up, I was exactly searching for these “goals to follow” in the value attitudes of young people.

- Tóth T (2009) *The value priorities of young adults who choose to pursue a paramedical career. New Medicine 13: 65–69.*
- Tóth T (2010) *Drawing value for young Hungarians selecting careers in the helping professions. New Medicine 14: 29–35.*
- Tóth T (2008) *Értékek dominanciája a pályaválasztásban. Az értékattitűd jellemzői a Semmelweis Egyetem Egészségtudományi Karának elsőéves hallgatói körében. Nővér 21: 10–18.*

## IBOLYA TULKÁN (2010)

### Measuring nursing competence with special regard to practical placement

*Supervisor: Sándor Hollós*

The education of nurses is significantly influenced by the strategic decisions of the European Union and by the needs of the labour market and students. One possible way to stand these demands is competence focused education which puts bigger emphasis on the effectiveness of training and the cooperation between training institutes and health care institutions providing for the local area practice. The concept of competence is not well-defined in Hungarian nursing practice. Although the contents of educational and output requirements established for nurses with an academic degree comply with international recommendations, there is lack of data enabling international comparison regarding the effectiveness of local area study practices. In my research, I intended to identify, with the application of EHTAN NCQ, the various competence fields which are valued more or less important by the test subjects, representing the nursing study practice, and point out related problems in local area study practices of nursing education at the level of higher education. In the international results of self-assessment the first four places were taken by team-working, professional and ethical practice, communication



and nursing care competence fields, while the last place was associated with research and development. The test conducted in Hungary brought similar results regarding the top four competences, but with a different order. Nursing care was assessed as the most important, while the competence field of research and development was assessed as least important in the order of priority as well. In the Hungarian ranking of competence fields, professional and ethical practice was classified as the second, communication as the third and teamwork as the fourth most important, however in international rankings the latter fields are associated with the least importance in different countries. This means that Hungarian nursing practice provides fewer possibilities to practice such competence fields. A significant difference could be established between various training institutions regarding research and development, and between various branches regarding the first phase in the process of the nursing, that is the competence field of assessment. Problems regarding the organisational conditions of cooperation between training and health care institutions and the effectiveness of local area study practices were indicated. Out of the criteria influencing the level of practical abilities, 4 factors could be defined which indicate the importance of cooperation between the educational and healthcare institutions.

- Tulkán I, Erdősi E, Pogány M, Helembai K (2009) Hungarian nurses' and nurse graduates competences in international context. *New Medicine* 13: 70–74.
- Tulkán I, Erdősi E, Pogány M, Helembai K (2009) A területi gyakorlatok hallgatói értékelése. *Nővér* 22(3): 3–16.
- Gábor K, Tulkán I, Helembai K, Csanádi L, Szögi Z, Unginé Kántor K (2008) At the European Union's door—a Hungarian team's experiences of participating in the Project Leonardo for better nursing care. *Int J Nurs Practice* 14: 329–334.

---

## PROGRAM 8/5.

---

## CLINICAL AND EXPERIMENTAL TRANSPLANTATION

### **Coordinator:**

**Róbert LANGER M.D., Ph.D.**

Department of Transplantation and Surgery

23 Baross st, Budapest H-1082

Tel.: +36 1 287 6000

E-mail: [lander@trans.sote.hu](mailto:lander@trans.sote.hu)

### **Program overview**

The Ph.D. School for Transplantation was founded in 2007. Transplantation represents an interdisciplinary area of medicine, which is a quite young discipline. It was only 50 years ago that the first successful operations were performed and the number of these was quite low at the beginning. The surgical technique has not changed much since then, but our knowledge of immunology and the evolution of intensive care changed the scenery enormously. The introduction of new immunosuppressive drugs and problems of the follow-up of the immunosuppressed patient represent a big everyday challenge. The background for these challenges is unimaginable without the laboratory work, the experimental and large

clinical studies and the dialogue between basic science and clinical science. Our Ph.D. School is the answer for these questions and gives possibility for the academically interested professionals.

### ***Titles of research projects***

Isotope diagnostics in transplanted patients  
 Significance of renal transplantation from living donors in Hungary  
 Anatomical and chirurgical basis of partial liver transplantation from living donor  
 Diagnostic method to study drugmetabolizing capacity in transplanted patients—the future of individual immunosuppression  
 Tolerance after renal transplantation  
 Hepatitis C before and after liver transplantation  
 Monitoring redox-homeostasis, graft function and therapeutic drug level in transplanted patients  
 Complications after renal transplantation with special attention to malignant diseases  
 Connection between immunosuppression and oncogenesis—immunosuppression as an oncogenic factor  
 The concerns of organ transplant and the immunosuppressive oncology treatment (experimental and/or clinical research)

### ***Supervisors***

Gabriella Dabasi  
 Jenő Járay  
 László Kóbori  
 László Kóbori  
 Róbert Langer  
 Balázs Nemes  
 Enikő Sárváry  
 Éva Toronyi  
 András Tóth  
 Gyula Végső

### ***Ph.D. students***

Pál Ákos Deák pt  
 Fanni Gelley ft  
 Petra Gombos ft  
 Mátyás Kiss pt  
 János Balázs Kovács pt  
 Zsolt Mészáros ft  
 László Piros pt  
 Gergely Zádori ft

### ***Supervisors***

Jenő Járay  
 Balázs Nemes  
 Róbert Langer  
 László Kóbori  
 László Kóbori  
 Róbert Langer  
 Gyula Végső  
 Balázs Nemes

### ***Ph.D. candidates***

Imre Fehérvári na  
 Heuer Matthias it  
 Georgios Sotiropoulos it

### ***Supervisors***

László Kóbori  
 László Kóbori  
 László Kóbori

### ***Ph.D. graduates***

Tamás Benkő na  
 Attila Doros na

### ***Supervisors***

László Kóbori  
 László Kóbori

ft, full-time; pt, part-time; na, not affiliated; it, international

## *Abstracts of Ph.D. theses successfully defended in 2010*

**TAMÁS BENKŐ (2010)**

### **Optimizing the living donor liver transplantation—effects of various donor pretreatments after partial hepatectomy in the rat**

*Supervisor: László Kóbori*

**Background:** Protection of the liver during the living donation is desirable to ensure that the remnant liver is able to maintain sufficient function. The aim was to analyze the effects of extended hepatectomy on liver regeneration in rats with or without pre-treatments with  $\alpha$ -tocopherol, silibinin and/or L-glycine, exogenous application of tri-iodothyronine (T3). In these extended liver resections the impact of venous outflow impairment and its consequences for liver regeneration and function was investigated. **Methods:** Male Wistar rats were pre-treated with L-glycine,  $\alpha$ -tocopherol and/or silibinin and T3 thereafter, 70% or 90% partial hepatectomy or a 70% liver resection with narrowing of the hepatic outflow of an additional 20% parenchyma (70%+PH) was performed. Untreated and sham-operated animals served as controls. Liver function, liver body weight ratio (LBWR), hepatic proliferation (Ki-67), apoptosis (TUNEL assay) and transcription factors (NF- $\kappa$ B, Stat3; ELISA) or cytokines (VEGF, HGF, TNF- $\alpha$ , IL-6, TGF- $\alpha$ , TGF- $\beta$ ) involved in liver regeneration were assessed by immunohistochemistry and by customized cDNA arrays and quantitative RT-PCR. **Results:** Glycine pre-treatment decreased transaminase release, serum ALP activity and serum bilirubin levels ( $p < 0.05$ ). Prothrombin time was reduced, and histologically, liver injury was also decreased in the glycine group. Liver resection induced HIF-1 $\alpha$  accumulation and it was decreased by glycine pretreatment. T3-treated rats showed an increased LBWR and Ki-67 index after 70%PH and 90% PH, T3-treated rats had an increased expression of VEGF which was associated with a higher expression of its receptor Flt-1, which reached statistical significance compared to placebo-treated rats ( $p < 0.05$ ). After 70% PH, NF- $\kappa$ B activation was detected 12 h after surgery, while there was no activation after 90% PH. These findings correlated with delayed induction of regenerative genes after 90% PH. **Conclusion:** The decrease of liver injury after pre-treatment with glycine suggests that glycine pre-treatment might be beneficial for living liver donors. Exogenous administration of T3 ameliorates liver regeneration after 70% PH and 90% PH, possibly due to stimulation of angiogenesis. Venous outflow obstruction leads to an impairment of liver regeneration and liver function. The molecular events involved in liver regeneration are significantly influenced by the extent of resection, which is associated with delayed activation of NF- $\kappa$ B and suppression of proregenerative cytokines.

- Benko T, Frede S, Gu Y, Best J, Baba HA, Schlaak JF, deGroot H, Fandrey J, Rauhen U (2010) Glycine pretreatment ameliorates liver injury after partial hepatectomy in the rat. *J Invest Surg* 23: 12–20.
- Bockhorn M, Frilling A, Benko T, Best J, Sheu SY, Trippler M, Schlaak JF, Broelsch CE (2007) Tri-iodothyronine as a stimulator of liver regeneration after partial and subtotal hepatectomy. *Eur Surg Res* 39: 58–63.
- Bockhorn M, Benko T, Opitz B, Sheu SY, Sotiropoulos GC, Schlaak JF, Broelsch CE, Lang H (2008) Impact of hepatic vein deprivation on liver regeneration and function after major hepatectomy. *Langenbecks Arch Surg* 393: 527–533.

**ATTILA DOROS (2010)****Interventional radiological treatment of complications after liver transplantation**

*Supervisor: László Kóbori*

End-stage liver disease is successfully treated with liver transplantation. The Hungarian LivEr Transplant Program has good results, however, complications can not be eliminated totally. Interventional radiology offers more tolerable therapeutic methods, compared to surgery, with favourable outcome. In Hungary interventional treatments for post-transplant complications are available from the beginning of the Liver Transplantation Program. This study tries to focus on the results achieved by interventional radiology in different types of complications, whether these methods are capable to help avoid retransplantation or serious surgical interventions. Besides diagnostic and therapeutic algorithm's were created according to our experience, technical and personal conditions, as a basis of further workout. Fortunately, the analyzed complication types occurred infrequently, however, this fact made statistical analysis difficult. According to the data collected, the following statements are done: in the early post-transplantation period arterial stenosis or occlusion can be safely and effectively treated with balloon dilatation, stent implantation and thrombolysis, as well as portal vein strictures with percutaneous transhepatic portal vein cannulation and stent implantation, vena cava inferior narrowing with balloon dilatation and stent implantation. The efficacy of these techniques are comparable to surgical methods, but they are less invasive. In non-anastomotic, or intrahepatic biliary strictures the interventional treatments are the only therapeutic options with the possibility to avoid retransplantation. In Hungary, the treatment of these complications were developed based on my work during the last 13 years. In cases with certain vascular or biliary complications interventional methods should be between the first-line treatment modalities. In this study therapeutic algorithm's were created, which, I hope, will help to achieve the earliest diagnoses and to choose the most ideal treatments.

- Doros A, Németh A, Deák AP, Hartmann E, Gerlei Z, Fazakas J, Kóbori L (2010) *Successful treatment with a covered stent and 6-year follow-up of biliary complication after liver transplantation. Cardiovasc Inter Rad 33: 425–429.*
- Doros A, Németh A, Hartmann E, Deák PÁ, Juharosi Gy, Lénárd Zs, Kozma V, Görög D, Gerlei Zs, Fehérvári I, Nemes B, Kóbori L (2009) *Öntáguló fémstentek a májátültetés után kialakult intrahepaticus epeúti szűkületek kezelésében. Magy Rad 83: 8–13.*
- Doros A, Nemes B, Fehérvári I, Görög D, Gerlei Zs, Németh A, Hartmann E, Deák PÁ, Fazakas J, Tóth Sz, Kóbori L (2009) *Percutan transhepaticus fémstent behelyezésével kezelt májkapuvéna-szűkületek májátültetés után. Orvosi Hetilap 150: 1231–1234.*

