Chronic progressive external ophthalmoplegia
More than meets the eye

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General introduction

CASE REPORT

A 47-year-old woman was evaluated for a 30-year history of bilateral ptosis and exercise intolerance. She also had complaints of hearing loss and was recently diagnosed with type II diabetes mellitus. Her family history was remarkable. She had four older brothers. Two brothers had died, supposedly from cerebral hemorrhage; both had ptosis and hearing loss. The third brother had hearing loss only. The fourth brother had a phenotype comparable to that of the patient. The patient's mother also had diabetes and hearing loss and died at age 77.

Physical examination revealed a divergent strabismus with bilateral restricted gaze in all directions. There was a mild generalized decrease in muscle strength. Sensory functions and coordination were normal.

Creatine kinase was normal, cerebrospinal fluid lactate was elevated (3200 µmol/l, normal < 1900). Electromyography revealed mild myopathic features. There was slight cerebellar atrophy on brain MRI. A skeletal muscle biopsy showed ragged red fibers and cytochrome-c-oxidase negative fibers (Figure 1). Mitochondrial complex I activity was moderately decreased. Genetic analysis revealed an m.03243A→G mutation in the skeletal muscle mitochondrial DNA (mtDNA). Based on the clinical features and results from the ancillary investigations, a diagnosis of chronic progressive external ophthalmoplegia (CPEO) was reached according to European Neuromuscular Centre criteria [1].

Most neuromuscular neurologists would not have much trouble reaching the diagnosis of CPEO in this particular patient. A bilateral, adult onset slowly progressive external ophthalmoplegia is highly suggestive of a mitochondrial disorder, which was readily confirmed by skeletal muscle histology and the detection of a known pathogenic mtDNA point mutation. Physicians in other specialties might however have reached alternative diagnoses in this particular patient, ranging from "mitochondrial encephalomyopathy", "complex I deficiency", "an m.03243A→G point mutation" to "MIDDS (maternally inherited diabetes-deafness syndrome)". None of these diagnoses are wrong, as they all describe a key characteristic of the patient. Conversely, all are incomplete and do not consider the complex relation between phenotype and genotype that characterizes most mitochondrial disorders.

The complexity of mitochondrial medicine is probably best illustrated by the m.03243A→G point mutation. With a prevalence of 16-236 /100.000, it is the commonest pathogenic mtDNA point mutation [2-5]. Originally described as the cause of MELAS, the m.03243A→G mutation was later found in patients with MIDDS, CPEO, Leigh syndrome, focal segmental glomerulosclerosis, sensorineural hearing loss, maternally inherited cardiomyopathy and myoclonic epilepsy with ragged red fibers (MERRF), as well as in a variety of overlap syndromes [6-8]. Conversely, the MELAS phenotype can be caused by 10 additional mtDNA mutations scattered across five different genes [6].

The **complex relation between phenotype and genotype** is only one of the features of mitochondrial disorders that make it difficult to conduct clinical research. Other difficulties are the low prevalence of each individual mitochondrial phenotype and the large variations in the rate of disease progression, even within a cohort of patients with a similar phenotype. Because of the difficulties associated with clinical studies, most of the scientific research has focused on the organelle (function, pathophysiology and genetics) rather than on the patient (phenotype, disease impact and treatment). This is clearly illustrated by the fact that the search term "mitochond*" yields 210.433 hits in Pubmed, while only 6 studies met the entry criteria for a 2006 Cochrane review on the treatment of mitochondrial disorders (which concluded that there was no evidence supporting the use of any intervention in mitochondrial disorders) [9].

Another factor that contributed to the **shift towards basic research** is the fact that better understanding of the function of the organelle is the likely key to the development of a medical treatment. Indeed, basic and translational research have led to several therapeutic strategies, including l-arginine in stroke-like episodes associated with MELAS, co-enzyme Q10 in primary co-enzyme Q10 deficiency and bone marrow transplant or dialysis in mitochondrial neurogastrointestinal encephalopathy (MNGIE) [10-12].

However, these strategies are applicable to specific disorders only and do not benefit the broader range of mitochondrial disorders. Although studies in cell lines and animals show some promising new therapeutic strategies, a causative treatment which fully ameliorates all symptoms, or even a treatment that prevents further decline is not expected in the near future for most mitochondrial disorders [13]. Therapeutic options for most present-day patients are therefore limited to **symptomatic management**. Symptomatic management however requires detailed knowledge about the patients' signs and symptoms, as well as their perception of the disease impact on daily life activities and social participation.

This thesis therefore focuses on signs, symptoms and impact of disease in one of the established mitochondrial disorders, **chronic progressive external ophthalmoplegia (CPEO)**. CPEO is very suitable for studying the disease impact in mitochondrial disorders, since it is a classical and readily recognizable mitochondrial phenotype, it is relatively common and it mainly affects adults. Moreover, CPEO is characterized by a large genetic heterogeneity, which allows for the identification of specific genotype-phenotype relations.

As most other medical specialties, mitochondrial medicine has its own basic principles and vocabulary. A short introduction to mitochondrial function and genetics is provided below in order to facilitate reading and understanding of this thesis.

Figure 1: Histologic and electromicroscopic sections of the quadriceps muscle biopsy
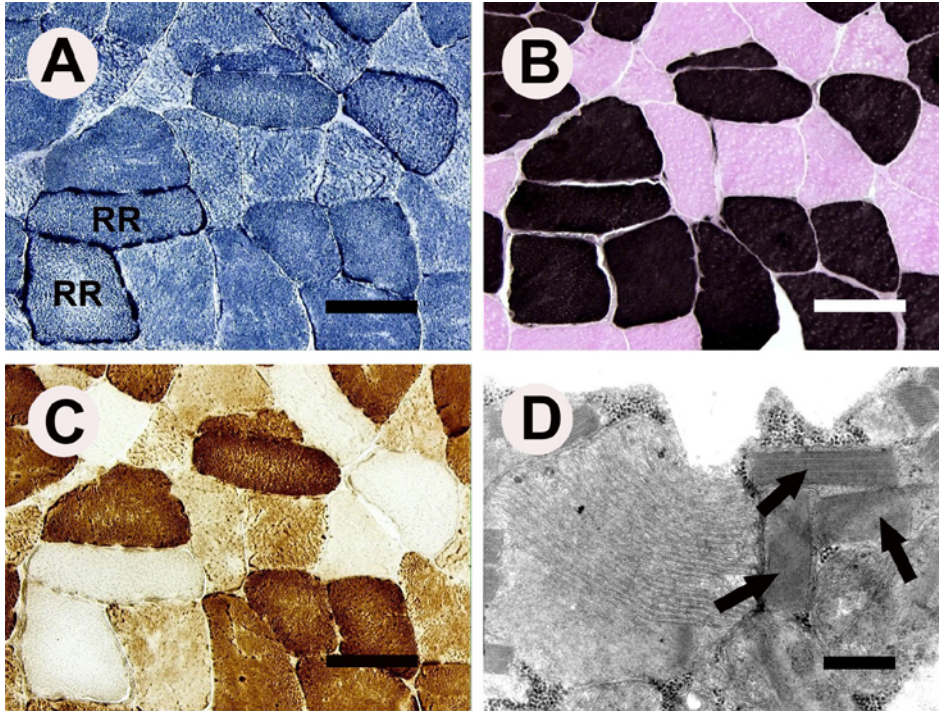
Adapted with permission from H. ter Laak

Quadriceps muscle tissue showing serial sections (**A**, **B**, and **C**) of ragged red fibers (RR) and COX-negative or COX-deficient fibers (see **C**). The electron microscopic image (**D**) shows a large mitochondrion with normal cristae at the left; arrows point to paracrystalline inclusions. Images **A**, **B**, and **C** respectively depict activity of succinic dehydrogenase (SDH), myofibrillar ATPase (preincubation at pH 4.2) and COX. Bars represent 100 micron (**A**, **B**, and **C**) and 0.5 micron (**D**).

MITOCHONDRIAL STRUCTURE, FUNCTION AND GENETICS

Mitochondria are sphere- or oval-shaped cellular organelles consisting of two membranes: a smooth outer membrane and an inner membrane, which is highly folded into cristae (Figure 2a). The space within the inner membrane is called the matrix, whereas the space between the inner and the outer membrane is called the intermembrane space.

The **main function of the mitochondria** is ATP production through oxidative phosphorylation. This process is catalyzed by four enzyme complexes (complex I-IV) of the respiratory chain located within the inner membrane. These complexes couple the transfer of electrons derived from glycolysis and fatty acid oxidation in the mitochondrial matrix to the transport of H^+-ions across the inner membrane into the intermembrane space. The resulting H^+-gradient serves as an energy source required for the transformation of ADP+phosphate into ATP by complex V (Figure 2d).

The **genetics of mitochondrial disorders** has some distinctive features. Most importantly, mitochondria contain their own strand of DNA (mtDNA), a single circular strand of 16.569 base pairs (Figure 2b), which is exclusively inherited from the mother [14]. It encodes 37 genes: 13 for subunits of complexes I, III, IV and V, 2 for rRNAs and 22 for mitochondrial tRNAs (Figure 2c). Interestingly, the "language" of mtDNA is somewhat different from nuclear DNA. For instance, the codon AUA codes for isoleucine in nuclear DNA, but

Figure 2: The deleterious effects of the m.03243A→G mutation on the mitochondrial respiratory chain
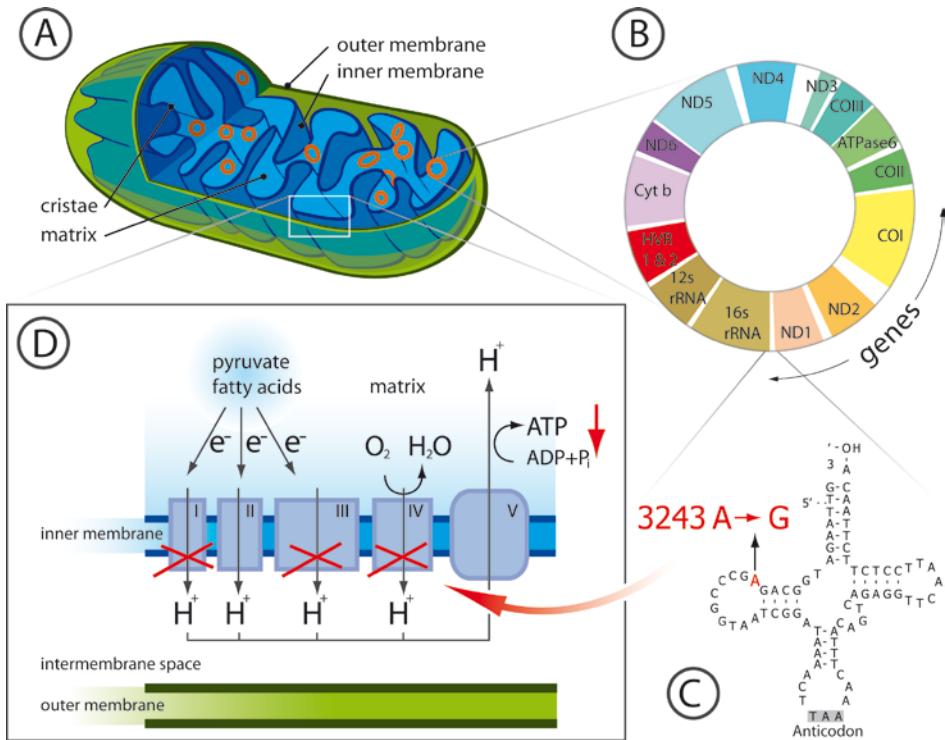
A a classic representation of a mitochondrion, with a smooth outer membrane and a highly folded inner membrane. **B** the mitochondrial DNA, a circular 16569 base pair DNA fragment. The largest genes are noted in the circle. **C** a mitochondrial transfer DNA (tRNA), in this case the tRNA that facilitates the incorporation of the amino acid leucine in various proteins. The base pair on position 3243 contains a mutation (the substitution of an adenosine (A) nucleotide by a guanine (G) nucleotide). **D** oxidative phosphorylation: pyruvate and fatty acids are metabolized inside the mitochondrial matrix. Electrons (e-) from their metabolites are transferred across respiratory chain complexes I - IV, which are located inside the inner membrane. The resulting H^+-gradient serves as an energy source for complex V, which coverts adenosine diphosphate (ADP) into adenosine triphosphate (ATP).
The red arrows and crosses represent the detrimental effects of the m.03243A→mutation. This mutation leads to impaired incorporation of leucine into complex I, III and IV, resulting in loss of function. Consequently, H^+-transport across the inner membrane is decreased, which ultimately results in a decreased capacity of complex V to convert ADP into ATP.

for methionine in mtDNA [15]. An explanation for this is provided by the endosymbiotic hypothesis, which states that mitochondria descend from aerobic bacteria that colonized primordial eukaryotic cells lacking the ability to metabolize oxygen [16].

Each mitochondrion contains several strands of mtDNA, while the number of mitochondria in individual cells can vary considerably, ranging from a few to several hundreds [17]. In a single cell, mutated mtDNA often coexists with normal (wild type) mtDNA, a concept called **heteroplasmy**. Higher levels of heteroplasmy (i.e. more mutated mtDNA) are generally associated with more severe cellular dysfunction. However, the percentage of heteroplasmy at which cellular function is impaired depends on rate of the oxidative phosphoralytion and therefore differs between various organs.

Mutation in the mtDNA can be divided into three categories:
1) point mutations in one of the 13 genes coding for a respiratory chain complex subunit. This type of mutation often results in severely decreased activity of the complex involved and is commonly associated with a severe phenotype
2) point mutations in of the 22 genes coding for a tRNA (Figure 2c+d). These can result in single or multiple respiratory complex deficiencies
3) point mutations in one of the two mitochondrial rRNA genes (12s and 16s rRNA)
4) large scale rearrangements: duplications or deletions, often affecting several thousand base pairs

MtDNA point mutations are maternally inherited. Heteroplasmy levels however can vary considerably within one family, as can disease severity and phenotype [18]. For unknown reasons, deletions and duplications are mostly sporadic.

MtDNA codes for only a minority of the proteins involved in mitochondrial function. **Nuclear DNA** encodes all other mitochondrial proteins. Consequently mitochondrial disorders can also result from nuclear DNA mutations. Most of these mutations affect genes coding for either respiratory complex subunits or for mtDNA maintenance proteins. Inheritance is Mendelian.

MITOCHONDRIAL DISORDERS

With an estimated **prevalence** of 1:5000, mitochondrial disorders are the commonest group of inborn errors of metabolism [19;20]. Mitochondrial disorders however comprise a genetically and clinically heterogeneous group of diseases, with manifestations ranging from adult-onset single organ dysfunction to severe neonatal multi-system failure.

In patients with mitochondrial disorders several seemingly unrelated organs or tissues can be affected. For example, skeletal muscles and the retina are commonly affected in

Figure 3: Random segregation of mutant and wild-type mitochondria during mitosis and its effect on phenotypic expression
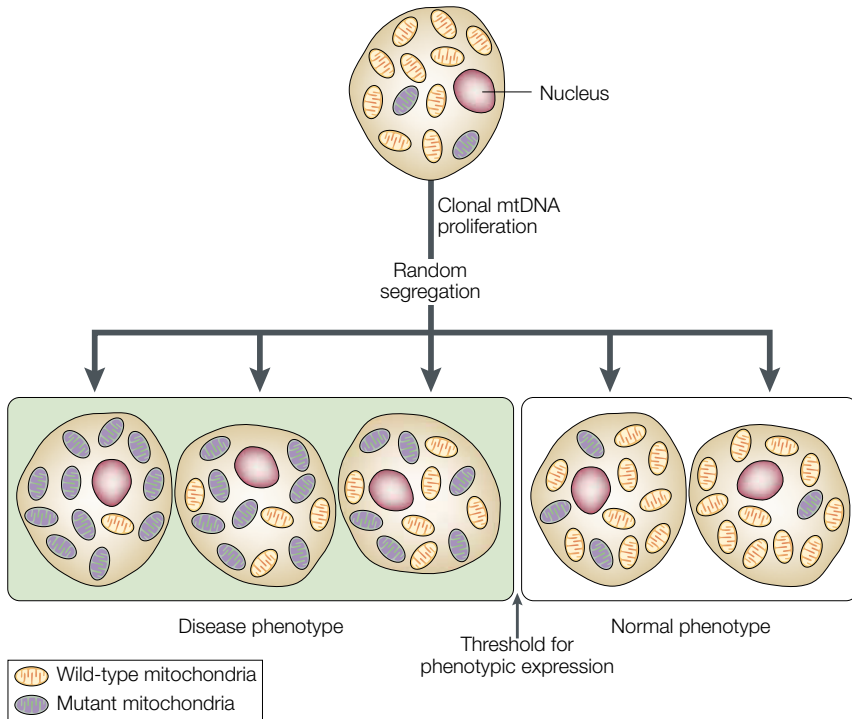
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Somatic mosaicism for mitochondrial disorders results from the random segregation of mutant and wild-type mitochondria during mitosis, which can result in daughter cells with different proportions of mitochondrial mutations. Somatic mosaicism in monozygotic twins (not shown) has also been observed. This could be due to intrauterine differences in the allocation of cells as a result of variations in the placental vascular supply, stochastic developmental events and other environmental influences [42].

mitochondrial disorders, whereas the skin and the lungs are not. As a rule of the thumb, the susceptibility of tissues and cells to mitochondrial dysfunction largely depends on two factors: both a high energy demand and a low mitotic activity make tissues prone to mitochondrial dysfunction [21]. The latter is explained by the fact that during mitosis, normal and mutated mtDNA copies are randomly distributed over the two daughter cells. If by chance a daughter cell receives only few mutated mtDNA copies, it is likely to survive whereas a daughter cell with a high percentage of mutated DNA is not. As a result, after several mitotic cycles, heteroplasmy decreases. This concept is called random segregation (Figure 3).

An overview of the organs most commonly affected in mitochondrial disorders is listed in Table 1 [22]. Patients with mitochondrial disorders often have more than one of these

Table 1: Overview of common signs and symptoms in mitochondrial disorders revealing a broad variety of organs and tissues that are possibly affected

Central nervous system	psychomotor retardation or regression
	epilepsy
	cerebellar ataxia
	myoclonia
	dystonia
	hemiparesis
	hemianopsia, cortical blindness
	migraine
Peripheral nervous system	external ophthalmoplegia
	proximal or bulbar myopathy
	exercise intolerance
	axonal neuropathy
	sensory neuronopathy
Ear, eye	sensorineural hearing loss
	pigmented retinopathy
	optic atrophy
Hematopoiesis	sideroblastic anemia
	pancytopenia
Heart	conduction block
	cardiomyopathy
Endocrine system	diabetes mellitus
	growth retardation, growth hormone deficiency
	hypoparathyroidism
Digestive tract	pancreatic insufficiency
	dysmotility, pseudo-obstruction
Kidney	focal segmental glomerulosclerosis
	Toni-Debré-Fanconi syndrome
	Bartter-like syndrome

symptoms. There are also several classical mitochondrial phenotypes, characterized by a fixed combination of symptoms. Some of these phenotypes are associated with specific mtDNA mutations (Table 2) [22]. With an estimated prevalence of 1:40.000, chronic progressive external ophthalmoplegia (CPEO) is one of the commonest mitochondrial phenotypes in adulthood [23].

Table 2: Overview of the commonest classical mitochondrial phenotypes and the associated gene mutations

Acronym /eponym	Phenotype	Common mutations
MELAS	Myopathy, Encephalopathy, Lactate Acidosis and Stroke-like-episodes	m.03243A→G
Leigh syndrome	psychomotor retardation, bilateral basal ganglia necrosis, lactate acidosis	m.08993T→C
LHON	Leber Hereditary Optic Neuropathy	m.11778G→A, m.03460G→A, m.14484T→C
MERRF	Myoclonic Epilepsy and Ragged Red Fibers	m.08344A→G
Kearns-Sayre syndrome	early onset CPEO, retinitis pigmentosa, cardiac conduction block, cerebellar ataxia	mtDNA deletion
CPEO	Chronic Progressive External Ophthalmoplegia	mtDNA deletion
NARP	Neuropathy, Ataxia and Retinitis Pigmentosa	m.08993T→C
MIDDS	Maternally Inherited Diabetes-Deafness Syndrome	m.03243A→G
Pearson syndrome	pancytopenia, pancreatic insufficiency	mtDNA deletion

CPEO

CPEO was first described by the ophthalmologist von Graefe in 1868 [24]. The main **clinical features** are bilateral slowly progressive ptosis and weakness of the muscles associated with ocular motility. Like in most mitochondrial disorders, there is a large phenotypical heterogeneity. Age at onset can vary from childhood to well into adult life. In addition to external ophthalmoplegia, involvement of other muscles groups (bulbar, limb girdle and heart) is common. Other common clinical features in CPEO patients are sensorineural hearing loss, pigmented retinopathy, axonal polyneuropathy, cerebellar ataxia and several non neurological features, such as a cardiac conduction block and endocrine disorders, mainly diabetes mellitus [25].

A specific combination of clinical features within the CPEO spectrum was described in 1958 by Kearns and Sayre [26]. This mitochondrial phenotype, now known as the **Kearns Sayre syndrome** (KSS), is characterized by external ophthalmoplegia, an early age of onset, the presence of a cardiac conduction block and pigmented retinopathy.

The exact definition underwent some changes over time, but is nowadays generally accepted as the combination of external ophthalmoplegia, pigmented retinopathy, an age of onset below 20 years, and at least one of the following symptoms: cardiac conduction block, cerebellar ataxia or elevated CSF protein content [27].

Progressive external ophthalmoplegia is also one of the main clinical features in two other mitochondrial phenotypes, SANDO (sensory atactic neuropathy, dysarthria and ophthalmoplegia) and MNGIE (mitochondrial neurogastrointestinal encephalopathy). As the acronyms indicate, the other prominent clinical features in SANDO are a severe sensory neuronopathy and dysarthria, while gastrointestinal symptoms (recurrent nausea, vomiting or diarrhea) due to intestinal dysmotility, axonal neuropathy and leukencephalopathy are part of the MNGIE phenotype [28;29].

CPEO was the first mitochondrial disorder for which the **genetic defect** was identified. In 1989, Moraes reported a 1.3 to 7.6 kb deletion in skeletal muscle mtDNA of 21 CPEO patients [30]. However, a single mtDNA deletion was also found in 11 KSS patients. Though the heteroplasmy levels and location of the deletion were later found to differ between KSS and CPEO, this does not fully account for the differences in age at onset, rate of progression and the distribution of organ involvement between the two disorders [31]. Consequently, there is ongoing debate about whether CPEO and KSS are distinct entities, or part of a continuous spectrum [32].

After the initial description of mtDNA deletions in CPEO, later studies revealed that one third to one half of all CPEO cases were not caused by a mtDNA deletion [30;33]. Since then, numerous other mutations have been identified (Table 3) [34]. Mutations in some of these genes are associated with a rather specific phenotype. For example, a large majority of SANDO cases is caused by mutations in the nuclear polymerase gamma 1 (*POLG1*) gene on chromosome 15q25 [35]. In contrast, MNGIE is mainly caused by mutations in the nuclear thymidine phosphorylase gene on chromosome 22q13.32-qter [36]. Despite these recent advances in the genetics of CPEO, there are still CPEO patients - albeit a minority - in whom no mutation can be identified.

The **workup** of a patient with suspected CPEO can be difficult. A muscle biopsy is often required for histological, biochemical and genetic analyses. A diagnostic algorithm is provided in the discussion of this thesis.

The **differential diagnosis** of CPEO is limited. Oculopharyngeal muscle dystrophy (OPMD) is an adult onset myopathy. Like in CPEO, clinical features include ptosis, myogenic dysphagia and limb girdle weakness. However, OPMD is mostly an autosomal dominantly inherited disorder and extra-muscular features are typically lacking. Moreover, extra-ocular weakness is mild in OPMD, while it is an early and severe symptom in CPEO. As a result of preserved extra-ocular muscle function, OPMD patients can compensate their ptosis by combinating retroflexion of the neck with downward gaze. This is referred to as the astrologist's posture. Since downward gaze is also often severely limited in CPEO,

Table 3: Mitochondrial and nuclear DNA mutations associated with CPEO

	Locus	Gene	Confirmed
MtDNA			
Large scale rearrangements	Variable	Multiple	+
Transfer RNA point mutations	m.00642T→C	tRNA Phe	-
	m.03243A→G	tRNA Leu (UUR)	+
	m.03243A→T	tRNA Leu (UUR)	-
	m.03249G→A	tRNA Leu (UUR)	-
	m.03250T→C	tRNA Leu (UUR)	-
	m.03254C→T	tRNA Leu (UUR)	-
	m.03255G→A	tRNA Leu (UUR)	-
	m.04267A→G	tRNA Ile	-
	m.04274T→C	tRNA Ile	-
	m.04285T→C	tRNA Ile	-
	m.04290T→C	tRNA Ile	-
	m.04298G→A	tRNA Ile	+
	m.04302A→G	tRNA Ile	-
	m.04308G→A	tRNA Ile	-
	m.04309G→A	tRNA Ile	-
	m.05628T→C	tRNA Ala	-
	m.05636T→C	tRNA Ala	-
	m.05692T→C	tRNA Asn	-
	m.05698G→A	tRNA Asn	-
	m.05703G→A	tRNA Asn	+
	m.07458G→A	tRNA Ser (UCN)	-
	m.07506G→A	tRNA Ser (UCN)	-
	m.08342G→A	tRNA Lys	-
	m.12276G→A	tRNA Ser (AGY)	-
	m.12283G→A	tRNA Ser (AGY)	-
	m.12294G→A	tRNA Leu (CUN)	-
	m.12311T→C	tRNA Leu (CUN)	-
	m.12315G→A	tRNA Leu (CUN)	+
	m.12316G→A	tRNA Leu (CUN)	-
	m.14723T→C	tRNA Glu	-
Coding and control point mutations	m.11232T→C	MT-ND4	-
	m.13094T→C	MT-ND5	-
Nuclear DNA	15q25	POLG1	+
	17q23-q24	POLG2	+
	10q24	Twinkle	+
	4q35	ANT1	+
	3q28-q29	OPA1	+
	22q13.32-qter	TYMP	+
	8q23.1	RRM2B	+

Adapted from www.mitomap.org

Figure 4: The patient with oculopharyngeal muscular dystrophy (left) compensates his bilateral ptosis with the astrologist's posture. Because of restricted downward gaze, the patient with chronic progressive external ophthalmoplegia (right) is unable to use this compensation.

CPEO patients rarely use the astrologist's posture to compensate their ptosis (Figure 4) [37]. Myasthenia gravis is an acquired neuromuscular disorder characterized by external ophthalmoplegia and dysphagia. Again, extra-muscular features are lacking. One of the main features of myasthenia gravis is exercise induced augmentation of weakness. As a result, muscle weakness shows marked variations during the day and the extent of extra-ocular weakness is often asymmetric. In CPEO, marked asymmetry and predominant exercise induced weakness are uncommon. Congenital myasthenic syndromes (CM) comprise a group of hereditary disorders characterized by muscle weakness, mostly due to a mutation in the postsynaptic acetylcholine receptor. Weakness can affect the extra-ocular, bulbar, limb and respiratory muscles, depending on the subtype [38]. Differentiation between CM and CPEO can be particularly difficult, although there are some distinctive features:

1) CM patients often present in infancy or early childhood, while disease onset in CPEO is often in adulthood or adolescence
2) muscle weakness is often the sole feature of CM, while extra-muscular features are common in childhood onset CPEO
3) although muscle weakness can fluctuate in CPEO, prominent fluctuations are rare
4) a decrement of the compound motor action potential (CMAP) after repetitive nerve stimulation suggests CM
5) elevated venous lactate and ragged red fibers or COX-negative fibers in a skeletal muscle biopsy suggest CPEO

The **prognosis** in CPEO is highly variable. Although there are no controlled studies on age of death in CPEO, available data indicate that life expectancy for the whole group is decreased to about 50 to 60 years [23]. The rate of disease progression is more severe in patients with a disease onset below 9 years and in patients with central nervous system involvement, including retinal and cochlear dysfunction [25].

Only one **therapy** has a proven beneficial effect in CPEO. An aerobic cycle training schedule was found to decrease fatigue and to increase physical endurance, daily physical activities and leg muscle strength [39]. However, the 12 weeks, four times weekly schedule is demanding and therefore not suitable for patients with advanced disease. Tetracycline 500 mg daily has been shown to delay progression of extra-ocular muscle weakness over a four year period in a single patient [40]. However, the effect of tetracycline has yet to be confirmed in a larger cohort. Last, a beneficial effect of nutritional supplements, most importantly co-enzyme Q10, has been reported in several uncontrolled trials [41].

OUTLINE OF THIS THESIS

The aim of this thesis is to delineate the CPEO phenotype, in order to more fully appreciate the multi-organ involvement. This helps to indentify the specific symptoms and disabilities in the individual patient, which ultimately allows for optimal personalized symptomatic management.

Patients' management generally starts with a diagnostic workup in order to reach the correct diagnosis. In **Part II** we provide several considerations for the workup of a suspected mitochondrial disorder: **Chapter 2** describes a study on the value of mitochondrial respiratory chain complex activities for the discrimination between mitochondrial disorders and the chronic fatigue syndrome, a condition of unknown etiology which can phenotypically resemble a mitochondrial disorder. **Chapter 3** describes the first case of CPEO caused by a specific point mutation in the mitochondrial isoleucine tRNA.

Part III provides a broad overview of the CPEO phenotype. This includes studies on the impact of disease (**Chapter 4**), sleep disturbances (**Chapter 5**), respiratory involvement (**Chapter 6**) and on renal involvement (**Chapter 7**).

All chapters are summarized in **Part IV**, which also contains a diagnostic algorithm, suggestions for patient management and a section on future perspectives.

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Part II

Diagnostic considerations

Chapter 2

Mitochondrial enzymes discriminate
between mitochondrial disorders
and chronic fatigue syndrome

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ABSTRACT

We studied the extent of mitochondrial involvement in chronic fatigue syndrome (CFS) and investigated whether measurement of mitochondrial respiratory chain complex (RCC) activities discriminates between CFS and mitochondrial disorders. Mitochondrial content was decreased in CFS compared to healthy controls, whereas RCC activities corrected for mitochondrial content were not. Conversely, mitochondrial content did not discriminate between CFS and two groups of mitochondrial disorders, whereas ATP production rate and complex I, III and IV activity did, all with higher activities in CFS. We conclude that the ATP production rate and RCC activities can reliably discriminate between mitochondrial disorders and CFS.

INTRODUCTION

Patients with non-specific neuromuscular complaints, such as fatigue, myalgia and exercise intolerance can pose a diagnostic dilemma. These complaints can either be the sole features of a mitochondrial disorder but can also occur in the absence of any recognizable somatic disorder [1]. In the latter case, the patient is often diagnosed with chronic fatigue syndrome (CFS) [2]. When in doubt between a mitochondrial disorder and CFS, a skeletal muscle biopsy can be performed for further differentiation [3].

Skeletal muscle is highly suitable for histological examination and is the tissue of choice for the measurement of the activities of the four mitochondrial respiratory chain complexes (RCC) involved in adenosine triphosphate (ATP) production. Decreased activity of one or more RCCs is suggestive of a mitochondrial disorder but is also reported in physically inactive, otherwise healthy subjects [4;5]. Since physical inactivity is common both in mitochondrial disorders and in CFS, the diagnostic value of measuring skeletal muscle RCC activities is unsure.

Here, we determined the diagnostic value of RCC activities for the discrimination between mitochondrial disorders and CFS. For this purpose, we first determined the extent of mitochondrial involvement in CFS by comparing skeletal muscle biopsies of CFS patients to those of healthy controls. Next, to determine the diagnostic value of RCC activities we compared muscle biopsies between CFS patients and two groups of patients with genetically confirmed mitochondrial disorders.

METHODS

CFS patients

To determine the extent of mitochondrial involvement in CFS we compared RCC activities in skeletal muscle biopsies between CFS patients and healthy controls. The CFS group was recruited from patients who had undergone a muscle biopsy with measurement of RCC activity for evaluation of a suspected neuromuscular or mitochondrial disorder between 2005 and 2007. All patients met the US Center for Disease Control (CDC) criteria for CFS [2;6;7]. According to these criteria patients have to be severely fatigued (operationalised as a score of ≥ 35 on the subscale fatigue of the Checklist Individual Strength) and the fatigue has to last longer than 6 months. The fatigue is neither the result of a somatic or psychiatric disease or of ongoing exertion, nor is it alleviated by rest. According to the CDC definition patients should also have four out of eight additional symptom criteria. These are myalgia, multi-joint pain, headaches, a sore throat, post-exertional malaise, unrefreshing sleep, concentration and/or memory impairments, and sensitive lymph nodes. The patients included in the present study should meet this criterion but at least

have myalgia and/or exercise intolerance. The CFS symptoms have to lead to substantial functional impairment, operationalised in the present study as having a score of 700 or higher on the Sickness Impact Profile (SIP8) [8;9].

All patients underwent a physical examination and laboratory tests to rule out an underlying medical condition that could explain the symptoms. Therefore, all CFS patients had:

1. Normal physical and neurological examination, performed by a neurologist specialized in neuromuscular disorders
2. No family history suggestive of a neuromuscular or mitochondrial disorder
3. Normal routine blood tests (serum blood count, liver, renal and thyroid function, glucose, creatine kinase, lactate)
4. Normal muscle histology
5. No newly diagnosed medical condition explaining the fatigue during a follow up period of at least two years

Controls

Healthy controls were recruited among medical students. None of these controls participated in professional or high intensity sports. Needle biopsies were taken from the right quadriceps muscle in all participants. All gave their written informed consent and the study was approved by local medical ethics committee.

Mitochondrial disorders

We compared the biopsies of CFS patients to those of two groups of mitochondrial disorders. One group consisted of all patients (n=22, 11 female) with genetically confirmed chronic progressive external ophthalmoplegia (CPEO) diagnosed at the Neuromuscular Center Nijmegen (mtDNA deletion in 14 patients, mtDNA point mutation in three, multiple mtDNA deletions associated with homozygote or compound heterozygote nuclear polymerase gamma 1 mutation in five). The other group consisted of all patients (n= 27, 15 female) registered at the Nijmegen Department of Human Genetics with an m.03243A→G mutation (A3243G) in skeletal muscle (mean heteroplasmy: 63.8%). All A3243G patients had phenotypes that were known to be associated with the m.03243A→G mutation. Two CPEO patients carrying the m.03243A→G mutation were included in the A3243G group only. All but three A3243G patients were previously described in detail, including correlations between heteroplasmy levels and RCC activities [10].

Muscle biopsy handling

Muscle biopsies underwent routine histological staining for light microscopy. Fiber typing was performed according to a standard procedure of determining ATPase activity under different pH-conditions (pH 4.3, 4.6 and 10.3) [11]. ATP production rate and mitochondrial RCC activities (complex I, II+III, III and IV) were measured in fresh muscle

according to methods described previously [12]. In addition, we determined the activity of the mitochondrial matrix enzyme citrate synthase (CS; in mU/mg protein) as a marker for mitochondrial content in the muscle biopsy samples [13]. Since RCC activities are notably affected by the total mitochondrial content in the muscle biopsy, all RCC activities were normalized to CS activity. Consequently, ATP production rate is expressed in nmol ATP/h/mU CS and RCC activities in mU/U CS.

Analysis

Mann-Whitney and Kruskal-Wallis tests were used to compare means, with p<0.05 (two-sided) indicating significance. We constructed receiver operating characteristic (ROC) curves to compare RCC activities of the CFS patients to the RCC activities of the healthy controls and of the two groups of mitochondrial disorders. We calculated an area under the curve (AUC) for all ROC curves as a determinant of discriminative value [14]. In general, an AUC of 0.5 indicates that the test has no discriminative value, whereas an AUC of 1 indicates 100% discriminative power. Here, we considered a test to have discriminating value when the AUC was above 0.7.

RESULTS

Sixteen patients (9 female) met the criteria for CFS. Mean Checklist Individual Strength fatigue score was 52.8 (range 44-56), mean SIP8 score 1265 (range 720-2288) and mean CDC symptom inventory score 6.6 (range 4-9). Eleven male healthy controls were recruited. Age at biopsy was 29.0 ± 10.8 years in controls, 38.3 ± 8.3 in CFS, 44.0 ± 10.1 in CPEO and 28.0 ± 16.1 in A3243G. The percentage of type I fibers did not differ between groups (controls 44%, CFS 44%, CPEO 43%, A3243G 49%, p=0.588). COX negative and ragged red fibers were only found in CPEO patients (23.4% and 3.4%) and in A3243 (2.7% and 5.2%) patients. Histological biopsies of 9 A3243G patients were performed in another center and could not be traced.

Mitochondrial involvement in CFS

CS activity, a marker for mitochondrial content, was decreased in CFS patients compared to healthy controls (97 ± 32 vs. 180 ± 49 mU/mg protein, p<0.001) (Figure 1). There were no differences in RCC activities or ATP production rate.

Diagnostic value of RCC activities in CFS vs. mitochondrial disorders

Complex I, III and IV activity and ATP production rate discriminated (AUC > 0.7) between CFS patients and both groups of mitochondrial disorders, with lower enzyme activities in the mitochondrial disorders (Table 1, Figure 1). In contrast, CS activity had no discrimina-

Figure 1: Scatter plots of the activity of mitochondrial respiratory chain complexes I, II+III, III and IV, the ATP production rate and citrate synthase activity.
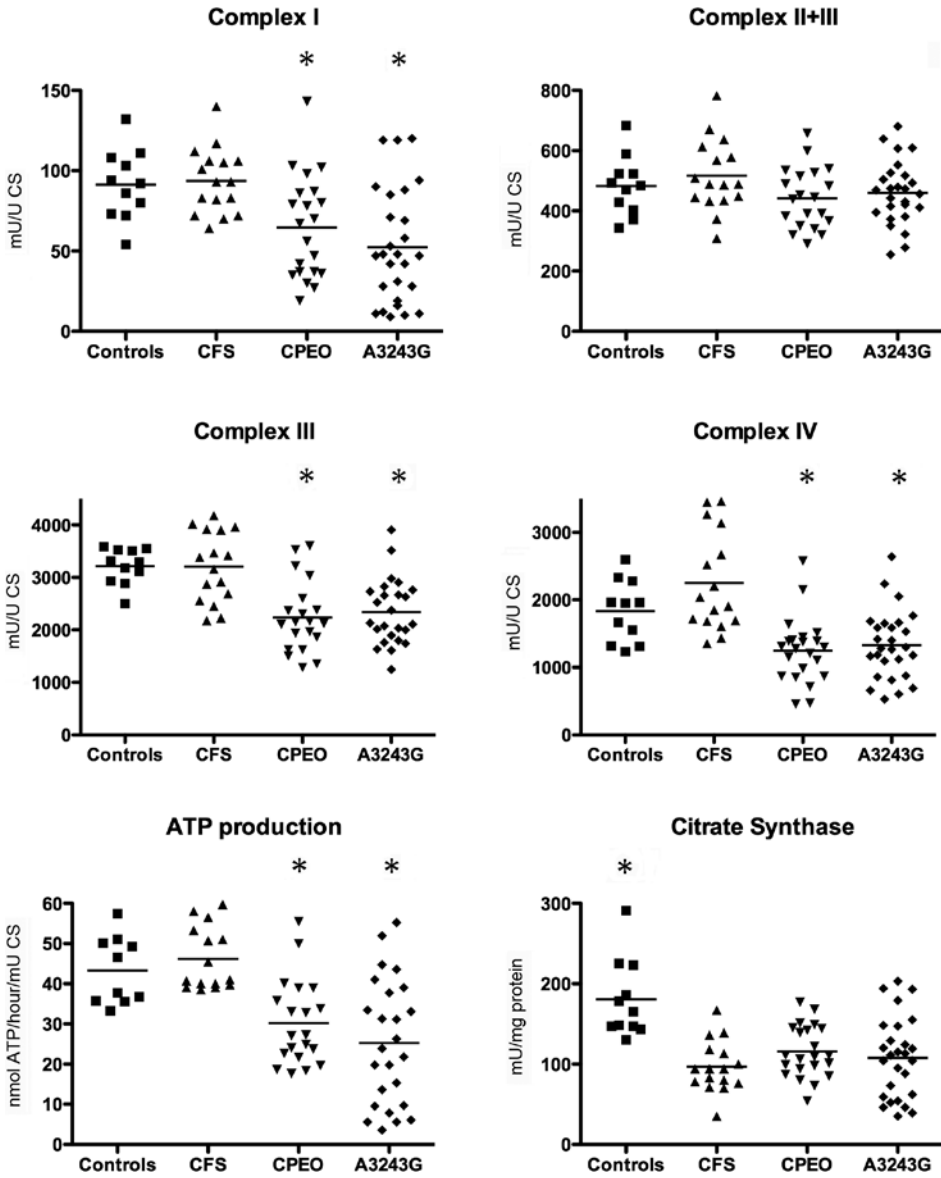
* area under the curve > 0.7 in comparison to CFS

tive value, nor did complex II+III. The latter is in agreement with the fact that CPEO and A3243G patients usually have deficiencies in the RCCs that mainly consist of mtDNA encoded subunits rather than in the entirely nuclearly coded complex II subunits.

Table 1: Areas under the receiver operating characteristic curves (AUC ROC) comparing the CFS patients to healthy controls and to CPEO and A3243G patients.

	CFS vs. Control	CFS vs. CPEO	CFS vs. A3243G
Complex I	0.52 (0.29-0.74)	**0.78 (0.63-0.93)**	**0.82 (0.69-0.95)**
Complex II+III	0.58 (0.36-0.80)	0.67 (0.49-0.85)	0.64 (0.47-0.81)
Complex III	0.52 (0.29-0.74)	**0.85 (0.73-0.98)**	**0.83 (0.71-0.95)**
Complex IV	0.66 (0.45-0.87)	**0.91 (0.82-1.0)**	**0.88 (0.78-0.98)**
ATP production	0.66 (0.42-0.90)	**0.90 (0.79-1.0)**	**0.87 (0.76-0.98)**
Citrate synthase	**0.96 (0.0-1.0)**	0.69 (0.51-0.86)	0.57 (0.39-0.74)

Tests with discriminative value (AUC > 0.7) are in bold.

Table 2: Cut off values with a 100% sensitivity for CFS and the associated specificities vs. CPEO and A3243G

	cut off value with 100% sensitivity for CFS	specificity for CFS vs. CPEO (%)	specificity for CFS vs. A3243G (%)
Complex I (mU/U CS)	60	48	67
Complex III (mU/U CS)	2153	50	52
Complex IV (mU/U CS)	1327	55	56
ATP production (nmol ATP/h/mU CS)	37.2	75	72

Complex II+III and citrate synthase activity did not have discriminative value and are therefore not included in this table.

The highest discriminative values (i.e. highest AUCs) were found for complex IV activity and ATP production rate. However, at cut off values with 100% sensitivity for CFS (meaning that no CFS patients are incorrectly diagnosed with a mitochondrial disorder), the specificity of the ATP production rate was much higher than the specificity of complex IV activity (Table 2).

In the four A3243G patients with the highest heteroplasmy levels (>87%), ATP production rate and RCC activities were below the cut off values in Table 2. In contrast, ATP production rate and RCC activities were above the cut off values in all three A3243G patients with the lowest heteroplasmy levels (< 34%), as described before [10].

DISCUSSION

Studying the extent of mitochondrial involvement in CFS, we found that mitochondrial *function* is unaffected in skeletal muscle of CFS patients whereas mitochondrial *content*

(indicated by CS activity) is notably decreased. Mitochondrial content has previously been related to the level of physical activity: mitochondrial content is low in physically inactive subjects and increases in response to training, both in otherwise healthy subjects as in patients with mitochondrial disorders [4;5;15;16]. This may suggest that low mitochondrial content in the present cohort of CFS patients is the consequence rather than the cause of physical inactivity. Nevertheless, regardless of cause or consequence, low mitochondrial content might be a perpetuating factor for complaints such as fatigue, myalgia and exercise intolerance in CFS, while an increase in mitochondrial content secondary to increased habitual physical activity can contribute to the beneficial effects of graded exercise treatment in CFS [17].

The present study is the first to directly compare mitochondrial function between patients with CFS and with mitochondrial disorders. However, there are previous studies comparing CFS patients to healthy controls, with conflicting results. Some studies found no differences between CFS and controls [18-21]. In contrast, others found evidence for mitochondrial dysfunction in CFS, consisting of a decreased anaerobic threshold to incremental exercise, or a decreased post exercise ATP recovery rate on skeletal muscle magnetic resonance spectroscopy [22-27]. However, incremental exercise tests or skeletal muscle magnetic resonance spectroscopy do not differentiate between decreased mitochondrial content and decreased mitochondrial function. Since the present results do not support the concept of primary mitochondrial dysfunction in CFS, we hypothesize that the findings of these previous studies might be attributed to decreased mitochondrial content. We found that ATP production rate was the most reliable test to discriminate between CFS and mitochondrial disorders. ATP production rate was within the normal range in all CFS patients whereas it was decreased in grossly three quarters of the patients with mitochondrial disorders. Patients with an ATP production rate below the proposed cut off value can therefore reliably be diagnosed with a mitochondrial disorder, without the risk of incorrectly diagnosing CFS patients with a mitochondrial disorder. However, an ATP production rate above the proposed cut off value does not discriminate between CFS and mitochondrial disorders.

CONCLUSIONS

The diagnosis of a mitochondrial disorder often relies on multiple laboratory diagnostic tests, such as mtDNA sequence analysis, metabolite profiling, and muscle histology. The present results however demonstrate that the ATP production rate and RCC activities can be used to discriminate between CFS and mitochondrial disorders in patients with non-specific neuromuscular complaints.

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Chapter 3

Chronic progressive external ophthalmoplegia
caused by an m.04267A→G mutation
in the mitochondrial tRNA^{Ile}

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ABSTRACT

We found a heteroplastic m.04267A→G mutation in the mitochondrial tRNAIle in a 52-year-old patient with a five year history of progressive external ophthalmoplegia and exercise induced muscle cramps. This specific mutation had been described only once before in a patient with deafness, cerebellar dysarthria, muscle ache, fatigue and mental slowing, but no signs of external ophthalmoplegia. The fact that we found the m.04267A→G mutation in a patient with CPEO, an established mitochondrial phenotype, provides additional evidence for its pathogenicity. Moreover, our findings support the approach of sequencing all relevant mitochondrial tRNA genes in cases of CPEO that are not due to large scale mtDNA rearrangements.

Chronic progressive external ophthalmoplegia (CPEO) is a mitochondrial disease, which mainly affects extraocular muscles. The majority of CPEO patients have single deletions or point mutations in the mitochondrial DNA (mtDNA) [1;2]. Pathogenic point mutations are almost exclusively located in tRNA coding regions, the isoleucine tRNA (tRNA^Ile) coding region being one of the hotspots. Mutation in four loci of this tRNA^Ile hotspot have been associated with CPEO, while mutations in nine other loci can cause a variety of mitochondrial disorders, mainly hypertrophic cardiomyopathy [1]. The m.04267A→G mutation in the tRNA^Ile has been reported once in a patient with mitochondrial myopathy [3]. Here, we present a patient with an isolated CPEO caused by the m.04267A→G mutation in tRNA^Ile.

A 52-year-old male had a five year history of double vision for which he was treated with prism glasses. He reported muscle cramps after intensive exercise, without muscle weakness, dysphagia or dysarthria. Family history was unrevealing. His medical history included surgery for a L5-S1 lumbar disc herniation and lactase deficiency, which was well under control with a lactose-free diet. On neurological examination, he had symmetrical ophthalmoparesis in all directions and bilateral ptosis. Both ptosis and ophthalmoparesis worsened after prolonged horizontal gaze. Further neurological examination was normal. Electromyography (EMG), including single fiber EMG, showed evidence of generalized myopathy with normal nerve conductions and neuromuscular transmission. Brain MRI and echocardiography were normal. Serum lactate was normal and creatine kinase was mildly elevated (391 U/l, normal <200). Antibodies against skeletal muscle and acetylcholine receptor were undetectable. Needle biopsy of the quadriceps muscle revealed 10% raggedred fibers, 50% cytochrome c oxidase (COX) negative fibers and abnormal mitochondria in electron microscopy (size differences, compact circular cristae, and paracrystalline inclusions) (Figure 1). Measured according to standard procedures, both complex I activity as well as complex III activity and ATP production rate from pyruvate were decreased (53, 58 and 64% of lowest reference value, respectively) in fresh skeletal muscle [4]. Activities of complex II, IV and V were normal. Molecular analysis of skeletal muscle mtDNA excluded large-scale rearrangements. Sequencing of several tRNA genes previously reported to be associated with CPEO revealed a heteroplasmic A to G mutation at position 4267 in the tRNA^Ile with a heteroplasmy level of approximately 50% in muscle [1].

This is the second report of a mitochondrial disease caused by an m.04267A→G mutation in the mitochondrial tRNA^Ile and the first report of an association with CPEO. Previously, Taylor et al. found the m.04267A→G in a patient with deafness, cerebellar dysarthria, muscle ache, fatigue and mental slowing since the age of 30 [4]. The level of heteroplasmy in muscle tissue in Taylor's patient was 88%, compared to 50% in our patient. It is tempting to speculate that the percentage of heteroplasmy of the m.04267A→G mutation in skeletal muscle correlates with the age of onset and the disease severity, although in

Figure 1: Quadriceps muscle sections showing ragged-red fibers (RR's) in **A** (hematoxylin-phloxine stain) and **C** (succinic dehydrogenase stain), abnormal mitochondria in **B** (electron microscopy), and COX negative fibers in **D** (cytochrome oxidase stain). The square inset in **B** shows an extra magnification of a mitochondrion with paracrystalline inclusions. **C** and **D** represent serial sections with one COX negative ragged-red fiber, two COX negative non-ragged-red fibers, and a longitudinal cut muscle fiber partly devoid of COX activity. Bars represent 50 micron (**A**, **C**, and **D**) and 0.5 micron (**B**).
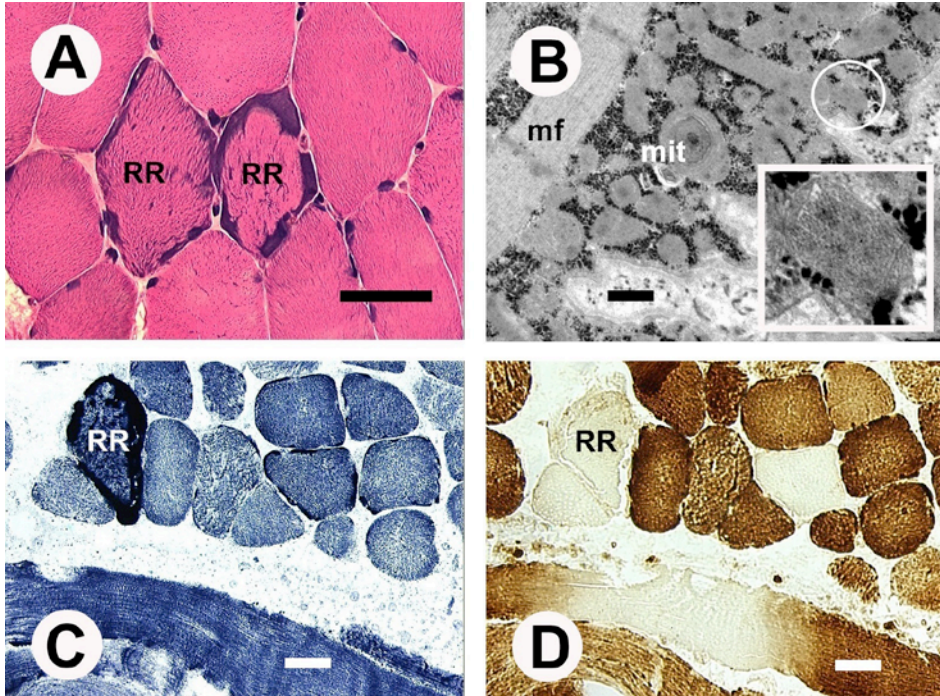
mf: myofibril; mit: region with mitochondria

general, genotype-phenotype correlations in mitochondrial disorders caused by mtDNA mutations are not clear.

We can only speculate about the link between the tRNA[Ile] mutation and the combined complex I and III deficiency. Since complex I and III both have a high isoleucine content, impaired incorporation of isoleucine due to the tRNA[Ile] mutation could have altered their assembly, thereby resulting in loss of function [1].

In conclusion, this is the first report of an association between CPEO and the m.04267A→G mutation in the mitochondrial tRNA[Ile]. Since CPEO is a typical mitochondrial phenotype, this study provides additional evidence for the pathogenicity of this mutation. Furthermore, our findings support the approach of sequencing all relevant mitochondrial tRNA genes in cases of CPEO that are not due to large scale mtDNA rearrangements.

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Part III

The phenotype of chronic progressive
external ophthalmoplegia

Chapter 4

Disease impact in chronic progressive
external ophthalmoplegia:
More than meets the eye

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ABSTRACT

We determined the extent of disease impact in 28 patients with genetically confirmed chronic progressive external ophthalmoplegia (CPEO) and compared the outcomes to those of matched myotonic dystrophy type I (DM1) patients.

CPEO patients reported a high frequency of severe fatigue (67.9%), pain (96.2%), depression (32.1%) and dependency in daily life (46.4%). The frequency and extent of depression were significantly higher than in DM1 patients (32.1% vs. 7.1%, p=0.040; mean Beck's depression inventory for primary care score 3.8 ± 3.5 vs. 1.3 ± 1.4, p=0.001), as were fatigue severity, pain intensity and extent of functional impairments.

CPEO patients with polymerase gamma 1 mutations reported more functional impairments than those with mitochondrial DNA mutations. Disease impact was however not influenced by most clinical features. The present results help physicians to identify and to treat the factors that influence quality of life in CPEO patients and to provide symptomatic treatment where needed.

INTRODUCTION

Chronic progressive external ophthalmoplegia (CPEO) is one of the most common mito-chondrial disorders in adults [1]. The defining symptom is a slowly progressive extraocular muscle weakness. