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The importance of electrophysiological and imaging methods in the diagnostics of inherited retinal degenerations: Genotype-phenotype correlations

PhD thesis

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Introduction

Inherited retinal dystrophies are a heterogeneous group of rare diseases affecting the posterior segment of the eye, including photoreceptors and retinal pigment epithelium (RPE). They all belong to the group of „rare dieases“ or „orphan diseases“, however, their prevalence is highly variable. The most common form of retinal degenerations, retinitis pigmentosa (RP) is reported in 1 case for each 3000-5000 individuals, while some rare conditions are described only in very few cases in the literature.

A particular hallmark of retinal dystrophies is the impressing genetic heterogeneity. More than 200 genes have been associated with inherited retinal degenerations so far, whose defects cause a stationary or progressive loss of photoreceptors or affect the RPE. Consequently, the underlying pathomechanisms are manifold and can affect phototransduction, synaptic signal transduction, specific metabolic features of photoreceptors or structural elements required in the visual cycle or in intracellular ion balance.

Up to date no established therapy is available for inherited retinal disorders, therefore, social and professional consequences are essential tasks to deal with. Inherited retinal disorders a major cause of visual disability and legal blindness in the working population and thus have a considerable socioeconomic impact. In recent years, enormous efforts have been made in research and the new therapeutic approaches are promising. Furthermore, with the help of improved molecular genetic and functional diagnostic tools an early recognition and differentiation has become possible.

Aims

The aim was to achieve a better clinical characterization of retinal dystrophies and to provide a better understanding of the nature of these disorders. The modern ophthalmological functional diagnostic tools enable a precise characterisation and early recognition of inherited retinal diseases. The detailed results can help to extend our understanding of the pathological mechanisms involved in these diseases. In my research studies, we were focusing on two essential diagnostic tools:

Electrophysiological measurements and their relevance in inherited retinal diseases

Electrophysiological measurements, such as the Ganzfeld and multifocal electroretinography (ERG) techniques enable non-invasive and objective measurements of the retinal function. Depending on recording conditions (i.e. scotopic or photopic), stimulus intensity, wavelenght, frequency or stimulus duration, the function of various retinal cells can be isolated. While the conventional Ganzfeld ERG is a mass response to diffuse illumination of the retina, the technique of the multifocal ERG (mfERG) allows determining a topographic measure of electrical activity of the central retina.

- Our aim was to present the usefulness of electrophysiological tools in the diagnostics of inherited retinal degenerations. We deal with the technical aspects of the mfERG and show its importance in genetic retinal disorders.

Retinal imaging techniques in inherited retinal disorders: genotype-phenotype correlations

FAF and OCT imaging have been shown to be useful with regard to understanding pathophysiologic mechanisms, diagnostics, genotype-phenotype correlation, identification of predictive markers for disease progression, and monitoring of novel therapies

- This work summarises the usefulness of functional and morphological diagnostic tools, like the FAF and OCT imaging in retinal disorders.

The better understanding of genotype-phenotype correlations reveals important information with respect to the likelihood of disease development and choices of therapy. The results provide valuable information for clinicians and can help to improve early and correct recognition and proper follow-up of various inherited retinal disorders.

- We present detailed clinical characterisation of families with cone-rod dystrophies due to known or novel mutations and focus on genotype-phenotype correlations.
- We present a very rare condition, the KCNV2-retinopathy and show detailed functional, electrophysiological and morphological findings of this disorder for better understanding of the disease mechanisms.
- Finally, the extremely rare Jalili syndrome is introduced in a case of a you child, who additionally also suffers from neurofibromatosis type 1. Our case points out the importance not only of thorough clinical examination, but also of interdisciplinary teamwork.

