Cognitive and psychiatric aspects of mitochondrial encephalomyopathies

Dissertation

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Budapest
2014

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1. Abbreviations

ADP: adenosine-diphosphate

AMP: adenosine-monophosphate

AMPK: AMP-dependent kinase

ANT: adenine nucleotide translocase

ATP: adenosine-triphosphate

BCL-2: B-cell lymphoma-2

BDI-SF: Beck Depression Inventory – Short Form

CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

cAMP: cyclic AMP

CI: confidence interval

CK: creatine-kinase

CMT: Charcot – Marie – Tooth phenotype of the PMP22 mutation

CNS: Central Nervous System

COX: cytochrome oxidase

CREB: cAMP response element-binding protein

CT: computer tomography

Cyt-b: cytochrome b

Cyt-c: cytochrome c

DCM: dilated cardiomyopathy

DM: diabetes mellitus

DSM: Diagnostic and Statistical Manual of Mental Disorders

EEG: electroencephalogram

EMG: electromyography

ENG: electroneurography

F: female

FSIQ: Full Scale Intelligence Quotient

GERD: Gastro-Esophageal Reflux Disorder

GSI: Global Severity Index of the SCL-90-R

HAQ-DI: Stanford Health Assessment Questionnaire 20-item Disability Index

HCM: hypertrophic cardiomyopathy

HDRS: Hamilton Depression Rating Scale

HN: Hereditary Neuropathy

HNPP: Hereditary Neuropathy with liability to Pressure Palsy phenotype of the PMP22 mutation

HTN: hypertension

HTTLPR: 5HT (serotonin) – Transporter – Linked Polymorphic Region

IgG: Immunoglobulin G

KSS: Kearns-Sayre Syndrome

LHON: Leber Hereditary Optic Neuropathy

L/L: long-long homozygous genotype of the 5HTTLPR

L/S: long-short heterozygous genotype of the 5HTTLPR

M: male

MAO: monoamine oxidase

MDD: Major Depressive Disorder

MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes

MERRF: Myoclonus Epilepsy with Ragged Red Fibers

MIDD: Maternally Inherited Diabetes and Deafness

MILS: Maternally Inherited Leigh's Syndrome

MNGIE: Mitochondrial Neuro-Gastrointestinal Encephalopathy syndrome

MOMP: Mitochondrial Outer Membrane Permeabilization

mPT: mitochondrial permeability transition pore

MI: myocardial infaction

MR: mental retardation

MRI: magnetic resonance imaging

MT-ATP6: mitochondrial ATP synthase

MTD: mitochondrial disorders

mtDNA: mitochondrial DNA

NAD: nicotinamide adenine dinucleotide

NARP: Neuropathy, Ataxia, Retinitis Pigmentosa

NCBI: National Center for Biotechnology Information

ND5: NAD dehydrogenase subunit 5

NOS: not otherwise specified

OXPHOS: oxidative phosphorylation

PCR: polymerase chain reaction

PDC: pyruvate dehydrogenase complex

PEO: Progressive External Ophthalmoplegia

PMP22: Peripheral Myelin Protein-22

POLG1: DNA polymerase gamma

PQ: Performance Quotient

Pt/Pts: patient/ patients

PTSD: Post-Traumatic Stress Disorder

RAVLT: Rey Auditory Verbal Learning Test

ROS: reactive oxygen species

SCID-I and SCID-II: Structured Clinical Interviews for the DSM-IV

SCL-90-R: Symptom CheckList – 90 – Revised

SDH: succinate dehydrogenase

SIDS: Sudden Infant Death Syndrome

SNHL: Sensorineural Hearing Loss

SNP: Single Nucleotide Polymorphism

S/S: short-short homozygous genotype of the 5HTTLPR

StroopC: Stroop Color Condition

StroopCW: Stroop Color-Word Condition

TCA: tricarboxylic acid cycle

TIA: Transient Ischemic Attack

TIM: Translocase of the Inner Membrane

TMTA, TMTB: Trail Making Test part A and B

TOM: Translocase of the Outer Membrane

tRNA: transfer RNA

$tRNA^{Leu}$: Leucine of tRNA

$tRNA^{Lys}$: Lysine of tRNA

VDAC: Voltage-Dependent Anion Channel

VQ: Verbal Quotient

WAIS: Wechsler Adult Intelligence Scale

2. Introduction

2.1. Mitochondria in health and disease

2.1.1. General characteristics

Mitochondria are intracellular organelles with possible common origin to bacteria (endosymbiotic theory). They are the sites ATP (adenosine triphosphate) production via oxidative phosphorylation (OXPHOS). Mitochondria are also involved in cell-specific functions such as steroid- (Miller, 2013) and amino acid synthesis (Araujo et al, 2012), neurotransmitter metabolism (Kann and Kovacs 2007), intracellular calcium homeostasis (Bygrave, 1978) and in the regulation of apoptosis (Mayer and Oberbauer 2003). These basic functions are summarized in Figure 1.

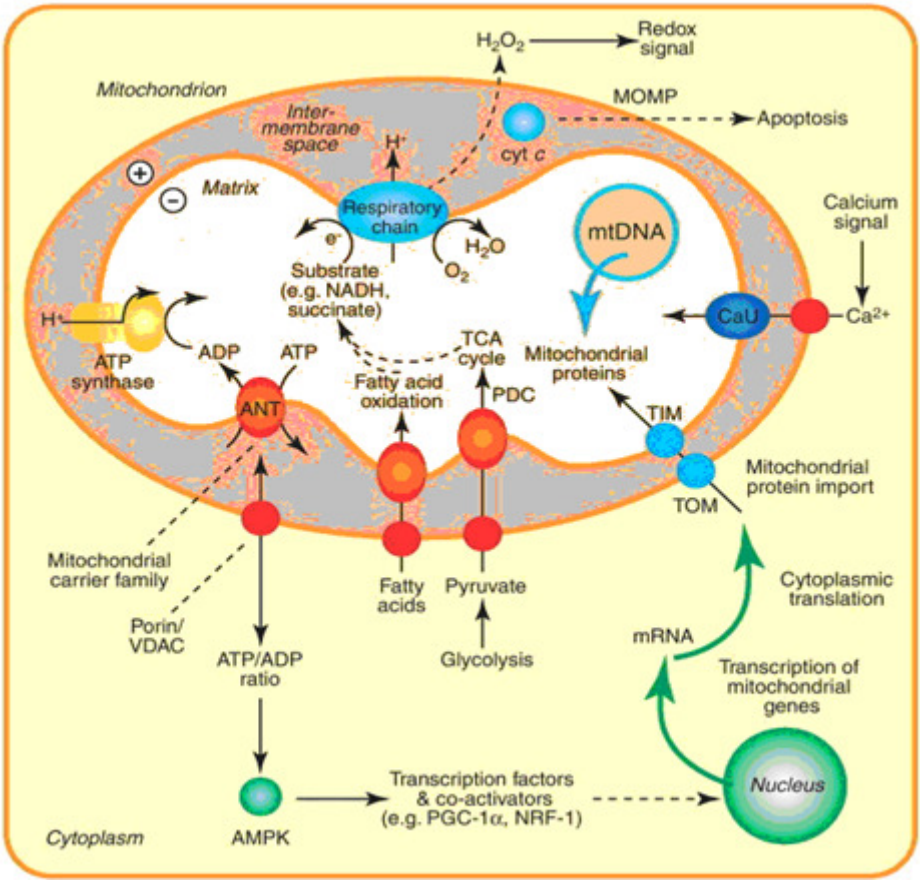

Figure 1. Mitochondrial function and biogenesis (www.sciencedirect.com, modified)

2.1.2. The mitochondrial genome

Mitochondria uniquely contain their own double-stranded 16.6 Kb circular genome (mtDNA) that is separate from the nuclear DNA but is in a complex interaction with it [nuclear-mitochondrial intergenomial communication]. MtDNA has only 37 genes; the majority of mitochondrial proteins are encoded by nuclear DNA. Thereby, mutations in either the mtDNA or the nuclear DNA can result in mitochondrial dysfunction. While clinically significant mutations can occur anywhere in the mitochondrial genome, common mutation sites, so called 'hot spots' has been identified. Probably the most frequent one is the m.3243 A>G mutation in the $tRNA^{Leu1(UUR)}$ present in around 80% of MELAS cases (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes, Goto et al, 1990). Another frequent tRNA mutation is the m.8344 A>G in the $tRNA^{Lys}$, identified as the cause of MERRF syndrome (Myoclonus Epilepsy with Ragged Red Fibers, Schoffner et al, 1990).

MtDNA mutations may also occur in genes coding for mitochondrial proteins, such as the most commonly studied primary LHON (Leber Hereditary Optic Neuropathy) mutations (m.3460 G>A, m.11778 G>A and m.14484 T>C) that result in a very specific, easily recognizable clinical phenotype. Another important mutation site is at position mt8993 in the ATP synthase 6 gene (MT-ATP6), which results in NARP (Neuropathy, Ataxia and Retinis Pigmentosa) (Tatuch and Robinson 1993) or MILS (Maternally Inherited Leigh's Syndrome), depending on the ratio of heteroplasmy (Degoul et al, 1995).

Another common cause of mitochondrial diseases is the rearrangement of mtDNA (Wallace et al, 1995). This can be single deletion, located between mt8470 and mt13447, or two or more deletions with different ranges, called 'multiple' deletions (Servidei et al, 1991) or mtDNA duplication (Poulton 1989). The 4977 base pair (bp) 'common' deletion, usually associated with Pearson syndrome in children and with PEO in adults, was detected in about 50% of the single deletion cases (Sadikovic et al, 2010). 'Multiple' mtDNA deletions are most frequently acquired (via aging, degenerative processes and autoimmune diseases) but they can also be due to intergenomial communication disturbances (Schröder and Molnar 1997).

2.1.3. Mitochondrial disorders

Mutations of the mtDNA cause primary mitochondrial dysfunction. Secondary dysfunction arises from extra-mitochondrial causes such as ischemia or neurodegeneration, among others. Energy depletion and chronic activation of AMP-dependent kinase (AMPK) shifts metabolism from anabolic to catabolic pathways (Bokko et al, 2007), shuts down or interferes with cellular functions (Bokko et al, 2007), results in further ROS production, disrupted calcium homeostasis and the induction of mitochondrial permeability via the mitochondrial permeability transition pore (mPT, Rasola and Bernardi, 2011) (Figure 2). These processes further damage mitochondrial function thereby establishing a vicious circle (Farrell et al, 2005).

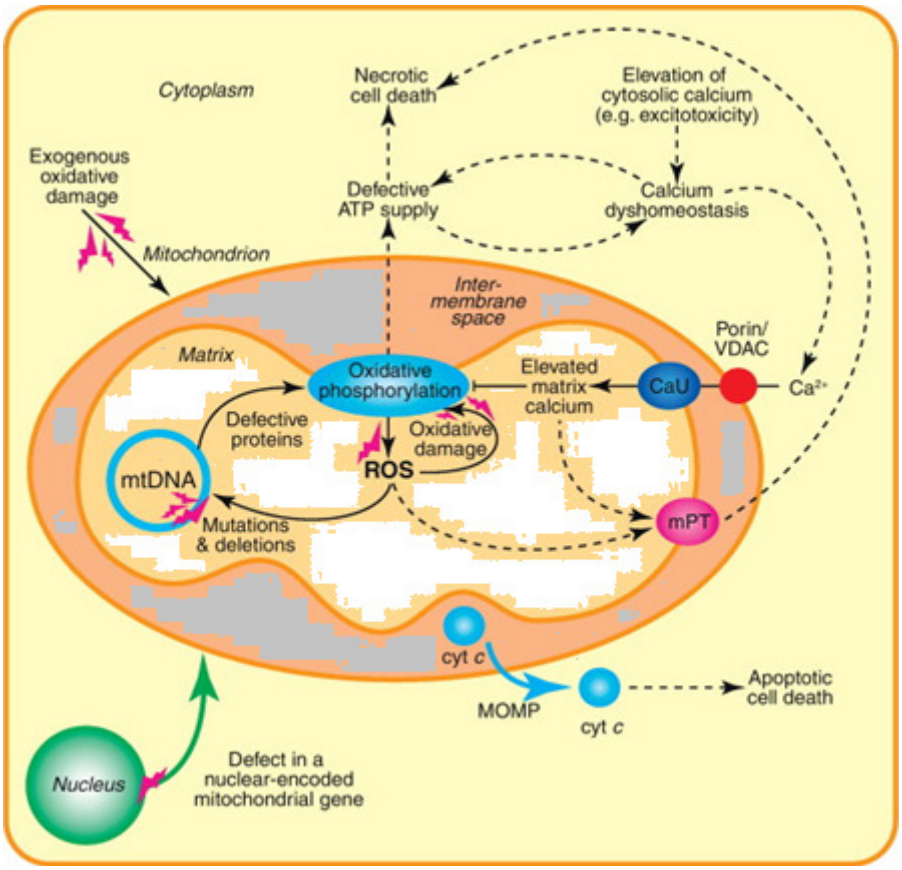

Figure 2. Mitochondrial dysfunction (www.sciencedirect.com, modified)

More than 200 genetic causes have been identified as potential causes of MTDs (Chinnery et al, 2012). The inheritance of the mtDNA and MTDs is maternal although paternal transmission has also been reported (Schwartz 2002). As we will demonstrate, the clinical picture can be very different in the offsprings and parents. This is due to heteroplasmy – the difference in mutation load on a tissue- and even cell level – and the genetic bottleneck that is a random shift and differences of mutated mtDNA load between generations (Taylor and Turnbull 2005) (Figure 3).

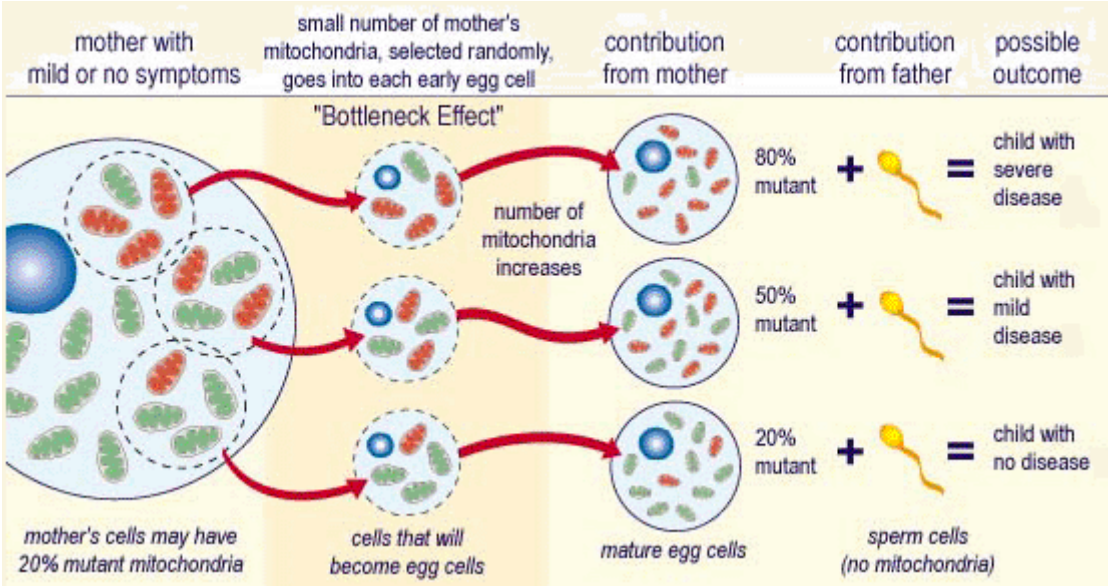

Figure 3. Inheritance of mtDNA mutations (http://medblog.medlink-uk.net/laurasmall/, modified)

Threshold effect means that the percentage of mutant mtDNAs must be above a certain threshold to produce symptoms. This threshold, just as the number of mitochondria per cell, varies from tissue to tissue due to differences in oxidative requirements of the tissue. In cells highly dependent on OXPHOS, low amounts of mutant mtDNA cause dysfunction making the brain, heart and skeletal muscle the most heavily affected organs. MTDs frequently cause multisystemic, seemingly unrelated symptoms (Figure 4), making diagnosis difficult.

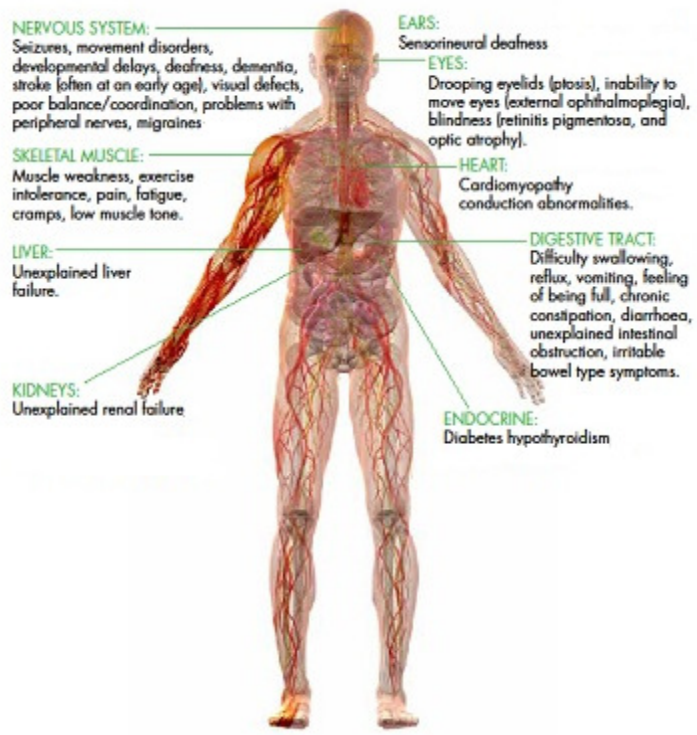

Figure 4. Symptoms of mitochondrial dysfunction in the human body (amdf.org.au, modified)

Manifestations of MTDs include classic syndromes, like the LHON, MELAS and MERRF as well as syndromes with high probability of mitochondrial involvement, such as PEO (Progressive External Ophthalmoplegia). Also, these mutations can be associated to common diseases or symptoms, like diabetes or hearing impairment (Taylor and Turnbull 2005). The inability to increase ATP production at times of higher energy demand explains why clinical symptoms typically worsen in association with physiological (Clay et al, 2010) or psychological (Gardner and Boles 2011) stress.

2.2. Epidemiology of mitochondrial disorders

Due to the multisystemic symptoms and multiple genetic causes, the diagnosis of MTDs is challenging and often requires referral to specialized research centers. Prevalence estimations thus vary widely and few systematic studies have been carried out so far. A prevalence of 6.57 - 9.2/100000 for symptomatic patients, 7.7 - 16.5/100000 including ‘at risk’ family members, a mean prevalence of 12.48 has been found in England

(Chinnery et al, 2000, Schaefer et al, 2008). The frequency of childhood mitochondrial encephalomyopathies has been estimated at 4.7/100000 in a Swedish study (Darin et al, 2001) while that of childhood respiratory chain diseases has been determined to be 6.2/100000 in Australia with a minimum birth prevalence estimated to be 13.1/100 000 for the total population (Skladal et al, 2003). Overall, mitochondrial dysfunction is probably the most prevalent metabolic abnormality considering the cardinal role mitochondria play in the metabolism.

2.3. Mitochondrial alterations in psychiatric disorders

With decades of research, the pathophysiology underlying psychiatric disorders seems more and more complex. Disease-associated genetic markers are being targeted for several psychiatric disorders by large-scale genome-wide association studies. Neurons critically depend on mitochondrial function for their metabolism, to establish membrane excitability and to execute the complex processes of neurotransmission and plasticity (Kann O and Kovacs R, 2007). In case of the chronic lack of ATP, reduced cellular resilience (Manji et al, 2012), synaptic plasticity (Manji et al, 2012) and abnormal neurotransmitter metabolism (Garcia-Cazorla et al. 2008) ensue. There is growing evidence that mitochondria are role players in the development of psychiatric conditions.

2.3.1. Biochemical and morphological data

Morphological changes (Inuwa et al, 2005), altered cellular location (Cataldo et al, 2010), decreased number (Kung and Roberts, 1999, Uranova et al, 2001) and function (Ben-Shachar and Karry, 2008, Andreazza et al, 2010) of mitochondria has been found in diverse psychiatric conditions. Downregulation of mtDNA genes (Konradi et al, 2004, Iwamoto et al, 2005, Sun et al, 2006, Shao et al, 2008, Washizuka et al, 2009), reduced expression profiles of electron transport chain subunits (Clay et al, 2010, Andreazza et al, 2010), impaired defense against oxidative stress (Erel, 2004, Naydenov et al 2007, Hovatta et al, 2010) and an overall dysfunction of brain metabolism at the mitochondrial level (Scaglia et al, 2010) has also been described in association with

psychiatric disorders. The most studied is bipolar disorder, where increased calcium signaling (Warsh et al, 2004), impaired molecular adaptation to energy stress (Naydenov et al, 2007), abnormal localization and size of mitochondria (Cataldo et al, 2010) as well as the global downregulation of mitochondrial genes in postmortem brain tissue has been described (Sun et al, 2006). Lactate levels were found to be significantly increased in subjects with bipolar disorder, primarily localized to the caudate and anterior cingulate cortices, suggesting that affective dysregulation may be related to metabolic abnormalities in the frontal-subcortical circuit (Chu WJ et al 2013). Post-mortem brain studies suggest that mitochondrial structure or function is altered in schizophrenia as well (Manji et al, 2012). In his interesting theory, Naviaux describes the "cell danger response" depicting the crucial role mitochondrial play in responding to harmful stimuli and how the resulting alteration in mitochondrial functioning can contribute to major psychiatric illnesses (Naviaux 2013).

2.3.2. Genetic data

Next generation sequencing of mtDNA did not yield significant mtDNA SNPs in association with bipolar disorder or schizophrenia. MtDNA common deletions have been the most consistent result in patients with bipolar disorder (Kato T et al 1997), schizophrenia (Sequeira et al 2010) and major depression (Gardner et al 2003). These deletions have been hypothesized to play a pathogenic role (Sequeira et al, 2010).

Point mutations and deletions (Kato M al, 2011), even polymorphisms of the mitochondrial DNA have been implied in the development of psychiatric conditions (Kato T et al 1997, Kato et al T 2000, 2001) and diverse personality traits (Kato C et al, 2004). Another group argued that it is a significantly higher cerebellar pH, associated with some mtDNA haplogroups (U, K and UK), that could be inherited through the mitochondrial genome and increase susceptibility to psychiatric diseases (Rollins et al, 2009).

2.4. Psychiatric symptoms in mitochondrial disorders

According to the above mentioned findings, patients with MTD frequently present with psychiatric symptoms. Before the publication of our work, only one systematic psychiatric assessment of mitochondrial patients has been published (Fattal et al, 2006). In a cohort of 36 patients, lifetime diagnosis was found to be 54% for major depressive disorder, 17% for bipolar disorder, and 11% for panic disorder, all representing a higher rate compared to the general population and to cohorts with cancer and epilepsy. Subjects with a comorbid psychiatric diagnosis were older (P=0.05), had more hospital admissions (P=0.02), more medical conditions (P=0.01), and lower quality of life (P=0.01) than subjects with MTD alone. Weakness of the study was that the diagnosis of mitochondrial disorder has not been confirmed by genetic investigation in all cases.
Since then, another study of 12 patients with depression, anorexia nervosa, bipolar disorder, and obsessive-compulsive disorder has been published (Anglin et al, 2012).

2.5. The role of mitochondria in neurodegenerative disorders

Mitochondria play a central role in apoptosis including the release of caspase activators, changes in electron transport, loss of mitochondrial transmembrane potential, alteration of cellular oxidation-reduction, and the release pro- and antiapoptotic Bcl-2 family proteins. Mutations in mitochondrial DNA and oxidative stress both contribute to aging, which is the greatest risk factor for neurodegenerative diseases (Perluigi et al 2013). Mitochondrial alterations has been assumed to play a role in the pathogenesis of complex, multifactorial neurodegenerative diseases, most straightforward in Parkinson's (Henchcliffe and Beal, 2008; Morais and De Strooper, 2010), but also clear in Alzheimer's (Galindo et al, 2010) and Huntington's disease (Turner and Schapira, 2010). In certain haplogroups, higher prevalence of dementia has been demonstrated (Tranah et al, 2012). On the other hand, neurodegeneration causes secondary mitochondrial dysfunction (Morais and De Strooper, 2010)

2.6. Cognitive symptoms in mitochondrial disorders

The chronic lack of ATP and CNS dysfunction results in neural dysfunction, manifesting not only in neurologic and psychiatric, but also in neuropsychologic symptoms (Finsterer 2006) ranging from mild cognitive impairment to full-blown dementia. Advanced neurodegeneration of mitochondrial origin is associated with structural abnormalities (Friedman et al, 2010), most commonly cerebral (Kim et al, 2009) or cerebellar (Scaglia et al, 2005) atrophy, also contributing to the development of cognitive symptoms.

Cognitive functions of adult patients with primary MTD have been evaluated so far in case reports of one single patient or family with MELAS (Sartor et al, 2002, Berg et al, 2011, Sharfstein et al, 1999, Inczedy-Farkas et al, 2011), NARP (Makela-Bengs et al, 1995, Gelfand et al, 2011) or MIDD (Maternally Inherited Diabetes and Deafness) (Lien et al 2001).

Studies assessing cohorts with distinct MTDs such as MELAS (Majamaa-Voltti et al, 2006), MERRF (Lorenzoni et al, 2011), PEO and KSS (Kearns-Sayre syndrome) (Bosbach et al, 2003) or MIDD (Fromont et al, 2009) also exist. Among further studies of more heterogeneous groups of MTD patients, some lack genetic diagnosis (Kartsounis et al, 1992), assessed a very limited number of patients (Lorenzoni et al, 2011), used only disease controls (Fromont et al, 2009, Kaufmann et al, 2004) or used no control group at all (Lang et al, 1995, Turconi et al, 1999). Some did not apply a comprehensive test battery (Lorenzoni et al, 2011) or did not specify it (Kaufmann et al, 2004), did not present the results in detail but reported ‘neuropsychological impairment’ (Kaufmann et al, 2004), or ‘dementia’ (Lorenzoni et al, 2011).

3. Objective

3.1. Complex clinical evaluation and assessment of psychiatric symptoms in a well-defined sub cohort of patients with genetically proven primary mtDNA mutations as compared to disease controls, harboring the CMT or HNPP type PMP22 mutation, living with similar level of disability.

3.2. The comprehensive neuropsychological assessment of the same cohort and compared the results to those of matched healthy controls.

3.3. Raising awareness to mitochondrial disorders in the international medical community.

3.4. Comprehensive assessment of the frequency rate of the most common mitochondrial mutations in Hungarian patients.

3.5. Biobank- and register-building activity (NEPSYBANK, SCHIZOBANK)

4. Patients and methods

4.1. Patients

4.1.1. Epidemiological study

A total of 1328 Hungarian patients were tested from the North-East, South-West and Central region of Hungary (Szabolcs-Szatmár-Bereg, Borsod- Abaúj-Zemplén, Hajdú-Bihar, Baranya, Pest counties and Budapest) between January 1999 and December 2012. A subcohort of 882 [485 female, 397 male; 740 adult and 142 children, mean age 38.8±19.1 years (female: 38.3±15.3 years, male 33.4±17.2 years) with a range of 1-75 years] patients were screened for m.3243 A>G, m.8344 A>G, m.8993 T>C, m.8993 T>G and mtDNA deletions. Patients were referred from county hospitals to the three genetic centers based on the symptoms and laboratory findings, for further evaluation and genetic analysis. All adult patients had a presentation consistent with mitochondrial disease i.e. the various combinations of symptoms such as short stature, epilepsy, ataxia, myopathy, lipomatosis, ophthalmoplegia externa, hypoacusis, exercise intolerance, myalgia, recurrent ischaemic stroke syndrome, cognitive dysfunction, psychiatric or endocrine disorder. In the case of young children, mitochondrial disease was suspected on the bases of muscle hypotonia, delayed psychomotor development, lactic acidosis and seizures.

We have screened 446 patients [217 male, 229 female, 366 adult and 80 children, 35.82±18.47 (male: 32.58±17.72 years, female: 38.98±18.64) with a range of 7-63 years] for the three primary LHON mutations (m.3460 G>A, m.17788 G>A, m.14484 T>C), based on the suggestive symptoms such as unilateral or bilateral decrease in visual acuity or sudden loss of vision. Written, informed consent was obtained from all participants. The study was carried out according to the Helsinki Declaration and was approved by the Research and Ethics committee of Semmelweis University.

4.1.2. Psychiatric and neuropsychologic assessment

Nineteen patients (Patient 1-19, 13 female, 6 male) with primary mutation of the mtDNA were selected for a detailed psychiatric and neuropsychologic assessment.
Selection was based on the ability and willingness of the patient to participate and the absence of severe ptosis, visual, hearing or mental disability that would have affected performance. Of the 19 patient, only 13 probands has been included in the statistical analysis in order to keep the variables independent. The results of the 6 family members are shown in the tables of the Results section.
Mean age for the 13 probands, included in the statistical analysis was 34±8.43 years (male: 27.25±6.8; female: 36.66±7.65), average years of education was 12.84±1.21 years (male: 13.25±1.5; female: 12.66±1.12).
As control for the psychiatric assessment, 10 patients (HN patients, Patient 20-29, 4 female, 6 male, mean age was: 40±10.99, average years of education was 14.2±3.85 years) with PMP22 mutation, causing CMT (Charcot-Marie Tooth) or HNPP type (Hereditary Neuropathy with liability to Pressure Palsy) sensorimotor neuropathy were examined. Diagnosis was based on clinical features supported by the presence of the PMP22 gene mutation in HN patients.
Thirteen healthy controls matched for age, sex and education (9 female, 4 male, mean age: 33.77±9.19, average years of education 14±0.9 years) were examined for the neuropsychological examination.
All participants of the study were Caucasian and visited the clinic within 1 year. Written, informed consent was obtained from all participants. These studies were carried out according to the Helsinki Declaration and were approved by the Research and Ethics committee of Semmelweis University.

4.2. Methods

4.2.1. General clinical evaluation

Basic neurological evaluation has been carried out by the primary neurologist where patients were referred to by the primary care provider. If the suspicion of MTD was

present, the patient was referred to one of the three specialized genetic centers for further evaluation. The diagnosis of MTD has been confirmed by further clinical, neurological evaluation and myopathological and/or genetic studies. In some cases, segregation analysis of the proband's family detected further patients with clinical symptoms.

4.2.2. Myopathology

Muscle biopsy have been performed in 312 cases and confirmed the mitochondrial disorder in 119 cases based on the presence of ragged blue/red and COX (cytochrome oxidase) negative fibers or on the presence of abnormal mitochondria on electron micrographs. Histopathological evaluation was carried out using standard methods. Ragged blue fibers were detected with the modified intense succinate dehydrogenase histochemistry (SDH) reaction, while ragged red fibers were searched for by the modified Gomori Trichrome stain. COX negative fibers were discerned by large accumulations of morphologically abnormal mitochondria with significantly reduced or undetectable COX activity. Ultrastructural changes were investigated in the case of 152 patients by electron microscope.

4.2.3. Genetic studies

4.2.3.1. Analysis of the mtDNA

Molecular genetic analysis was performed for diagnostic purposes from the biological sample of all investigated patients. DNA was extracted from blood (in 570 cases) or if the blood sample yielded no result but the suspicion of MTD was high, skeletal muscle tissue (in 312 cases) by QIAamp DNA Blood or Tissue kit, according to the manufacturer's (QIAgen, Hilden, Germany) instructions. The m.3243 A>G mutation in the $tRNA^{Leu\ (UUR)}$, m.8344 A>G mutation in the $tRNA^{Lys}$ gene and the m.8993 T>C and m.8993 T>G mutations in the ATP synthase F0 subunit 6 (ATPase6) of mtDNA were screened based on a method described earlier using restriction fragment length polymorphism (RFLP) with HaeIII (m.3243 A>G), BanII (m.8344 A>G), HpaII

(m.8993 T>C), AvaI (m.8993 T>G) BSAHl (m.3460 G>A), SfaNl (m.11778 G>A) and Bccl (m.14484 T>C) restriction endonucleases after PCR amplification (Shoffner et al, 1990). The ratio of heteroplasmy was measured by Quantity One Software (Bio-Rad Corp. Hertfordshire, UK). The mtDNA deletion was screened for by long PCR methods using Phusion DNA Polymerase (Finnzymes). The entire mtDNA tRNA sequence has been investigated in 200 cases by bidirectional sequencing using the ABI Prism 3500 sequencing machine. The sequences were compared with the human mitochondrial complete reference genome (NC_012920.1) using the NCBI Blast program.
The entire mtDNA was resequenced with MitoChip v2.0 microarray (Affymetrix) in 17 patients, and the supposedly pathogenic alterations were validated by Sanger sequencing.

4.2.3.2. Analysis of the PMP22 gene

The PMP22 deletion and duplication in the HN group was detected with real time PCR according to earlier published methods (Aarskog et al, 2000).

4.2.3.3. Determination of the 5HTTLPR genotype

The 5HTTLPR (serotonin-transporter-linked polymorphic region) genotypes of both MTD and HN patients were analyzed according to earlier published methods (Heils et al, 1996).

4.2.4. Focused clinical evaluation

In the case of the 19 selected patients, functional ability was assessed using the Hungarian validated version of the Stanford Health Assessment Questionnaire 20-item Disability Index (HAQ-DI) (Ponyi et al, 2005). Patients' charts were reviewed for duration of disease, medication and brain CT or MRI scans in case of the 19 patients selected for further evaluation.

4.2.5. Psychiatric examination

Detailed psychiatric assessment used the Symptom Checklist-90-Revised (SCL-90-R) (Derogatis et al, 1994) and the Beck Depression Inventory-Short Form (BDI-SF, Beck and Beck, 1972) self tests. The BDI-SF includes cognitive-affective but not somatic items to avoid spuriously high scores and overreporting of depression in somatic patients (Furlanetto et al, 2005). The clinician-administered 21-item Hamilton Depression Rating Scale (HDRS, Hamilton, 1960) and the clinical version of the Structured Clinical Interview for the DSM-IV axis-I (SCID-I) (First et al, 1996) and axis-II disorders (SCID-II) (First et al, 1997) were also used. The SCL-90-R helps evaluate a broad range of psychopathological symptoms. It yields 9 scores of primary symptom dimensions (somatization, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism) and additional item subscale referring to sleep and memory problems. The mean of these subscales yields the global severity index (GSI). The BDI-SF is a 13-item questionnaire scored on a 4-point scale, from 0 to 3, with overall scores ranging from 0 to 39. The BDI-SF has been found to have a good correlation with the standard 21-item BDI ($r = 0.96$, $p = 0.001$) and relates the clinical depth-of-depression ($r = 0.61$) (Beck and Beck, 1972). The emphasis of the 21-item HDRS is on melancholic and physical symptoms of depression. In order to control for the confounding effect of cognitive impairment, we included patients with an FSIQ of 70 and above, as assessed by the Hungarian validated version of the Wechsler Adult Intelligence Scale (WAIS-III-R version, Kun and Szegedi 1996). Scales and interviews were administered by trained clinicians.

4.2.6. Neuropsychological assessment

Memory functions were evaluated by the Rey Auditory-Verbal Learning Test (RAVLT1 for immediate recall, RAVLT2 – RAVLT5 for learning ability over successive trials and RAVLT6 for delayed recall. The Stroop Color (Stroop C) and Color-Word (Stroop CW) tests assessed selective attention, cognitive flexibility and interference control, both the number of words read in 60 sec and the number of errors made. The Trail Making Tests (TMTA, TMTB) were used as a measure of visuo-spatial abilities,

attention and psychomotor speed and – the part B – of flexibility, working memory and executive functions in general (see for Maruta et al, 2011). With the category (aka semantic) and letter Fluency Tests (Rosen, 1980), we tested fluency of verbal associations under restricted conditions, executive functions and psychomotor speed. Intelligence was assessed using the Hungarian version of the Wechsler Adult Intelligence Scale (WAIS, Kun and Szegedi, 1996).

In the case of Patient 16 we had the chance to do a longitudinal follow-up by retesting the patient 6 months apart. In her case, a more complex test battery was used comprising of the RAVLT, the Raven test (Raven Progressive Matrices, Raven 1936), the Toulouse-Pieron Attention Test (Toulouse et al, 1986), the Bells Test (Gauthier et al, 1989), the Benton Visual Retention Test (Benton Sivan A, 1992), the Rey-Osterrieth Complex Figure Test (Shin et al, 2006).

In all cases, neuropsychological assessment took place in one session of approximately two hours, with breaks if requested by the examinee. The tests were scored according to standard guidelines. Results have been correlated with the type of mtDNA mutation, age, duration of disease as well as scores on the Global Severity Index (GSI) of the Symptom Checklist-90-R (SCL-90-R) detecting the presence and severity of psychiatric symptoms.

4.2.7. Statistical analysis

In the epidemiological study, frequency of the mutations was calculated from the number of patients with pathogenic substitutions divided by the total number of investigated patients. The 95% confidence interval (CI) was calculated according to the standard method.

In the other studies, independent variables were obtained selecting only the proband (index case) from each family from the cohort of MTD patients. HN patients were all unrelated, statistics were thus performed using data from 13 MTD (Pt 1, 2, 3, 5, 8, 10, 11, 13, 14, 15, 16, 18, 19) and 10 HN patients. In the statistical analysis of the cognitive study, data of the same 13 MTD patients as well as 13 healthy controls, matched for age, sex and education was used. Correlation of total scores in GSI, BDI and HDRS with HAQ-DI in both groups was evaluated using Pearson's correlation. Differences

between the patient and control groups were assessed using Chi-Square test for categorical variables and parametrical (t-test) or non-parametrical tests (Wilcoxon Mann-Whitney test) for continuous variables (depending on the distribution of the variables). The normality of the data was checked by Shapiro-Wilk test (data not shown). All tests were two tailed and p values ≤ 0.05 were deemed significant.

SCL-90-R was analyzed with SAS System for Windows (Release 9.1 TS Level 1 M3, Statistical Analysis System, SAS-Institute USA).

5. Results

5.1. Epidemiology of mitochondrial disorders

In the epidemiology study, mutations of the mtDNA have been categorized into three groups; mutations in genes of the mitochondrial tRNA, mutations in genes coding for mitochondrial proteins and rearrangements of the mtDNA. The ratio of heteroplasmy was between 22% and 95% in the 570 blood sample, and between 25% and 79% in the 312 muscle sample (Remenyi et al, 2014).

5.1.1. Mutations in genes of the mitochondrial tRNA

5.1.1.1. The m.3243 A>G point mutation

The most commonly investigated m.3243 A>G substitution in the $tRNA^{Leu1(UUR)}$ was found in a heteroplasmic form in 11 index patients and further 12 family members from the investigated 882 patients. The ratio of heteroplasmy varied between 22 and 80%. From the positive cases, the mtDNA was isolated from blood in 20, and muscle in 3 cases. In one of the cases the mutation was found only in muscle tissue. All probands and family members carrying the mutation had clinical symptoms, mostly sensorineural hearing loss (SNHL) and diabetes mellitus (DM). Five cases had history of stroke. One family member had psychiatric symptoms whereas others were asymptomatic. The frequency of the m.3243 A>G mutation was 2.61 % (95% CI: 0.0207-0.0315).

5.1.1.2. The m.8344 A>G point mutation

The heteroplasmic m.8344 A>G mutation of the $tRNA^{Lys}$ gene was present in 13 patients (8 males, 5 females) from 3 families of the investigated 882 patients. The ratio of heteroplasmy varied between 25 and 82 %. The pathogenic mutation was detected from blood in 8 and from muscle in 5 cases. These patients also had diverse symptoms, mostly not the classic myoclonus epilepsy. The frequency of m.8344 A>G mutation was 1.47% in the investigated cohort (95% CI: 0.0106-0.0187).

5.1.1.3. Other mutations of the mitochondrial tRNA

With sequence analysis of the tRNA, we found 6 further pathogenic mutations in 17 (11 female, 6 male) patients out of the 200 investigated cases (1.93%). The m.3250 T>C substitution in the $tRNA^{Leu1}$ was present in 3 females of a family with myopathy and idiopathic pulmonary hypertension. The proband with PEO and myopathy had the homoplasmic, her mother and grandmother the heteroplasmic form. The heteroplasmic m.4298 G>A substitution in the $tRNA^{Ile\ in}$ was present in a male patient with hypertension and exercise intolerance and a m.5698 G>A in the $tRNA^{Asn}$ in one female patient with familial PEO. In both cases, multiple deletions were also present. Two mutations were present in the $tRNA^{Ser1(UCN)}$, both presenting with bilateral SNHL; a heteroplasmic m.7445 A>G in 6 patients and the m.7510 T>C in a homoplasmic form in 3 patients with hearing impairment.

We found the new pathogenic m.8332 A>G mutation in the $tRNA^{Lys}$, in a heteroplasmic form in two brothers and their mother with familial dystonia and juvenile stroke like syndrome (Gál et al). By sequencing the entirety of mtDNA tRNA genes 3-8 homoplasmic SNPs (single nucleotide polymorphisms) were detected per patient.

The overall frequency of tRNA mutations was found to be 6.35% (56/882 cases) in the Hungarian population.

5.1.2. Mutations in genes coding for mitochondrial proteins

5.1.2.1. m.8993 T>C and m.8993 T>G point mutations

We also screened for the m.8993 T>C and m.8993 T>G substitution that were present in 4 cases among 882 patients. With familial segregation analysis, the m.8993 T>C pathogenic mutation has been found in a heteroplasmic form in further 3 patients (2 male, 1 female). The heteroplasmic m.8993 T>G substitution was detected in further 1 female patient. Heteroplasmy varied between 58 and 95% in blood cells. The prevalent symptom of affected patients was cerebellar ataxia. The frequency of m.8993 T>C was 0.34% that of m.8993 T>G was 0.11% with an overall frequency of 0.45% for the nt8993 substitutions (95% CI: 0.00038-0.0086).

5.1.2.2. The m.3460 G>A, m.11778 G>A and m.14484 T>C point mutations

The three primary LHON mutations were detected altogether in 80 cases (41 male, 39 female) in homoplasmic form. The m.3460 G>A mutation was found in 9 cases (6 male, 3 female). The m.11778 G>A mutation was detected in most cases in 66 cases (34 male, 32 female) and the m.14484 T>C was found in 5 cases (2 male, 3 female) from the 446 patients. The frequency of each single mutation was as follows: 2.02% for the m.3460 G>A (95% CI: 0.0135-0.0269), 14.80% for the m.11778 G>A (95% CI: 0.1312-0.1648) and 1.12% for the m.14484 T>C (95% CI: 0.0062-0.0162), yielding an overall frequency of 17.94% (95% Cl: 0.1612-0.1976).