**PROGRAM 8/6.****HEALTH SCIENCES*****Coordinator:*****István Szabolcs M.D., Ph.D., D. Sc.**

Department of Dietetics and Nutrition Sciences

17 Vas st, Budapest H-1088

Tel.: +36 1 486 4820; fax: +36 1 486 4830

E-mail: [szabolcsi@se-etk.hu](mailto:szabolcsi@se-etk.hu)**Program overview**

To provide research facilities of high standard for students in a wide range of topics in the field of health sciences. Health sciences became an internationally acknowledged branch of science in the last quarter of the 20th century. This is partly due to the rapid technical development, considerable differentiation and increased costs of biomedicine, and partly to the “pathogenetic” role of socio-economic processes and the development of prevention on the level of society (international and national prevention programs). Health sciences, as part of medicine (Government edict nr. 169/2000 IX. 29.), study principles and development facilities of human health maintenance in a system-based interdisciplinary approach. The main aim of health sciences is to study the biological and social factors of the population’s state of health and quality of life. Health maintenance strategies include providing facilities for the choice of a healthy lifestyle, supporting health maintenance attitudes and avoiding a health-damaging lifestyle. Research in health sciences is characterized by an interdisciplinary approach and the application of methods of genetics, epidemiology, biostatistics, dietetics, behavioural sciences and health economics. However, research methods of health sciences differ from research methods of biomedicine in that besides methods of natural sciences they also apply methods of social sciences to a great extent.

***Titles of research projects***

Role of mission in health occupations

Prevention of pediatric obesity

The relationship of childhood obesity to adulthood cardiovascular diseases with special respects to comorbidity

Development of an intelligent telediabetology system

The examination of the long-term life quality and rehabilitation possibilities of patients with systematic autoimmune diseases (SLE, PSS, RA, etc.)

The examination of the long-term life quality and rehabilitation possibilities of thrombophilic patients with thrombotic disorders

The examination of the long-term life quality and rehabilitation possibilities of hematological patients with Myeloma multiplex

The examination and preservation of nutritive value of greenery and fruits through modern storing and production processes

The significance of monitoring in acute treatment

Identity of profession and counselling in nursing

***Supervisors***

Péter Balázs

Mária Barna

Antal Czinner

Tibor Deutsch

Gyula Domján

Gyula Domján

Klára Gadó

Mária Pankotai

Gillingerné

Tibor Gondos

Kornélia Helembai

Development of teaching in higher education of health—	Sándor Hollós
Paradigms in nursing and teaching at XXI century	
The effect of selenium replacement on subclinical hypothyroidism	Gábor László Kovács
Analysis of effects in health education and nursing	Judit Mészáros
The significance and treatment methods of food allergies and intolerances	Kristóf Nékám
Psychosocial characteristics of addictive disorders	József Rácz
Interaction of iodine and selenium supply in elderly ages	István Szabolcs
Quality of life in subclinical hypothyreosis	István Szabolcs
Food production by fermentation for patients with food allergy and intolerance	Zsuzsa Varga
The sociocultural context of the health-status, health-culture, and the health care system	István Vingender
Deviant behaviour and sociopathology	István Vingender

### ***Ph. D. students***

Eszter Borján	pt
Judit Bozóki	pt
Róbertné Csajbók	a
Róbert Csák	pt
Mihály Dió	pt
Andrea Fogarasi-Grenczer	pt
Henriett Éva Hirdi	pt
Ferenc Horváth	pt
László Kádár	pt
Ibolya Krémer (Lipienné)	pt
Zsuzsanna Liptai-Menczel	pt
Erika Nagy	pt
Ákos Novotny	pt
Éva Relle (Nyergesné)	a
Márk Oravecz	pt
Melinda Péntes	pt
Melinda Zsófia Rados	pt
Veronika Rajki	pt
Ágnes Sima	pt
Katalin Simon	ft
András Terebessy	pt
László Ternyik	pt
Tímea Tóth	pt
Péter Váry	a

### ***Ph. D. candidates***

Sándor Bollók	pt
Judit Kormos-Tasi	pt
Edit Király	pt
Mariann Csernus (Raskovicsné)	pt

a, absolutorium; pt, part-time; ft, full-time

### ***Supervisors***

Judit Mészáros
Péter Balázs
István Szabolcs
József Rácz
Tibor Deutsch
Péter Balázs
Judit Mészáros
István Szabolcs
Péter Balázs
Judit Mészáros
István Vingender
Kornélia Helembai
István Szabolcs
István Szabolcs
Judit Mészáros
Péter Balázs
Judit Mészáros
Judit Mészáros
Péter Balázs
József Rácz
Péter Balázs
Péter Balázs
István Szabolcs
Sándor Hollós

### ***Supervisors***

István Vingender
Antal Czinner
Tibor Gondos
Sándor Hollós