Methods

Electrophysiological measurements and their relevance in inherited retinal diseases

Clinical psychophysical and electrophysiological measurements have been used to provide objective information regarding changes of the retinal function. While examinations, like visual acuity testing, perimetry, etc., rely on subjective criteria, electrophysiological methods are used to quantify retinal function in a more objective way. The Ganzfeld-ERG allows recording of electrical responses originating from the entire retina when stimulating with a Ganzfeld globe. In contrast, the multifocal ERG (mfERG) allows assessment of a "map" of electrical activity. With disease progression in the Ganzfeld-ERG the waveforms may no longer be detected while still some residual visual field could be measured. In such cases localized responses may still be obtained using the mfERG.

Modern retinal imaging techniques

Inherited retinal disorders exhibit a huge variability of retinal changes, mainly affecting the outer retina and RPE cells. With the help of high resolution imaging, like optical coherence tomography (OCT) the integrity or abnormality of RPE cells, photoreceptor inner/outer segments and external limiting membrane can be analyzed even during early stages of disease, when the fundus appears normal. Furthermore, OCT has become one of the most important tools in clinical testing of experimental therapeutic strategies (surgical procedures, gene therapy trials).

FAF imaging gives information above and beyond that obtained by conventional imaging methods, such as fundus photography, fluorescein

angiographyor even OCT. Its clinical value coupled with its simple, efficient, and noninvasive nature is increasingly appreciated.

Genotype-phenotype correlations

Despite the relatively high rate of occurrence of this heterogeneous pathologic category, the various forms of inherited retinal degenerations belong to the group of rare diseases. The phenotyping of isolated or syndromic forms of retinal disorders can be a very challenging task, that should be preferentially carried out in a specialized center, where possibilities of extensive clinical examination and interdisciplinary collaboration are present. During the routine clinical practice, a standardized patient's phenotyping and monitoring should be realized to maximize the chance of successful characterization of inherited retinal degenerations. Considering the extraordinary variability of retinal phenotypes, an extensive and standardized functional and morphological diagnostic protocol is required.

Results and Conclusions

Electrophysiological measurements and their relevance in inherited retinal diseases

Comparison of three types of multifocal ERG:

In our first study, we introduced a novel technique, which combined a fundus camera with a stimulation display allowing for compensation of eccentric fixation and direct fundus control. Our study showed that the fundus-controlled multifocal ERG is a convenient and precise technique for monitoring fixation during measurement. One major advantage of the system is the continuous fundus imaging while mfERGs are recorded and fundus controlled compensation for eccentric fixation, which makes it an ideal device for mfERGs in patients with macular disease. Additionally, it allows for accurate overlay of functional data on fundus images.

In a second study, we described the similarities and differences and the clinical relevance between three multifocal ERG systems – including the fundus-controlled mfERG system. Therefore, measurements in healthy subjects have been carried out using three different stimulation and recording systems. Response amplitudes showed a strong correlation between all three systems, Even though the stimulation, response recording and filtering follows ISCEV recommendations, hardware differences (especially those of the amplifiers and stimulating monitor) lead to significant waveform and response parameter variability. Therefore, a comparison of clinical data obtained with either system is restricted to larger effects with the central hexagon showing the largest variations.

Long-term follow-up in retinitis pigmentosa using the mfERG:

The aim of our study therefore was to investigate the usefulness of the mfERG among other clinical psychophysical and electrophysiological techniques. Furthermore, we also provided a review of the characteristics and natural course of retinitis pigmentosa. Emphasis was placed on determining the yearly progression of the mfERG responses. Twenty-three patients (9 male and 14 female) with clinically defined RP were included in the study. Disease progression was monitored during a period of up to 10 years using psychophysical techniques, Ganzfeld ERG and multifocal ERGs recordings. In our results, the progression of visual field loss is fairly described by an exponential decay, a yearly progression of approximately 14.5 % can be calculated in patients affected with RP every year. Regarding multifocal ERG values, approximately 6-10 % of the amplitude is lost every year in the outer three rings. MfERGs could be recorded even in cases, where the Ganzfeld ERGs did not show reproducible responses. In conclusion, the mfERG is well-suited for observation and long-term follow-up in disease development and – besides other psychophysical methods - it could be used as an objective outcome measure in upcoming treatment studies involving patients with advanced retinal diseases.

Modern imaging techniques in inherited retinal disorders: genotype-phenotype correlations

The wide heterogeneity of inherited retinal dystrophyies is presented in several clinical studies and genotype-phenotype correlations.

Autosomal dominant cone-rod dystrophies:

We conducted a study to investigate whether the two different genotypes—mutations either in retGC-1 (*GUCY2D* gene) or its activating protein, GCAP1 (*GUCA1A* gene)—make a difference in the phenotype, although they both act at the same step of the visual signal transduction cascade. Our aim was to compare the retinal function and morphology in patients affected by autosomal dominant cone or cone-rod dystrophies carrying either a mutation in *GUCY2D* or *GUCA1A* using the same psychophysical, electrophysiological, and morphologic methods. Or results suggested that mutations in the *GUCA1A* gene cause a less severe phenotype and less involvement of rod photoreceptors than *GUCY2D* mutations. The dominance and gene expression differencies in cones and rods might explain the more preserved rod function in cases with *GUCA1A* mutations.

In a second study we reported the identification of a new *CRX* gene mutation in a family affected by autosomal dominant retinal dystrophy characterised by a highly variable disease expression, including cone dystrophy in some subjects and cone–rod dystrophy with a negative ERG in others. We identified a novel disease-associated mutation in the *CRX* gene, c.636delC, which segregates with disease in all affected family members, and a sequence variant, c.100+12 C.T, which is only present in the more severely affected family members. We suggested that this polymorphism might have a modifying effect of the disease phenotype. In addition, we proposed that a negative ERG in autosoaml dominant cone-rod dystrophies might be an indicator of *CRX* gene mutations.

KCNV2 retinopathy:

We presented detailed psychophysical and electrophysiological testing as well as spectral domain optical coherence tomography (OCT) and fundus autofluorescence (FAF) to reveal novel insights into disease-specific functional changes in KCNV2 retinopathy. Additionally, we explore differences of disease specific functional aspects in the phenotype that correlate with the underlying *KCNV2* gene alterations. In summary, KCNV2 retinopathy is considered a very rare retinal disorder associated with high but often normal mixed rod-cone response amplitudes, a marked prolongation of b-wave implicit times and a delayed, almost sudden, steep amplitude-versus-intensity relationship under scotopic conditions. Furthermore, while rod phototransduction is intact, there is a constant delay of the responses, which suggests changes in the synapse or in postreceptoral signaling pathway. Inner retinal involvement is also probable, since oscillatory potentials are almost absent. These findings are diagnostic and are exclusively linked to *KCNV2* mutations.

Jalili syndrome:

Here we reported an unusual case of a girl with neurofibromatosis type 1, who additionally also suffers from a rare disease, Jalili syndrome, which is a combination of cone-rod dystrophy and amelogenesis imperfecta. We performed molecular genetic testing of the gene *CNNM4*, responsible for the syndrome, a homozygous mutation c.1312dupC p.Leu438ProfsX9 could be detected in our patient. Our case points to the importance not only of thorough clinical examination, but also of interdisciplinary teamwork.

Summary

This thesis aimed to show the importance of the previously discussed electrophysiological and imaging methods in the diagnostics and follow-up of inherited retinal degenerations. With the help of standard and extended protocols of electrophysiology and with the innovative imaging techniques of retinal morphology, a much more detailed characterisation of clinical phenotypes has become possible.

- We showed that a special setup, the fundus-controlled multifocal ERG is a convenient and precise technique for monitoring fixation during measurement. Even though the stimulation, response recording and filtering follows ISCEV recommendations, hardware differences lead to significant waveform and response parameter variability when using different setups. Therefore, a comparison of clinical data obtained with different systems is restricted to larger effects with the central hexagon showing the largest variations.
- In a second study, we demonstrated the usefulness of mfERG in retinitis pgmentosa. The mfERG is well-suited for observation and long-term follow-up in disease development and could be used as an objective outcome measure in upcoming treatment studies involving patients with advanced retinal diseases.
- Based on genotype-phenotype correlations, we suggested that mutations in the *GUCA1A* gene in autosomal dominant retinal dystrophies cause a less severe phenotype and less involvement of rod photoreceptors than *GUCY2D* mutations.
- We identified a novel disease-associated mutation in the *CRX* gene, c.636delC, which segregated with disease in all affected family members of a large

family, and a sequence variant, c.100+12 C.T, which was only present in the more severely affected family members. We suggested that this polymorphism might have a modifying effect of the disease phenotype. In addition, we proposed that a negative ERG in adCRD might be an indicator of *CRX* gene mutations.

- We studied a very rare condition, the KCNV2-retinopathy, which is known for its one-of-a-kind electrophysiological findings and strict association to mutations in the *KCNV2* gene Beyond identifying novel mutations, we presented a very detailed clinical characterization of the disease, which can help to better understand the underlying pathomechanisms.
- Finally, we reported a very rare case of Jalili syndrome, which occured in combination with neurofibromatosis type 1.

In summary, we can conclude, that with the help of improved molecular genetic and functional diagnostic tools an early recognition and differentiation of inherited retinal disorders has become possible. The detailed results and information can help to extend our understanding of the pathological mechanisms involved in these diseases. Up to date, no established therapies are available, however, enormous efforts have been made in research in recent years and the new therapeutic approaches are underway and look very promising. Therefore, precise clinical and genetic characterization plays a crucial role in not only managing diseases, but also in patient counselling regarding future therapeutic options.

Publications

Publications relevant to the PhD thesis:

Nagy D, Schönfisch B, Zrenner E, Jägle H. Long-term follow-up in retinitis pigmentosa using the multifocal ERG. IOVS 2008 Oct;49(10):4664-71.

Zobor D, Zrenner E. [Retinitis pigmentosa - a review. Pathogenesis, guidelines for diagnostics and perspectives]. Ophthalmologe. 2012 May;109(5):501-14;quiz 515. German. PubMed PMID: 22581051.

Zobor D, Zrenner E, Wissinger B, Kohl S, Jägle H. *GUCY2D*- or *GUCA1A*-related autosomal dominant cone-rod dystrophy: is there a phenotypic difference? Retina. 2014 May 28. [Epub ahead of print] PubMed PMID: 24875811.

Kitiratschky VB, **Nagy D**, Zabel T, Zrenner E, Wissinger B, Kohl S, Jägle H. Cone and cone-rod dystrophy segregating in the same pedigree due to the same novel CRX gene mutation. Br J Ophthalmol. 2008 Aug; 92(8):1086-91. doi: 10.1136/bjo.2007.133231. PubMed PMID: 18653602.

Zobor D, Kohl S, Wissinger B, Zrenner E, Jägle H: Rod and cone function in patients with KCNV2 retinopathy. PLoS One. 2012;7(10):e46762. doi: 10.1371/journal.pone.0046762. Epub 2012 Oct 15. PubMed PMID: 23077521; PubMed Central PMCID: PMC3471896.

Wissinger B, Schaich S, Baumann B, Bonin M, Jägle H, Friedburg C, Varsányi B, Hoyng CB, Dollfus H, Heckenlively JR, Rosenberg T, Rudolph G, Kellner U, Salati R, Plomp A,De Baere E, Andrassi-Darida M, Sauer A, Wolf C, **Zobor D**, Bernd A, Leroy BP, Enyedi P, Cremers FP, Lorenz B, Zrenner E, Kohl S. Large deletions of the KCNV2 gene are common in patients with cone dystrophy with supernormal rod response. Hum Mutat. 2011 Aug 31. doi: 10.1002/humu.21580. [Epub ahead of print] PubMed PMID: 21882291.