5.1.2.3. Mutations detected with the Mitochip v.2.0.

We resequenced the entire mtDNA, isolated from muscle tissue of 17 patients (with family history characteristic of MTD) using the Mitochip v.2.0. microarray (Affymetrix). This yielded the m.12770 A>G substitution in the NAD dehydrogenase subunit 5 (ND5) with a low rate of heteroplasmy, in a patient who was selected for further evaluation.

5.1.3. Rearrangements of the mtDNA

Single mtDNA deletions were detected in 132 out of 882 cases representing 14.97% (95% CI: 0.1377-0.1617) of the investigated cohort. Forty percent of these cases harbored “common” deletion. Multiple deletions were present in 53 cases which is 6.01% (95% CI: 0.0521-0.0681). The majority (60.3%, 32 out of 53) of the cases had positive family history. Among the 132 cases with single deletion, 30 patients (22.7%) had PEO and 48 patients (36.3%) had myopathy. Out of the 132 single deletion cases, 74 were detected from muscle tissue and 58 from blood sample. Results of the epidemiological study are summarized in Table 1.

Table 1. Mutation frequency of the most common mtDNA mutations in Hungary

mtDNA mutation type	positive cases	number of investigated patients	frequency
tRNA mutations			
m.3243 A>G	23	882	2.61%
m.8344 A>G	13	882	1.47%
m.3250 T>C	3	200	0.34%
m.4298 G>A	1	200	0.11%
m.5698 G>A	1	200	0.11%
m.7445 A>G	6	200	0.68%
m. 7510 T>C	3	200	0.34%
m. 8332 A>G	3	200	0.34%
Mutations in genes coding for mitochondrial proteins			
m.8993 T>C	3	882	0.34 %
m.8993 T>G	1	882	0.11%
m.3460 G>A	9	446	2.02%
m.11778 G>A	66	446	14.8%
m.14484 T>C	5	446	1.12%
Rearrangements of the mtDNA			
single deletion	132	882	14.97%
multiple deletion	53	882	6.01%

5.2. Genetic background of the psychiatric study

5.2.1. MtDNA mutation types of patients selected for further evaluation

We selected patients for further psychiatric evaluation from the three groups identified in the epidemiological study and one further group consisting of patients with coexisting nonsynonymous mtDNA SNPs previously associated with symptoms suggestive of MTD (Table 2).

Mutations in genes of the mtDNA tRNA: Pts 1-8, 14. Nine MTD patients had common mutation of mtDNA. Four of them (Pt 1-4) harbored the m.3243 A>G substitution. Interestingly, none of them had a history of stroke-like episodes. Four patients (Pt 5-8) had the m.8344 A>G substitution in $tRNA^{Lys}$. Among them, only Pts 7 and 8 (twin brothers) had the classic myoclonus epilepsy. Pt 14 had the m.8332 A>G base substitution in the $tRNA^{Lys.}$ Pts 9, 10, 15 had mutations in protein-coding genes; Pts 9

and 10 carried the m.8993 T>G, but only the son had the classic NARP symptoms. Pt 15 had the m.12770 A>G substitution (detected with Mitochip sequencing as outlined above) resulting in a Glu - Gly substitution in the ND5 gene. Pts 11-13 and 18 had common deletion of the mtDNA; Pts 11 and 12 had PEO and Pt 13 had KSS. Pt 18 had cardiomyopathy and cognitive impairment.

The Mitochip sequencing yielded no pathogenic mutation but the combination of different nonsynonymous SNPs, previously associated to multisystemic symptoms characteristic of MTD (www.mitomap.org). Laboratory and myopathological results of these patients indicated mitochondrial dysfunction. In case of Pts 16 and 17, a C-T base substitution at nucleotide 14771 was present resulting in Pro-Ser substitution in the mitochondrial cytochrome b (Cyt-b) gene. In the case of Pt 19 - beside the A2706G polymorphism which is responsible for linezolid-induced severe lactic acidosis - 14 single nucleotide polymorphisms were present, previously associated with LHON, potentially exerting synergistic effect.

5.2.2. PMP22 gene analysis in control (HN) patients

As a disease control group patients with HN were used. In the HN group, 9 patients harbored a duplication (Charcot-Marie-Tooth phenotype, CMT), and one of them (Pt 22) had a deletion (Hereditary Neuropathy with Liability to Pressure Palsy phenotype, HNPP) in the PMP22 gene (Table 3).

5.2.3. Analysis of the 5HTTLPR gene

To rule out the confounding effect the 5HTTLPR gene might have, we obtained our patients' 5HTTLPR genotype. In the MTD group, 5 patients harbored the long-long (L/L), 10 patients harbored the long/short (L/S) while 4 patient had the short-short (S/S) genotype (Pt 6, 7, 8, 19) (Table 5). In the HN group, 3 patients had the L/L, further 3 patients the L/S, and 4 patients the S/S polymorphism (Pt 22, 23, 27, 28) (Table 7).

5.3. Clinical evaluation

The MTD and HN groups did not differ significantly in gender (χ^2=1.9652; p=0.1610), age (t= -1.42; p=0.1711) or education (t= -1.20; p=0.243). Mean HAQ-DI score was 0.82 in the MT (range: 0 - 1.625) and 0.71 in the HN group (range: 0 - 1.625) which did not differ significantly (p=0.6076) implying comparable level of disability in the two groups (Table 9).

5.3.1. Neurological symptoms

Hypoacusis, ataxia, myopathy, neuropathy and exercise intolerance were the most common neurological symptoms in the MTD group (Table 2). HN patients mostly had distal type paresis and muscle atrophy (Table 2).

5.3.2. Medication

Medication for the MTD comprised of Coenzyme Q10, Vitamin E and Vitamin C. Some patients were taking psychiatric drugs at the time of the assessment. Monotherapies were clonazepam (Pts 7, 10, 14), sertraline (Pt 12) and mirtazapine (Pt 13). Combination therapies were quetiapine and trazodone for Patient 16, clonazepam, sertraline and quetiapine for Pt 18 and aripiprazole with duloxetine and clonazepam for Pt 19. Anticonvulsant treatment was valproate for Pts 7 and 8, carbamazepine for Pt 14 and lamotrigine for Pt 16 (Table 2). In the HN group, prescribed CNS drugs were duloxetine and pregabalin for Pt 23, chlordiazepoxide for Pt 25 and gabapentin with duloxetine for Pt 28. (Table 3). Gender, age, duration of disease, mtDNA genotypes, clinical symptoms and medications of MTD patients (patient group) are outlined in Table 2. Gender, age, mtDNA genotypes, clinical symptoms and medications of of HN patients (control group) are outlined in Table 3.

Table 2. Gender, age, duration of disease, mtDNA genotypes, clinical symptoms and medication of MTD patients (patient group). Grey color fill indicates unrelated patients (independent variables) included in the statistical analysis.

ID	Gender, age, disease duration (years)	mtDNA mutation type	Clinical symptoms and findings	Medication (with total daily dose)
1	F 34 (20)	m.3243 A>G MELAS	Hypoacusis, ataxia, myopathy, endocrine dysfunction	-
2	F 51 (30)	m.3243 A>G MELAS	Ataxia	-
3	F 34 (20)	m.3243 A>G MELAS	PEO, myopathy (mother of Patient 4)	-
4	F 17 (10) [daughter of Pt 3]	m.3243 A>G MELAS	PEO	-
5	M 32 (4)	m.8344 A>G MERRF	Migraine	-
6	F 61 (10) [mother of Pt 8]	m.8344 A>G MERRF	Mild tremor in the upper limbs (mother of Pt 7 and 8)	-
7	M 34 (17) [twin brother of Pt 8]	m.8344 A>G MERRF	Myoclonus epilepsy, thrombocytopenia (twin brother of Pt 8)	levetiracetam (1000 mg), clonazepam (2 mg), valproate (1500 mg), vinpocetine (20 mg)
8	M 34 (17)	m.8344 A>G MERRF	Myoclonus epilepsy, ataxia, cognitive dysfunction, thrombocytopenia (twin brother of Pt 7)	levetiracetam (2500 mg), valproate (2100 mg), vinpocetine (90 mg), betaxolol (10 mg), atorvastatine (10 mg), allopurinol (200 mg)
9	F 59 (30) [mother of Pt 10]	m.8993 T>G NARP	Developmental abnormality of the right arm, hypothyreosis	-
10	M 23 (18)	m.8993 T>G NARP	NARP	clonazepam (1 mg) vinpocetine (10 mg)
11	F 34 (20)	mtDNA del	PEO, iron-deficient anemia, hypercholesterolemia	-

12	F 40 (14) [sister of Pt 11]	mtDNA del	Myalgia, exercise intolerance	sertraline (50 mg)
13	F 38 (20)	mtDNA del	Kearns-Sayre syndrome	clonazepam (1mg) vinpocetine (20 mg) mirtazapine (30 mg)
14	M 20 (15)	m.8332 A>G m.8270 C>T	Dystonia, early onset stroke-like symptoms	levetiracetam (1000 mg), clonazepam (1.25mg) carbamazepine (1000 mg)
15	F 22 (9)	m.12770 A>G	Ataxia, hypoacusis, spastic paraparesis	-
16	F 39 (20)	m.15326 A>G m.750 A>G m.1438 A>G m.8860 A>G m.15326 A>G	Hypoacusis, limb tremor, myalgia, apraxia (mother of Pt17)	trimetazidine (60 mg), metformin (850 mg), bisoprolol (5 mg), quetiapine (400 mg), lamotrigine (50 mg), trazodone (300 mg)
17	M 20 (7) [son of Pt16]	see Pt 16.	-	-
18	F 37 (23)	m.3720 A>G m.3849 G>A m.13020 T>C m.13734 T>C m.12308 A>G m. 8473 T>C	Severe cardiomyopathy, cognitive dysfunction	trimetazidine (40 mg), enalapril (5 mg), molsidomine (4 mg), quetiapine (400 mg), lamotrigine (100 mg), sertraline (100 mg), clonazepam (1.5 mg)
19	F 41 (17)	m.14766 C>T m.709 G>A m.73 A>G m.16126 T>C	Peripheral neuropathy	tolperisone (300 mg), aceclofenac (200 mg), aripiprazole (15 mg), duloxetine (30 mg), clonazepam (1 mg)

Table 3. Gender, age, mtDNA genotypes, clinical symptoms and medication of of HN patients (control group)

ID	Gender and age (years)	PMP22 mutation type	Clinical symptoms and findings	Medication (with total daily dose)
20	M, 41	CMT	Atrophy of the hand and feet muscles	-
21	M, 17	CMT	Pes equinovarus, paresis in the distal muscles of the legs	-
22	M, 49	HNPP	Sensory disturbances in the lower limbs, neuropathy to pressure palsy	-
23	F, 33	CMT	Generalized muscle weakness, walking difficulty, distal type hypesthesia, excavated feet	pregabalin (300 mg) duloxetine (30 mg)
24	M, 34	CMT	Paresthesia, distal type hypesthesia in the limbs, muscle cramps, gait instability	-
25	F, 46	CMT	Distal type muscle weakness in the limbs, tremor in the hands	chlordiazepoxide (5 mg), tolperisone (150 mg) ,
26	F, 31	CMT	Excavated feet, distal paresis of the limbs, ataxia	-
27	M, 42	CMT	Mild paresis in the hand and feet muscles	-
28	F, 55	CMT	Diabetes mellitus, hyperlipidaemia, distal weakness of the limbs, distal type hypesthesia, head tremor, gait instability	duloxetine (60 mg) gabapentin (600 mg) rosuvastatin (20 mg) ezetimibe (10 mg)
29	M, 47	CMT	Bronchial asthma, atrophy and paresis in the hand and feet muscles, gait instability	-

5.3.3. Neuroimaging

Various changes have been found on neuroimaging studies (Table 7). Multiple cerebral (Pt 2) or cerebellar (Pt 10) parenchymal lesions, focal contrast enhancing lesion at the left insula (Pt 5) and near the left occipital horn (Pt 18), paraventricular (Pt 18) and basal ganglia (Pt 2) calcifications were detected. Pt 3 had cortical, Pt 15 had cerebellar atrophy and Pt 1, 7 and 8 had both. Pt 2 and 18 had occipital lesions. MRI spectroscopy results showed low levels of choline, elevated glutamate for Pt 1, low levels of N-AcetylAspartic acid, creatine, choline for Pt 8 and elevated lactate peak for both of them. Pt 4 (the m.3243 A>G mutation with PEO symptoms), 6 (MERRF), 9 (NARP), 11-13 (mtDNA deletion), 14, 17, 19 (mt DNA polymorphism) had normal MRI findings. The mean HAQ-DI and GSI score of the probands in this subgroup did not differ significantly from those of the rest of the group (0.62 vs 0.93, $p = 0.417$ and 0.98 vs 1.65, $p = 0.178$, respectively). In this subgroup, only Pt 12 and 17 were free of physical symptoms. Pt 18 did have MRI alteration, but only exercise intolerance as physical symptoms and a high GSI score. Neurological symptoms, CT or MRI findings and WAIS FSIQ scores of MTD patients are presented in Table 4.

Table 4. Neurological symptoms, CT or MRI findings and FSIQ scores of MTD patients. Grey color fill indicates unrelated patients (independent variables) included in the statistical analysis.

ID	Neurological symptoms	CT/MRI finding	VQ	PQ	FSIQ
1	Hypoacusis,ataxia, lower limb paresis	Cerebellar and cortical atrophy	97	53	74
2	Hypoacusis, myopathy, neuropathy, exercise intolerance, ataxia	Multiple demyelinating lesion in the occipital lobe, at the sides of the lateral ventricles, calcifications in basal ganglia	93	92	92
3	PEO, severe myopathy	Slight cortical atrophy	81	72	74
4	PEO	Normal findings	93	81	86
5	Migraine	9 mm contrast-enhancing lesion at the left insula	118	119	120
6	Mild limb tremor	Normal findings	119	119	119
7	Myoclonus epilepsy, ataxia (MERRF)	Slight cortical and cerebellar atrophy	95	96	94
8	Myoclonus epilepsy, severe ataxia (MERRF)	Cerebellar and cortical atrophy	87	71	77
9	Limb deformation	Normal findings	121	121	122
10	NARP, dysarthria, severe ataxia	Multiple parenchymal lesions in both cerebellar hemispheres	68	47	55
11	PEO	Normal findings	115	140	129
12	-	Normal findings	126	132	131
13	KSS	Normal findings	104	123	114
14	Dystonia, early onset stroke-like symptoms	Normal findings	77	76	75
15	Ataxia, hypoacusis, spastic paraparesis	Hypoplasia, cerebellar atrophy, Wider posterior scala and 4th ventricle	108	101	105
16	Hypoacusis, limb tremor, apraxia	Left frontal cortical dysgenesis with venous dilation	96	109	103
17	-	Normal findings	120	135	129
18	Exercise intolerance	contrast accumulating lesion at the left occipital horn, paraventricular calcifications temporally and frontally	100	96	98
19	Severe peripheral neuropathy, distal type weakness in the limbs	Normal findings	117	124	122

5.4. Psychiatric findings

The MTD and HN groups' BDI-SF and HDRS score differed significantly (12.85 vs 4.40, $p<0.031$, and 15.62 vs 7.30, $p<0.043$, respectively). Statistical difference was also found in the GSI score (1.44 vs 0.46, $p<0.013$) and the nine subscales of the SCL-90-R scale (Table 9). These subscales were obsession-compulsion ($p<0.008$), interpersonal sensitivity ($p<0.008$), depression ($p<0.031$), anxiety ($p<0.031$), hostility ($p<0.143$), phobic anxiety ($p<0.031$), paranoid ideation ($p<0.01$) psychoticism $p<0.000$) and additional items ($p<0.013$). No significant difference was found between the two groups' somatization score (Figure 5).

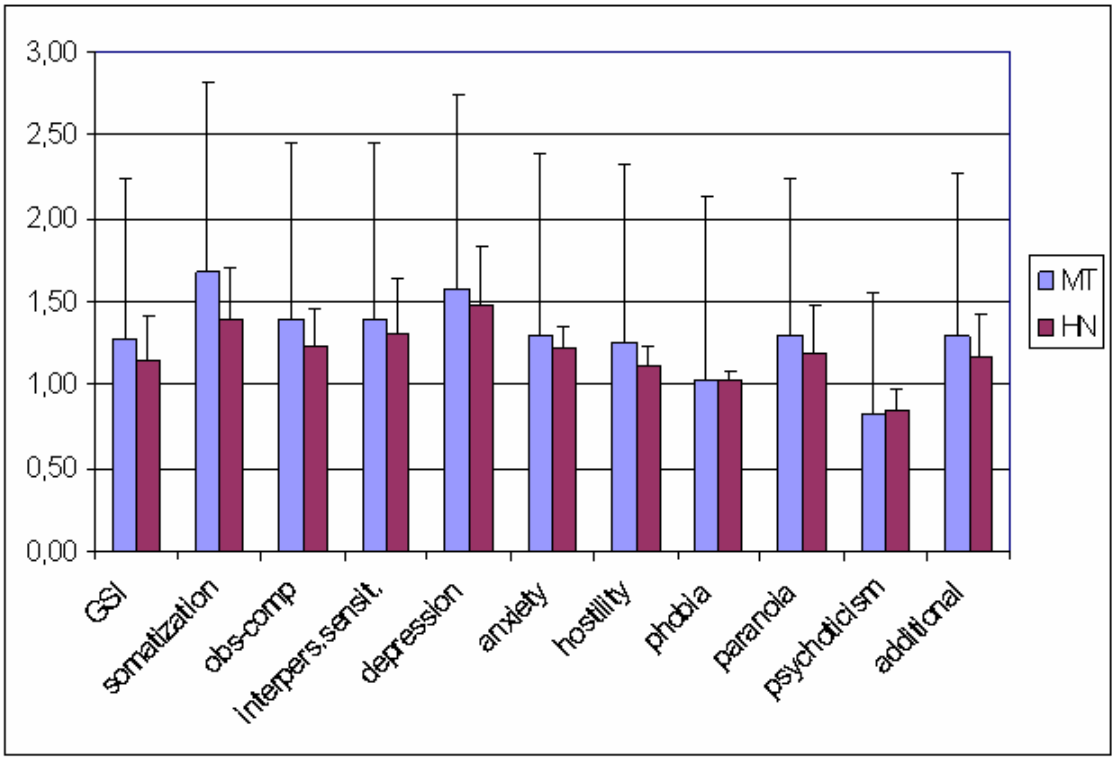

Figure 5. Results of the SCL-90-R questionnaire with the GSI and subscales.

Patients harboring the S/S genotype had lower levels of depression than the rest of the group (BDI average score of 4.5 vs 11.8, see Table 5). BDI-SF, GSI, HAQ-DI scores and 5HTTLPR genotype of MTD patients are presented in Table 5.

Table 5. BDI-SF, GSI, HAQ-DI scores and 5HTTLPR genotype of MTD patients

ID	BDI	GSI	HAQ-DI	5HTTLPR genotype
1	22	1.92	1.375	L/S
2	8	1.09	0.875	L/S
3	8	1.09	1.25	L/S
4	14	1.24	0.125	L/S
5	21	2.46	0	L/L
6	0	0.33	0	S/S
7	2	0.31	0.375	S/S
8	3	0.87	1.625	S/S
9	8	0.59	0	L/S
10	7	0.44	1.375	L/L
11	10	0.86	0	L/L
12	2	0.22	0	L/S
13	16	1.19	0.375	L/S
14	4	0.78	0.125	L/S
15	5	0.74	1.625	L/S
16	30	3.02	0.625	L/L
17	2	0.25	0	L/L
18	20	3.2	0.625	L/S
19	13	1.08	0.75	S/S

A variety of psychiatric disorders; current diagnosis in 6 (31%), past diagnosis in 8 (42%), lifetime prevalence in 9 MTD cases (47%) were diagnosed with SCID-I (Table 6). Lifetime prevalence was 10% (2 patients) for major depressive disorder, for dysthymia, for bipolar II and for mood disorder due to general medical condition, 5% (1 patient) for major depression with psychotic features, for bipolar I, for mixed anxiety-depressive disorder, for postpartum depression and for PTSD. Three avoidant, 2 obsessive-compulsive personality were diagnosed in the MTD group. In 3 cases, personality disorder not otherwise specified (NOS) was detected referring to depressive

personality in case of Pt 5, and mixed personality disorder in case of Pts 16 and 18. Personality disorder was found in 8 MTD cases (42%, Table 6).

Table 6: SCID-I and SCID-II results of MTD patients. Grey color fill indicates unrelated patients (independent variables) included in the statistical analysis.

ID	Past diagnosis SCID I.	Current diagnosis SCID I.	Personality disorder SCID II.
1	Mixed anxiety-depressive disorder	Major depressive disorder	Avoidant
2	Dysthymia	-	-
3	-	-	-
4	-	-	-
5	-	Dysthymia	Personality disorder NOS
6	-	-	-
7	Adjustment disorder with depressed mood	-	-
8	Adjustment disorder with depressed mood	-	Avoidant
9	-	-	Obsessive-compulsive
10	-	-	-
11	-	-	-
12	-	-	-
13	Bipolar II disorder	Bipolar II, current episode depressive	-
14	-	-	-
15	-	-	Obsessive-compulsive
16	Major depression with psychotic features	Major depressive disorder	Personality disorder NOS
17	-	-	Avoidant
18	PTSD	Bipolar II, current episode depressive	Personality disorder NOS
19	Postpartum depression	Bipolar I, latest episode depressive	-

In the HN group, depression was more present in those with the S/S (BDI score of 6.75 vs 2.83, Table 7.). Correlation analysis was not done due to the small number.

Table 7. BDI-SF, SCL-90-R GSI, HAQ-DI scores and 5HTTLPR genotype of HN pts

ID	BDI	GSI	HAQ-DI	5HTTLPR genotype
20	1	0.22	0	L/L
21	1	0.32	0.125	L/L
22	11	1.3	0.375	S/S
23	2	0.18	0.875	S/S
24	2	0.12	0.5	L/S
25	2	0.26	1.25	L/S
26	11	0.37	1.125	L/S
27	0	0	0	S/S
28	14	1.59	1.25	S/S
29	0	0.25	1.625	L/L

Three patients had past and current diagnosis. Lifetime prevalence was 20% (2 patients) for dysthymia, 10% (1 patient) for MDD, bipolar II, mood disorder due to general medical condition and alcohol abuse (Table 8). No personality disorder was found

Table 8. SCID-I and SCID-II results of HN patients.

ID	Past diagnosis SCID I.	Current diagnosis SCID I.
20	-	-
21	-	-
22	Adjustment disorder with depressed mood	Dysthymia
23	-	-
24	-	-
25	-	-
26	-	-
27	-	-
28	MDD	Dysthymia
29	Alcohol abuse	Bipolar II, latest episode hypomanic

Table 9. Results of GSI and the ten subscales of SCL-90-R, BDI-SF and HDRS in the group of MTD vs HN patients. Adjusted_p: p values with Bonferroni-Holm correction. *Asterisks refer to statistically significant differences between the two groups.

	MTD group		**HN group**		
Measured items	**mean**	**SD**	**mean**	**SD**	**p-value (adjusted)**
GSI	1.44	0.91	0.46	0.53	0.0130*
Somatization	1.77	1.10	0.98	0.83	0.0817
Obsessive-compulsive	1.65	1.04	0.47	0.68	0.0079*
Interpersonal sensitivity	1.55	0.97	0.40	0.51	0.0079*
Depression	1.90	1.21	0.75	1.03	0.0309*
Anxiety	1.32	1.14	0.42	0.63	0.0309*
Hostility	1.26	1.00	0.48	0.50	0.0428*
Phobia	1.14	1.11	0.29	0.56	0.0309*
Paranoia	1.42	1.05	0.28	0.39	0.0101*
Psychoticism	0.92	0.60	0.13	0.24	0.0002*
Additional items	1.48	0.96	0.43	0.66	0.0130*
BDI	12.85	8.33	4.40	5.36	0.0309*
HDRS	15.62	8.62	7.30	5.52	0.0428*
HAQ-DI	0.82	0.59	0.71	0.59	0.6076

5.5. Cognitive symptoms

5.5.1. Results of the neuropsychological assessment

Results of the RAVLT show that both groups learned new words with each repetition from trial1 (RAVLT1) to trial 5 (RAVLT5) (Inczedy-Farkas - Trampush et al, 2014). There is positive correlation between the number of words retained and the number of trials, which is slightly stronger in the control group ($r=0.603$, $p<0.0001$ for patients vs. $r=0.748$ $p<0.0001$ for controls). Patients performed below controls and also below normative data. We detected impaired short-term (RAVLT1, mean of patients = 5.46 ± 2.1062, mean of controls = 8.0 ± 1.354, $p = 0.0015$) and delayed recall (RAVLT6, mean of patients: 7.15, mean of controls = 12.15, $p = 0.0001$).

The number of read words differed significantly on both the Stroop C and the Stroop CW (Stroop C_60 Pt: 65.77±27.7, controls: 87.08±8.36, $p = 0.018$, Stroop CW_60 Pt: 42.77±22.9, Controls: 61±11.68, $p = 0.021$) No difference has been found in the number of errors on either test (Stroop C_error Pts: 0.77 ± 1.16, Controls: 0.75±1.35, $p = 0.97$, Stroop CW_error Pts: 3±3.96, Controls: 1. 08±1.44, $p = 0.127$). Controls performed close to normative data. No significant difference of the Stroop errors (StroopCW_error minus StroopC_error) was found in either group (Pts: 2.23, $p = 0.064$, controls: 0.33, $p = 0.601$).

Results on TMT show that patients' performance is over the cut-off values for abnormality (Lezak 2004) (TMTA: 96.39 vs. 86 (1st percentile), TMTB: 186.08 vs. 155 (10th percentile). Five patients could not complete the TMTB task within 300 seconds, which was discontinued and the TMTB time recorded as 300 ms. Error rate of these patients could not be recorded and were thus excluded from further calculations. Errors were supposed to be reflected in the overall completion time of the test.

Motor function was found to be impaired, although patients could perform the task without any mistakes – only in a much slower way (TMTA time Pts: 96.39 ± 62.5 msec (SD), controls: 34.15 ± 8.1 msec (SD), $p = 0.0016$). The difference was greater when motor and executive functions were assessed simultaneously a in a more complex task (TMTB time Pts: 186.08 ± 109.3 msec, controls: 64.39 ± 26.8 msec, $p = 0.0007$).

Patients with TMTA time exceeding 100 sec performed significantly worse on the WAIS Block Design subscale (mean of Pts with a TMTA_time>100 sec: 71.6, mean of Pts with TMTA_time<100 sec: 96.25, p = 0.0497).

In both groups, scores were higher for category than for letter fluency (Pts: 48.77 ± 21.8 vs 23 ± 11.9 (p = 0.0001), controls 67.77 ± 12 vs 38.8 ± 10.1 (p = 0.0001) showing better performance. Difference of means (mean of category fluency minus mean of letter fluency) was not found to be significant between the groups (mean of difference for Pts: 25.77. mean of difference for controls: 31.5, p = 0.4166) showing a similar pattern in both groups. Patients' performance was 71.9% of the controls' on the letter fluency, while it was 59.3% on the category fluency test (Figure 6).

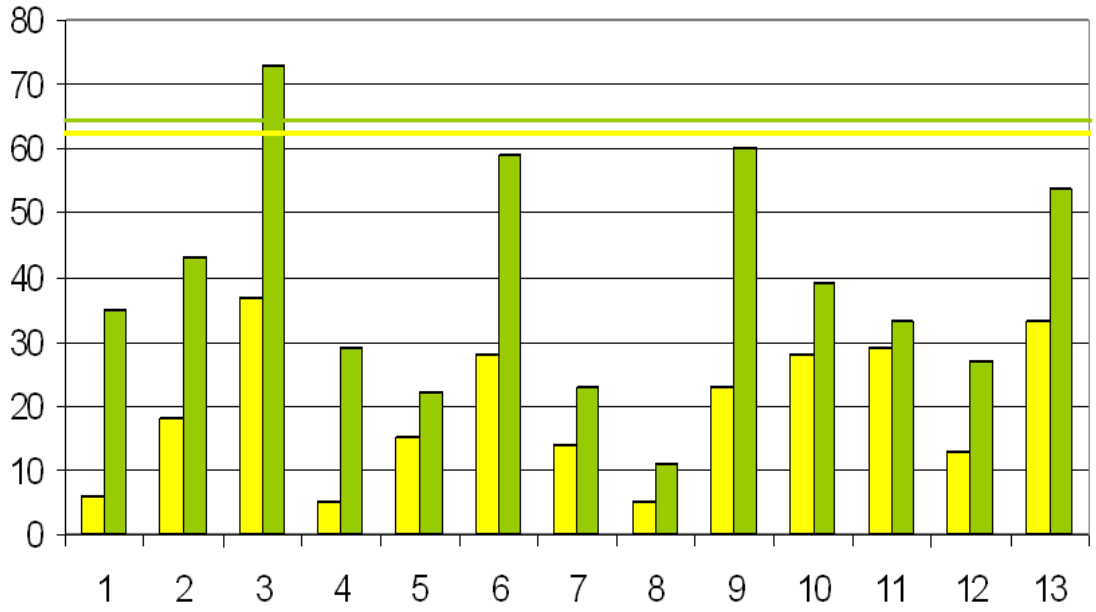

Figure 6. Patients' results on the fluency tests as compared to controls' scores; semantic fluency (green): 71.9%, letter fluency (yellow): 59.3%.

General intelligence, assessed with the WAIS, was in a lower zone of the normal range for the Pt group (FSIQ Pts: 95.2 ± 22.8, controls: 123.7 ± 8.6, p = 0.0003, patients' mean is 76.9% of the controls' mean). Only one Pt exhibited mental retardation (FSIQ<70). The difference between the two groups was significant for both the VQ and the PQ, although a greater impairment was detected in the latter component showing primarily nonverbal impairment (VQ Pts: 97.00 ± 15.7, controls: 117.62 ± 9.9, p =

0.0007, patients' mean is 82.5% of the controls), PQ Pts: 94.1 ± 28.8, controls: 127.23 ± 8.3, p = 0.0006, patients' mean is 73.9% of controls). Results of the WAIS are shown in Table 10. Scores of the 19 patients as well as scores of the 13 probands as percentage of controls' are shown in Figure 7.

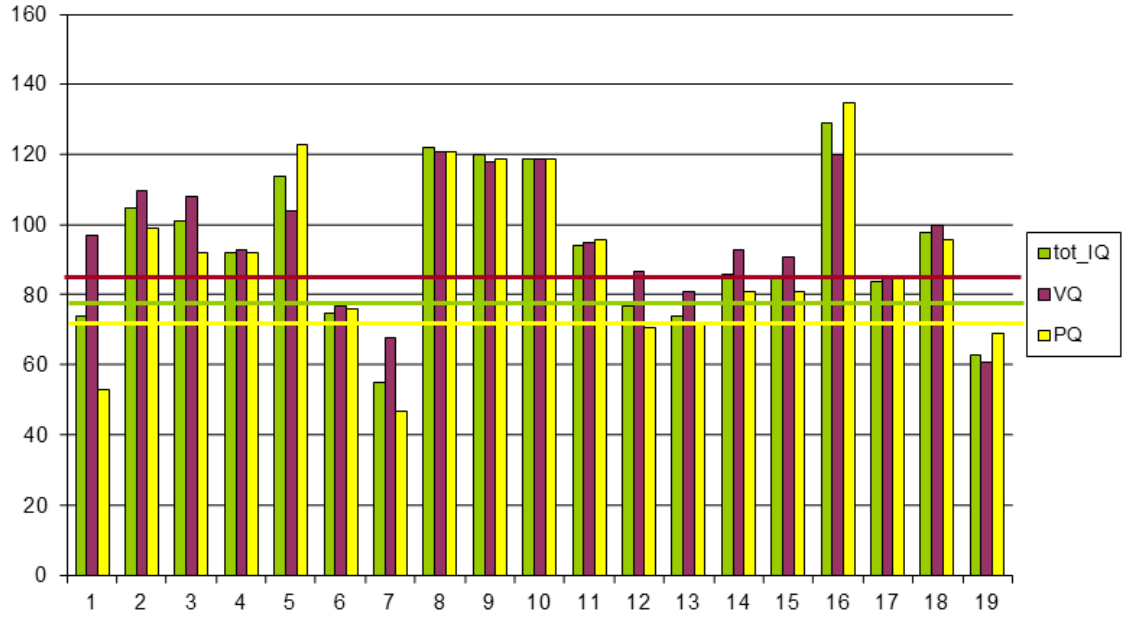

Figure 7. Patients' scores are shown as percentage of controls' results. FSIQ: 76.9%, VQ: 82.5%, PQ: 73.9%.

Patients performed significantly weaker on most subscales, including the following VQ domains: Information (102.9 ± 15.54 vs 118.8 ± 12.1, p = 0.008), Digit Span (94.3 ± 13.4 vs 112.7 ± 12.7, p = 0.001), Arithmetic (86.2 ± 21.3 vs 104.4 ± 15.1, p = 0.021), Comprehension (92.9 ± 10.9 vs 114.8 ± 11.3, p = 0.000). PQ domains with significant difference were: Picture completion (109.6 ± 27.2 vs 130.3 ± 11.5, p = 0.018), Block design (86.7 ± 21.6 vs 117.1 ± 10.3, p = 0.00), Object assembly (84.2 ± 23.1 vs 107.9 ± 5.5, p = 0.001), Digit symbol (99.3 ± 22.9 vs 133.5 ± 8.6, p = 0.00). Subscales where no difference was found were Similarities (110.7 ± 13.4 vs 118.6 ± 8.5, p = 0.089) and Picture arrangement (101.5 ± 7.6 vs 112.6 ± 10.2, p = 0.136 Results of the RAVLT, Stroop, TMT, Fluency and FSIQ tests are summarized in Table 10.

Table 10. Raw data of the neuropsychological tests used with means and standard deviations for MT patients and controls and the cognitive domains tested. *asterisks refer to statistically significant alterations

Test applied	**Patient**	**Control**	**p-value**	**Cognitive domain tested**
RAVLT 1-5	0.60 0.000*	0.75 0.000*		Learning ability
RAVLT1	5.46±2.16	8.0±1.35	0.001*	Immediate recall
RAVLT6	7.15±3.05	12.15±1.67	p=0.000 *	Delayed recall
StroopC_60	65.77±27.7	87.08±8.36	p=0.018*	Selective attention, Cognitive flexibility, Interference control
StroopC_error	0.77±1.16	0.75±1.35	p=0.97	
StroopCW_60	42.77±22.9	61±11.68	p=0.021*	
StroopCW_error	3±3.96	1.08±1.44	p=0.127	
TMTA time (msec)	96.4±62.5	34.15±8.1	p=0.001*	Visuo-spatial abilities, Attention, psychomotor speed Flexibility, working memory
TMTB time (msec)	186.1±109.3	64.4±26.8	p=0.000*	
Category Fluency Sum	48.77±21.8	67.77±5.9	p=0.006*	Executive functions Psychomotor speed
Letter Fluency Sum	23±11.9	38.8±9.6	p=0.001*	
FSIQ	**95.2±22.8**	**123.7±8.6**	**p=0.003***	**General intelligence**
VQ	**97±15.7**	**117.6±9.9**	**p=0.007***	**Verbal skills**
Information	102.9±15.54	118.8±12.1	p=0.008*	Knowledge base Premorbid functioning
Comprehension	92.9±10.9	114.8±11.3	p=0.000*	Social norms, judgment
Digit Span	94.3±13.4	112.7±12.7	p=0.001*	Short-term memory, attention
Arithmetic	86.2±21.3	104.4±15.1	p=0.021*	Calculation, problem solving
Similarities	110.7±13.4	118.6±8.5	p=0.089	Abstract reasoning, logic
PQ	**94.1±28.8**	**127.2±8.3**	**p=0.006***	**Perceptual organization**
Digit Symbol	99.3±22.9	133.5±8.6	p=0.000*	Visuo-motor speed and coordination, attention
Picture Completion	109.6±27.2	130.3±11.5	p=0.018*	Visuo-perceptual abilities
Picture Arrangement	101.5±7.6	112.6±10.2	p=0.136	Social skills, planning
Block Design	86.7±21.6	117.1±10.3	p=0.000*	Visuo-spatial construction
Object Assembly	84.2±23.1	107.9±5.5	p=0.001*	Visual synthesis

Significantly lower score on the WAIS Block Design has been found for those with a long TMTA time (greater than 100 sec) (71.6 vs 96.25, p = 0.0497).

None of these results showed correlation with duration of disease or GSI scores. However, positive correlation was found between age and StroopC_60 score (r = 0.601, p= 0.029), as well as between age and TMTA time (r = 0.609, p = 0.027). Negative correlation between HAQ-DI FSIQ was also significant (r = -0.594 p = 0.032).

Group I. Pts had significantly worse performance than all other patients on the RAVLT5 (p = 0.01) and sum of letter fluency (p = 0.009).

Patients with normal neuroimaging findings had a higher mean FSIQ (110 vs 91.33), VQ (103.25 vs 94.22) and PQ (115.75 vs 84.44) than the rest of the group, although these were not significant differences (p-values of 0.246, 0.427, 0.106, respectively). Patients with structural alteration in the cerebellum (atrophy, parenchymal lesions, Pt 1, 7, 8, 10, 15) had significantly lower PQ (68 vs 105, p = 0.042) and greater VQ-PQ difference (22 vs 5, p = 0.029), than the rest of the group. The VQ and FSIQ scores of the same subcohorts did not differ (p = 0.035 and p=0.088, respectively).

The performance of our control group was close to normative data in the RAVLT, Stroop, TMT and Fluency Tests as well as on WAIS.

5.5.2. Longitudinal follow-up of Patient 16

In case of Patient 16 we had the opportunity to carry out a more complex neuropsychological test procedure with a follow-up examination 8 months apart (Inczedy-Farkas – Trampush et al 2014).

On the first test, Raven test yielded an average level of FSIQ (46 points), which means that the reasoning in visual modality was intact. The quality of sustained attention in visual modality was good, but it was very slow and signs of fatigue were detectable (Toulouse-Pieron Attention Test). The visual scanning was not well-organised enough (Bells Test).

In her memory functions she had difficulties mainly in visual modality. Her performance on the Benton Visual Retention Test was very weak: the visual immediate memory and the visuospatial constructional abilities were damaged. The incidental immediate visuospatial recall (memory part of the Rey-Osterrieth Complex Figure Test)

was close to normal. As per verbal memory; the immediate memory span, learning of new verbal material, and delayed recall of the verbal items were intact. There were no signs of interference [proactive and retroactive inhibition] (Rey Auditory-Verbal Learning Test). Short-term memory and sequential processing (forward and backward digit-span) worked on average level (6 and 5 items). Story recall was intact.

In her spontaneous speaking mild difficulties were observed in word finding. Comprehension of complex grammatical phrases and sentences was difficult. She could carry out continuous loud reading of longer words only by spelling. In writing of dictated material omissions and perseverations of letters were observed in case of long words. Her dynamic praxis was damaged – the patient reported inability in typing and continuous writing. Ideomotor praxis was also injured. Constructional praxis was nearly intact. There were signs of finger-agnosia and mistakes in right-left discrimination.

She carried out basic mathematical operations well, but not complex mathematical tasks. Functions of planning, self-control and self-correction were intact.

On the second test the quality of sustained attention in visual modality was invariably good, but its speed was also very slow and more signs of fatigue were detectable (Toulouse-Pieron Attention Test). Attention deficit was evident during solution of diverse tasks. Her verbal memory functions – found intact on the first test – showed signs of deterioration (Rey Auditory-Verbal Learning Test) and the visuo-spatial memory functions worsened further (Benton Visual Retention Test). Short-term memory and sequential processing (forward and backward digit-span) worsened too (4 and 3 items). However, story recall remained intact.

In her spontaneous speaking mild word finding difficulty was observed. Comprehension of complex grammatical phrases and sentences proved to be complicated for her. Continuous reading and writing of dictated material showed more omissions and perseverations of letters compared to the first test, and sometimes she could differentiate letters b-d and p-d only with difficulty. The dynamic and ideomotor praxis were damaged to the same extent as on the first session with a nearly intact constructional praxis. There were signs of finger-agnosia and mistakes in right-left orientation. She performed basic mathematical operations well, but had difficulty comprehending more complex mathematical tasks. Planning was intact, but self-control, self-correction started to show signs of deterioration.

5.6. La belle indifference - general characteristic or individual trait?

In the topic of the frequently observed lack of concern about the disease process in our MTD patients, we presented the case of Patient 2, a now 50-year-old female in detail (Inczedy-Farkas et al, 2011).

Her symptoms began at age 6 with a few months' period of frequent vomiting. Lab results showed ketosis and iron-deficiency anemia. In her teenage years, she was treated for arthritis. Around the age of 20 she had two car accidents, both resulting in multiple limb fractures and a head concussion. After the second accident, the patient complained of hearing impairment and seizure-like episodes consisting of severe episodic headaches associated with vertigo and nausea. An X-ray revealed block formation of the C5/C6 vertebrae. A CT scan detected calcification of the basal ganglia; slightly decreased levels of parathyroid hormone and of serum calcium were also found. An abnormality in calcium metabolism was suspected but was not further explored, and the hearing impairment was considered as part of a post-concussion syndrome. Nootropics were administered without effect. Although the patient achieved secondary level education, she was unable to keep even undemanding jobs because of fatigue and headache, for which the adrenergic vasopressor pholedrine proved to be the only effective treatment. The patient was found eligible for an incapacity benefit.

A few years later, still in her twenties, inflammation of multiple organs was observed, including pyelonephritis, chronic hepatitis, pancreatitis, jejunitis and terminal ileitis resulting in the atrophy of the intestinal mucosa. She was losing weight due to lactose intolerance and malabsorption. Results of routine blood, liver, and kidney function tests were normal. The heterozygotic form of cystic fibrosis was hypothesized but was challenged by a negative sweat test. The patient also complained of dysmenorrhea. Her endocrinologic profile was normal except for an elevated prolactine level that normalized spontaneously. Multiple adnexitis was found and—despite hydrotubation and abrasion of the occluded tubes—total amenorrhea and postmenopausal osteoporosis developed. Dysthymia was also diagnosed. In her thirties, progression of the conductive hearing impairment, as well as transient paralysis of the right arm was observed. Neurological examination revealed hypotrophic limb muscles, mild truncal ataxia, and decreased deep tendon reflexes on the lower extremities.

When the patient was 40, the incapacity benefit was reevaluated and the revising physician gave her the summarizing diagnosis of somatoform disorder. No psychiatric treatment was offered, but the patient's incapacity benefit was reduced.

A few years later, apraxia and resting tremor of the hands developed and she underwent an extensive workup. A CT scan showed progression of the basal ganglia calcification with confluating ischemic lesions periventricularly. An MRI scan found multiple demyelinating lesions in the occipital lobe, the frontal horn, and the trigone of the lateral ventricles. Immunological investigation revealed elevated levels of albumin and IgG both in the serum and the cerebrospinal fluid, but oligoclonal bands were not present. EEG revealed theta-beta paroxysms with frontal lobe dominance and occasional spikes, provoked by hyperventilation. Based on these findings, the possibility of Fahr-syndrome and CADASIL arose. The patient was put on long-term disability status with financial support and was referred to our center for further investigation.

On assessment in our center, sequence analysis did not find alteration in the NOTCH3 gene. However, an elevated level of resting serum lactate (4 mmol/l), creatine-kinase (CK, 305 U/l) and lactate dehydrogenase (LDH, 490 U/l) were found. A neurological examination found bilateral ptosis, dysarthria, diffusely hypotrophic muscles, latent paresis of the right arm, hypesthesia in the extremities, generalized areflexia and marked truncal ataxia. Psychiatric assessment showed subclinical depression with the BDI-SF and HDRS (scores of 8 and 9, respectively), SCID-I and SCID-II detected no psychopathology. Neuropsychological assessment measured an FSIQ of 92, a PQ and a VQ of 93, which is a balanced but subnormal profile. Task performance was slowed, short- and long-term memory were both impaired. Her family history was positive for head tremor in her mother, beginning at the age of 70. Revision of the patient's medical history, the clinical picture together with laboratory data suggested mitochondrial disorder. The diagnosis was confirmed by myopathological and genetic investigation that revealed the m.3243 A>G nucleotide substitution (with a heteroplasmy rate of 35%), causing MELAS syndrome, which explained the diverse nature of the patient's symptoms.

5.7. NEPSYBANK

NEPSYBANK is a disease-based biobank collecting both phenotypical and environmental data and biological materials such as DNA/RNA, whole blood, plasma, cerebral spinal fluid, muscle / nerve / skin biopsy, brain, and fibroblast. The target of the diseases is presently stroke syndromes, dementias, movement disorders, motoneuron diseases and mitochondrial disorders. NEPSYBANK coordinates the biobanking activities of the neurological and psychiatry departments of the four medical universities in Hungary. Anamnesis, childhood development, family history, medical and neurological status, ECG, electrophysiological records, neuroimaging, laboratory data, pathological records, respiratory chain activities and medication of 79 patients with MTD has been uploaded to the registry.

Another biobank focusing on the neurodevelopmental disease schizophrenia (SCHIZOBANK) has also been built with the coordination of our Center. Mitochondrial dysfunction is frequent in schizophrenia and thus this biobank has been a valuable source of our research. Between 2009 and 2013, detailed clinical data and biological samples of 535 patients with schizophrenia have been uploaded (www.schizobank.hu) by the participating institutions (Departments of Psychiatry at Semmelweis University, Nyiro Gyula Hospital in Budapest, the Health and Science Center of the University of Debrecen, of BAZ County Hospital and the University of Szeged).

6. Discussion

6.1. Epidemiology of mitochondrial disorders

Our group performed the first systematic assessment of the frequency rate of the most common mtDNA mutations - m.3243 A>G, m.8344 A>G, m.8993 T>C and m.8993 T>G tRNA mutations and the common mtDNA deletions - in Central-Eastern Europe.
In our study, the frequency of the m.3243 A>G mutation was calculated to be 2.61%. Frequency of the m.3243 A>G mutation in Hungary has previously been reported to be 2.22%, based on a smaller cohort (631 patients) (Gal et al, 2010). The frequency of the m.8344 A>G substitution was found as 1.47% which fits into the hitherto published wide range in the Caucasian race. The result of 0.45% for the m.8993 T>C and m.8993 T>G substitutions in MT-ATP6 is in the lower range of hitherto published data.
The "common" mtDNA deletion most commonly results in PEO and KSS (diMauro et al, 2003). The most prevalent clinical symptoms in our subgroup of patients with mtDNA deletion were myopathy and PEO. Confirming literature data, the majority of our cases with single deletion harbored the so called 4977 bp-long "common" mtDNA deletion, located between nts 8470 and 13447. Our frequency results are similar to those published in other Caucasian populations. The marked variability of published prevalence data of mitochondrial mutations in different countries is possibly due to the difference in selectional criteria and the examined tissue (blood or muscle) making it difficult to compare results of different studies. It is important that similar prevalence studies be carried out in different populations in the future in order to accurately assess the importance and impact of mitochondrial diseases and to adequately manage these patients.

6.2. Mitochondrial psychiatry?

6.2.1. Affective spectrum disorders

In MTDs, mitochondrial encephalopathy has been presented as part of the multisystemic symptoms. CNS impairment would logically result not only in neurologic but also, in

psychiatric symptoms. However, very few systematic studies have been carried out so far, only case reports have been published reporting mostly depression (Suomalainen et al 1992). Our results of the BDI-SF, HDRS and SCID-I revealed a higher prevalence of psychiatric symptoms, especially affective spectrum disorders in the MTD group as compared to a disease control group. We checked the 5HTTLPR polymorphism from which the S/S has been implied in the genetic background of depression (Cervilla et al, 2006, Middeldorp et al, 2007, Gonda et al, 2009). In the control group, the subgroup with the S/S polymorphism had much higher levels of depression (reflected in a mean BDI score) than the rest of their group. This was not the case in the MTD group where patients harboring the S/S alleles had lower level of depression than the rest of the group.

Mitochondrial dysfunction is supposed to be one factor in the multifactorial development of mood disorders, beside monoamine transmission, hypothalamus-pituitary-adrenal axis function, immune function, neurogenesis dysfunction, and neuropeptide signaling (McClung, 2013)

6.2.2. Other psychiatric symptoms

Significant differences were found in 9 SCL-90-R subscales suggesting that MTD patients are more prone to develop not only affective spectrum disorders but a variety of other psychiatric symptoms as well. The only subscale without significant difference was somatization probably because HN patients also exhibit prominent physical symptoms.

Personality disorders were also highly represented in the MTD group. The most prevalent was avoidant personality possibly reflecting the fact that mitochondrial patients have debilitating symptoms already in childhood predisposing them to peer rejection and its longterm psychological consequences. Obsessive personality disorder was present in 2 MTD cases. MELAS syndrome has so far been associated with obsessive-compulsive disorder (Lacey et al, 2008), but not obsessive personality disorder.

6.2.3. The concept of mitochondrial psychiatry

Although more data is needed in the future to elucidate the causal relationship between mitochondrial dysfunction and the development of psychiatric disorders, the "mitochondrial psychiatry" model has been proposed by Gardner and Boles (Gardner and Boles, 2005), based on the growing clinical, genetic, ultrastructural, and pharmacologic evidence about the involvement of mitochondria in the multi-factorial development of psychiatric illnesses.

According to our observations, psychiatric symptoms of MTD patients show unique features. They often precede and do not correlate with the severity of neurologic or other somatic symptoms. These symptoms do not have a classic course (Fattal et al, 2007). They are frequently treatment-resistant and may even get worse when treated with psychotropic medications. This is partly due to the effect of various psychiatric medication on mitochondrial function and also, to the fact that MTD patients have abnormal autonomic regulation; pulse, blood pressure and gastrointestinal tract motility may all be affected, making them more prone to side effects. Tricyclic antidepressants, MAO inhibitors and certain antipsychotics should be avoided. Drugs with the shortest half-life are recommended.

Growing data suggest that psychiatric symptoms are independent manifestations of mitochondrial dysfunction and not the consequence of the chronic somatic disease. In our opinion, "mitochondrial psychiatry" is a valid concept but causal relationship between mitochondrial dysfunction and the development of psychiatric symptoms must be further elucidated by future, large-scale studies.

6.3. Mitochondrial dementia?

6.3.1. Involvement of the frontal lobe/ prefrontal cortex

We detected the predominance of executive dysfunction in our patients possibly due to the increased metabolic demand and a higher susceptibility to energy deficit of the prefrontal cortex (Ihara et al, 2010). We detected short-term (working) memory impairment on the RAVLT1 and the WAIS Digit Span subtest, confirming previous

results (Majamaa-Voltti et al, 2006, DiMauro and Hirano, 2005, Rosen 1980, Kun and Szegedi, 1996). Low performance on the RAVLT and tests measuring verbal working memory may indicate bilaterally decreased activation of the dorsolateral and posterolateral prefrontal cortex (*?*). Our patients displayed the ability to learn new words, but this ability was stronger in the control group. Prevalent impairment has also been found in delayed recall (RAVLT6). Previously a relatively intact verbal learning, delayed recall, recognition of learned words (Turconi et al, 1999) and verbal working memory (Sartor et al, 2002) has been reported in MTDs.

Weaker performance on the Stroop test implies decreased activation of the right anterior cingulate and dorsolateral prefrontal cortex (Ottowitz et al, 2002). Similarly, decreased activation of the dorsolateral and medial cortex of the right frontal lobe results in lower TMTA performance, and those of the dorsolateral prefrontal cortex-orbitofrontal cortex-striatum-thalamus-prefrontal cortex circuit in lower TMTB scores (Ottowitz et al, 2002).

Psychomotor slowing (Neargarder et al, 2007, Turconi et al, 1999, DiMauro and Hirano, 2005), a generally reduced attentional capacity (Turconi et al, 1999; DiMauro and Hirano, 2005; Inczedy-Farkas et al, 2012), with impairments of both divided (TMTA) (Neargarder et al, 2007, Turconi et al, 1999, DiMauro and Hirano, 2005) and selective attention (Sartor et al, 2002) have been found with impaired interference control on the Stroop test. However, the error rate on the Stroop test did not differ significantly, implying that although patients carry out tasks slower, they are not less accurate, contrary to normal aging (Ahn et al, 2011). Actually, age was found to be in positive correlation not only with the TMTA time but also with the StroopC_60 score implying that psychomotor slowing might positively affect attention deficits, ultimately improving performance.

Fluency also requires an intact dorsolateral prefrontal cortex with letter fluency activating mostly frontal, and category fluency mostly temporal areas (Munoz et al, 1999), besides memory stores. The dominance of general frontal lobe dysfunction might underlie the finding of lower letter than category fluency scores, although a similar pattern was observed in the control group as well.

6.3.2 Belle indifference

The involvement of greater frontal areas might also underlie the frequent presence of moria (Rief and Isaac, 2007) and impaired judgment (also referred to by the WAIS Comprehension subscale). In our presented case, it was very clear that the patient did not seek further evaluation and comforted herself in the physicians' negative attitude.
The lack of concern about the disease process in MTD patients is most similar to the "belle indifference" described in multiple sclerosis and the frontal lobe variant of frontotemporal dementia possibly originating in the involvement of the frontal lobe.

6.3.3 Involvement of non-frontal areas

Interestingly, in a study, cerebral glucose uptake in mitochondrial cytopathies has been found impaired prevalently in the occipital and temporal lobes, regardless of the presence of CNS symptoms (Molnar et al, 2000). With the WAIS subscales we demonstrated the impairment of cognitive abilities associated with non-frontal brain areas such as visuo-spatial performance, reflecting superior parietal and occipital functions and visuo-perception, related to fusiform, the parahippocampal, and the middle occipital gyrus based on observations in a cohort with Parkinson's disease (Pereira et al, 2009). Turconi et al studied PEO and KSS patients and detected hypoperfusions prevalently in the temporal lobes. They assumed deficits in both the visuo-constructive functioning of parietal lobes and in the temporal lobes involved in the processing of visual information (Turconi et al, 1999) and proposed ocular motility problems in the background of both visuo-spatial cognitive and attention deficits (Turconi et al, 1999).

6.3.4. Neurological symptoms as confounding factors

The presence of neurological symptoms can indeed be a confounding factor in our results. Ataxia can play a role not only in the PQ but in the low performance on the TMT tests as well, although patients with the most prevalent ataxia (Pt 1, 8, 10) had the lowest performance not only on the TMT but also on the RAVLT and Fluency Tests.

Patients 3, 4, 11, 13 had ophthalmoparesis that could also affect performance on the TMT. Likewise, dysarthria can impede performance on many tests such as the Stroop and the WAIS, and although the only patient with dysarthria (Pt 10) had the lowest performance on these tests, the overall low performance of the group indicates that dysarthria accounts only partly for these results.

6.3.5. Intelligence

We could also confirm the results of previous studies in that patients with MTD tend to have intelligence profile in the lower zone of the normal range (Kaufmann et al, 2004; DiMauro et al, 2005; Inczedy-Farkas et al, 2012; Kun and Szegedi, 1996) with the PQ more decreased than the VQ referring to deficits in verbal (Sartor et al, 2002; Neargarder et al, 2007; Inczedy-Farkas et al, 2012), but mostly in nonverbal functions (Turconi et al 1999). VQ impairment comprised of knowledge base, memory and attention, calculation (Sartor et al, 2002; Rosen et al, 1980) and practical knowledge, but not abstract reasoning and logic, which was intact, contrary to previous reports (Turconi et al, 1999; DiMauro and Hirano, 2005). It has been reasoned that the lower verbal intelligence does not account for specific neuropsychological deficits (Turconi et al, 1999). Impairment in perceptual organization (PQ) comprised of focal cognitive deficits not only in visuo-perception (Turconi et al, 1999) and visuo-spatial abilities (Sartor et al, 2002; Neargarder et al, 2007; Majamaa-Voltti et al, 2006) as previously reported, but also, visuo-motor coordination and slowing of visuo-motor speed. Social skills and planning were intact, although the understanding of social norms seemed to be defective.

We saw the progression of cognitive symptoms in the case of Patient 16 in which we had the chance to longitudinally follow the patient.

A negative correlation was found between FSIQ and the HAQ-DI score implying that the cognitive decline does not correlate with and/or progress independently of the somatic symptoms.

6.3.6. Correlations of cognitive performance and structural alterations

It has been demonstrated that focal cortical thinning is a pathological marker of certain cognitive deficits (Ahn et al, 2011). Although we have not tested for the specific tests, we failed to find a difference in intelligence scores between subgroups with and without atrophy possibly supporting the approach that subcortical dysfunction mediates basic cognitive processes, shared by multiple tests (Ahn et al, 2011). Similarly, no such difference was found in GSI or HAQ-DI scores implying that significant cognitive, psychiatric symptoms and functional alteration can exist even in the absence of detectable structural alterations. This is also in contradiction with a previous report stating that normal neuroimaging findings are characteristic of early stages of the disease (Munoz et al, 1999). PQ scores of the two subgroups, however, differed more than the VQ, possibly implying that performance problems are more closely associated with structural alterations than verbal findings. The subgroup of probands with cerebellar pathology had indeed significantly lower PQ scores and greater VQ-PQ difference, possibly also due to ataxia. Despite cerebellar findings and ataxia in some patients, the VQ and PQ means of the entire group were evened out and are very similar at the group level. This is interesting if we consider that our patients had higher intersubject variability on all tests than controls, referred to by higher SD values.

6.3.7. Patients with tRNA mutations are the most affected

In two domains, learning (RAVLT5) and letter fluency, the performance for patients harboring mutations of the tRNA, including the most common point mutations of MELAS and MERRF syndrome, was significantly weaker than for the rest of the mitochondrial cohort. Patients with the m.3243 A>G mutation have already been described as most severely affected (Rosen, 1980, Maruta et al, 2011). Although in MELAS, besides cerebral lactic acidosis (Maruta et al, 2011) and seizures (Perez-Lopez et al, 2006), vascular impairment might have a role in neuronal injury, the progression of cognitive symptoms has been observed in the absence of further stroke-like episodes (Sartor et al, 2002). Our patients had no history of stroke-like episodes but they still had the weakest performance.

In rare studies of NARP cases, signs of dementia (Gelfand et al, 2011), such as slowed processing speed, impaired visuospatial copying, recall and verbal fluency (Majamaa-Voltti et al, 2006) have been described. Of our NARP patients, Pt 10 was severely affected having the lowest FSIQ of the cohort, but his mother, Pt 9, had intact cognition. KSS cases have so far been found to be free of cognitive symptoms and also to perform better than PEO and MELAS Patients (Lang et al, 1995) and to be equally impaired as PEO Patients (Majamaa-Voltti et al, 2006). Our cases with mtDNA deletion, despite relatively high muscle heteroplasmy levels, had superior performance.

6.3.8. The concept of mitochondrial dementia

In MTDs, a variety of cognitive symptoms, from mild impairment to full-blown neurodegenerative disorder, has been demonstrated. The severity generally is greater than predicted on clinical grounds on by neuroimaging. Targeted neuropsychological testing helps to recognize the organic cause, to detect latent abnormalities and the progression of symptoms. Mitochondria play a central role in apoptosis. Clonal expansion of mtDNA replication errors is an important factor of aging in general (Bratic and Larsson, 2013). Mitochondrial dysfunction and neurodegeneration might be reciprocal processes by enhancing each other. In mitochondrial disorders, CNS dysfunction and chronically, degeneration has been described, creating a variety of focal cognitive deficits with a potential to progress to 'mitochondrial dementia' (Finsterer, 2008). Due to the chronic metabolic disturbance (Sartor et al, 2002), cognitive symptoms progress independently of MTD, although they are provoked by the same factors (Berg et al, 2011). Current metabolic state might account for the marked fluctuation (Neargarder et al, 2007) and eventual absence (Lien et al, 2001) of cognitive symptoms. We speculate that primary or secondary mitochondrial dysfunction; the morphologic, biochemical and molecular changes of mitochondria might be a risk factor for variable level of cognitive impairment, even dementia. The causal relationship must be elucidated by future studies.

6.3.9. The importance of the awareness to MTDs

Due to the multisystemic symptoms and the multiple genetic causes, MTDs are frequently underdiagnosed or misdiagnosed. One frequent misdiagnosis is somatization. In the same time, patients with true somatoform disorder - conversion, somatization, pain disorders and hypochondriasis - often present to general medical settings rather than mental health settings and undergo multiple workups, pharmacological treatments, and even surgeries (Gardner and Boles, 2011). The diagnostic categories of somatoform disorders are being radically modified for the DSM-V (Schoenberg et al, 2012) as there has been a great heterogeneity in how physicians identify and manage this patient population (Kanaan et al, 2011). Frustration, the inability to synthesize the clinical findings might have been one factor in misdiagnosing the presented MTD patient. Teamwork, consultation between different specialists in complicated multisystemic diseases, is essential.

Mental Health Problems are the major cause of Years Lived with Disability (YLD) (Murray et al, 2010), thus, it is very important to find the organic cause if one exists. Psychiatrists should consider mitochondrial diseases as a possible diagnosis in patients with atypical presentation of psychiatric symptoms resistant to normal doses of pharmacologic treatment and/or psychotherapy, especially in the presence of comorbid physical symptoms and a maternal inheritance pattern. Early diagnosis is crucial not only in the optimal disease management but also in the identification of affected family members, in genetic counseling, and in avoiding empirical pharmacotherapy and multiple workup The awareness of the role of mitochondrial dysfunction can give rise not only to more effective diagnosis but to novel therapeutic approaches as well. The demonstrated effects psychotropic medication exerts on mitochondria may help regulating neuronal circuits that mediate complex brain functions such as cognition, mood and behavior. Improving mitochondrial function may gain an important role in the long-term treatment of various neurodegenerative disorders as well as psychiatric and cognitive symptoms of patients with MTD.

7. Conclusion

7.3. Using a comprehensive clinical assessment, we demonstrated that psychiatric symptoms, especially mood disorders are more frequently present in patients with MTD compared to HN patients, who live with comparable level of disability.

7.4. We elucidated hitherto unknown aspects of cognitive decline in a well-defined cohort of patients with MTD. Our results indicate a decreased but balanced intelligence profile with a variety of focal cognitive deficits present in these patients. Cognitive decline is greater than predicted on clinical grounds or neuroimaging and tend to progress, as demonstrated with the case of Patient 16. Mitochondrial disease is a multisystemic process in which neurodegeneration seems to be present irrespective of the mutation type.

7.5. In order to raise awareness to MTDs in the international medical community, we reported a case of a woman with multisystemic symptoms where the signs of somatoform disorders were present with laboratory abnormalities and a positive family history, and emphasized that in similar cases mitochondrial workup is warranted to avoid misdiagnosis.

7.6. We carried out the first genetic epidemiologic study systematically investigating the frequency of the most common mtDNA mutations - m.3243 A>G, .8344 A>G,.8993 T>C and.8993 T>G tRNA mutations and the common mtDNA deletions - in Central-Eastern Europe. The mutation frequency in Hungarian patients was similar to other Caucasian populations for the hot spot mutations.

7.7. We established the registry of mitochondrial disorders (NEPSYBANK, data of 79 patients uploaded) and schizophrenia (SCHIZOBANK, biological samples and clinical data of 535 patients uploaded).

8. Summary

Mitochondrial disorders represent a major challenge in medicine. Tissues with high energy requirement, including the CNS, are the most affected giving rise to the notions "mitochondrial dementia' and 'mitochondrial psychiatry' in the literature. 1. With the present work we aimed to perform the frequency assessment of mitochondrial mutations in Hungary 2.-3. We assessed the presence of psychiatric and neuropsychological symptoms in a subcohort of 19 selected patients. 4. We aimed to raise awareness to mitochondrial disorders in the international medical community. 5. We also established a registry of patients with mitochondrial disorder (NEPSYBANK) and built a schizophrenia biobank (SCHIZOBANK). 1. The frequency was found to be 2.71% for the m.3243 A>G, 1.45% for the m.8344 A>G mutation, a total of 5.52% for all tRNA mutations. In the protein coding genes, the frequency of the m.8993 T>C was 0.34 % and that of m.8993 T>G was 0.11%. Single mtDNA deletions were detected in 15.3%, multiple deletions in 6.2% of the investigated cohort. 2. The BDI-SF, HDRS, SCL-90-R and the SCID interviews yielded a variety of affective spectrum and personality disorders in the mitochondrial group. Atypical course, treatment resistance, increased susceptibility to adverse side effects is frequent. The severity of psychiatric symptoms does not correlate with that of the neurological and other physical symptoms. 3. Patients performed significantly weaker on the neuropsychological tests with prevalent nonverbal impairment. 4. We published a case presentation to emphasize the importance of correct and early diagnosis. 5. We uploaded data of 79 patients with mitochondrial disorders to NEPSYBANK and biological samples coupled with clinical data of 535 patients with schizophrenia to SCHIZOBANK between 2009 and 2013. We propose that in mitochondrial disorders, psychiatric and cognitive symptoms are independent manifestation of CNS dysfunction and not the consequence of the chronic somatic disease. "Mitochondrial psychiatry" and "mitochondrial dementia" might be valid terminologies but causal relationships between mitochondrial dysfunction and clinical symptoms must be further elucidated by future, large-scale studies. Clinicians should be aware of the most common presentations of mitochondrial disorders and the high prevalence of psychiatric and cognitive symptoms which has both etiologic and therapeutic relevance.

9. Összefoglalás

A mitokondriális betegségek az orvostudomány nagy kihívásait jelentik. A multiszisztémás tünetek és a szerteágazó genetikai háttér miatt a diagnózis komplikált, a prevalencia-becslések pedig széles skálán mozognak. Az igen gyakran leírt kognitív károsodást "mitokondriális demencia"-ként, a gyakori pszichiátriai tüneteket, illetve a mitokondriumok fontos szerepét különböző pszichiátriai betegségek kialakulásában "mitokondriális pszichiátria"-ként emlegeti újabban a szakirodalom. 1. Célunk volt a leggyakoribb mitokondriális mutációk gyakoriságának felmérése hazánkban 2.-3. Részletes vizsgálattal mértük fel a pszichiátriai és neuropszichológiai tüneteket egy 19 beteget tartalmazó szubkohortban. 4. Célunk volt az orvostársadalom figyelmének felhívása a mitokondriális betegségekre. 5. További célunk volt a 4 hazai orvosegyetem regiszter és biobank építési tevékenységének koordinálása, a NEPSYBANK bővítése, illetve a SCHIZOBANK felépítése.

1.Az M.3243 A>G mutáció frekvenciáját 2.71%-nak, az M.8344 A>G-ét 1.45%-nak, a tRNS mutációk frekvenciáját összesen 5.52%-nak mértük. A protein kódoló gének közül a m.8993 T>C 1.34%-ban, a m.8993 T>G pedig 0.11%-ban volt jelen a vizsgált betegek körében. Az egyes deléciókat 15.3%, a multiplex deléciókat 6.2%-ban detektáltuk. 2. A BDI-SF, HDRS, SCL-90-R skálák és SCID interjúk az affektív zavarok és személyiségzavarok szignifikánsan magasabb előfordulását mutatták ki a mitokondriális betegek csoportjában. A mitokondriális betegségekben a pszichiátriai tünetek lehetnek a betegség első manifesztációi. Gyakori az atípusos lefolyás, a terápia-rezisztencia és a mellékhatásokra való fokozott hajlam. A pszichés tünetek súlyossága nem korrelál a neurológiai és egyéb szomatikus tünetek súlyosságával. 3. Betegeinknél szignifikánsan nonverbális károsodást detektáltuk. 4. Egy olyan betegünk esettanulmányát közöltük, akit tévesen szomatoform zavarral diagnosztizáltak 5. Regiszter-és biobank építési tevékenységünk 79 mitokondriális beteg adatainak a NEPSYBANK-ba, illetve 535 schizophren beteg biológiai mintáinak és részletes klinikai adatainak SCHIZOBANK-ba való feltöltését foglalta magában 2009 és 2013 között. Véleményünk, hogy a "mitokondriális pszichiátria" és a "mitokondriális demencia" érvényes fogalmak, de a kauzalitást további kutatásoknak kell eldöntenie.

10. References

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11. List of publications

11.3. Papers relevant to the dissertation

1. Reményi V, Inczédy-Farkas G, Komlósi K, Gál A, Pentelényi K, Karczagi V, Melegh B, Molnar MJ: Retrospective assessment of the most common mitochondrial DNA mutations in a large Hungarian cohort of suspect mitochondrial cases. Mitochondrial DNA. 2014 Jan 17. (Impact factor: 1.7)

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11.4. Other papers

1. Nyiro G, Inczedy-Farkas G, Remenyi V, Gal A, Pal Z, Molnar MJ: The effect of the CYP 2C19*2 polymorphism on stroke care. Acta Physiol Hung. 2012 Mar 99(1):33-9. (Impact factor: 0.821)

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3. Makkos Z, Fejes L, Inczedy-Farkas G, Kassai-Farkas A, Faludi G, Lazary J: Clinical characteristics of cannabis-induced schizophrenia spectrum disorder Neuropsychopharmacol Hung. 2011 13(3):127-38

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12. Acknowledgement

I am thankful to my supervisor **Maria Judit Molnar** for all her support and guidance.

I also want to thank my colleagues for their contribution to the presented work; **Viktoria Remenyi, Aniko Gal** and **Klara Pentelényi** for the molecular genetic studies, **Agnes Udvardy-Meszaros** and **Monika Andrejkovics** who carried out the neuropsychological assessment of patients and controls, **Zsofia Varga**, who carried out the statistical analysis. To **Petra Balla** who participated in the psychiatric assessment of the selected patients. To **Benjamin Bereznai** who performed the muscle biopsies. To **Györgyi Báthory, Marianna Markó, Metta Stralendorff, Mónika Sáry, Andrea Tóth, Péter Balicza** and the staff of the Institute of Genomic Medicine and Rare Disorders. I also want to acknowledge the contribution of county hospital neurologist and physicians who referred their patients to us for further clinical evaluation and genetic analysis.

I would also like to thank **Joey W Trampush** and **Danielle Beech** at the Zucker Hillside Hospital for their contribution to the neuropsychological study.

In the SCHIZOBANK project, it was **György Németh, Szilvia Magyarósi, Krisztián Nagy** at Gedeon Richter Inc. as well as **Zoltán Makkos**, **Mária Maurer** and **Ákos Kassai-Farkas** and the staff of the I. Department of Psychiatry at Nyiro Gyula Hospital whose help was much appreciated.

We want to thank **Blaskó Görgy** for his referral of the presented case.

I am grateful to Edina Varga for her remarks and guidance with regards to my dissertation.

I am thankful to **Lajos Simon** and **István Bitter** for recommending me to the PhD fellow position.

I am grateful to our patients for their consent, cooperation and patience that enabled us to perform these studies.