Zobor D, Kaufmann DH, Weckerle P, Sauer A, Wissinger B, Wilhelm H, Kohl S. Cone-rod dystrophy associated with amelogenesis imperfecta in a child with neurofibromatosis type 1. Ophthalmic Genet. 2011 Jul 5. [Epub ahead of print] PubMed PMID: 21728811.

Other publications:

Zobor D, Balousha G, Baumann B, Wissinger B. Homozygosity mapping reveals new nonsense mutation in the FAM161A gene causing autosomal recessive retinitis pigmentosa in a Palestinian family. Mol Vis. 2014 Feb 7;20:178-82. eCollection 2014. PubMed PMID: 24520187; PubMed Central PMCID: PMC3919667

Leitritz MA, Ziemssen F, Voykov B, Dimopoulos S, **Zobor D**, Bartz-Schmidt KU, Gelisken F. Early postoperative changes of the foveal surface in epiretinal membranes: comparison of 23-gauge macular surgery with air vs. balanced salt solution. Graefes Arch Clin Exp Ophthalmol. 2014 Feb 4. [Epub ahead of print] PubMed PMID: 24492933.

Shao Y, Keliris GA, Papanikolaou A, Fischer MD, **Zobor D**, Jägle H, Logothetis NK, Smirnakis SM. Visual cortex organisation in a macaque monkey with macular degeneration. Eur J Neurosci. 2013 Nov;38(10):3456-64. doi: 10.1111/ejn.12349. Epub 2013 Aug 23. PubMed PMID: 24033706; PubMed Central PMCID: PMC3834013.

Perrault, A. Estrada-Cuzcano, I. Lopez, S. Kohl, S. Li, F. Testa, R. Zekveld, X. Wang, E. Pomares, J. Andorf, N. Aboussair, S. Banfi, N. Delphin, A. den Hollander, C. Edelson, R. Florijn, M. JeanPierre, C. Leowski, A. Megarbane, C. Villanueva, B. Flores, A. Munnich, H. Ren, **D. Zobor**, A. Bergen, R. Chen, F. Cremers, R. Gonzales-Duarte, R. K. Koenekoop, F. Simonelli, E. Stone, B. Wissinger, Q. Zhang, J. Kaplan, JM. Rozet: Union Makes Strength: A worldwide collaborative genetic and clinical study to provide a comprehensive survey of RD3 mutations and delineate the associated phenotype. PLoS One. 2013;8(1):e51622. doi: 10.1371/journal.pone.0051622. Epub 2013 Jan 7. PubMed PMID: 23308101; PubMed Central PMCID: PMC3538699.

Pach J, Kohl S, Gekeler F, **Zobor D**. Identification of a novel mutation in the PRCD gene causing autosomal recessive retinitis pigmentosa in a Turkish family. MolVis 2013 Jun 13;19:1350-5. Print 2013.

Fischer MD*, **Zobor D***, Keliris GA, Shao Y, Jägle H, Logothetis NK, Smirnakis SS. Juvenile macular degeneration in a rhesus macaque: structural and functional description. Doc Ophthalmol 2012 Aug 26. [Epub ahead of print] PubMed PMID:

22923360.

Jägle H, **Zobor D**, Brauns T: Accomodation limits induced optical defocus in defocus experiments. Doc Ophthalmol 2010 Oct;121(2):103-9. doi: 10.1007/s10633-010-9237-y. Epub 2010 Jun 11. PMID: 20544259

Becirovic E, Ebermann I, **Nagy D**, Zrenner E, Seeliger MW, Bolz HJ: Usher syndrome type 1 due to missense mutations on both CDH23 alleles: investigation of mRNA splicing. Hum Mutat. 2008 Mar;29(3):452.

Horsch, Wanka: Das Usher-Syndrom – eine erworbene Hörsehbehinderung. Kapitel: Das subretinale elektronische Implantat zur Wiederherstellung von Seheindrücken: künftig Einsatz auch beim Usher Syndrom möglich? Old.70-81. Ernst Reinhardt Verlag München Basel, 2012 (könyvfejezet)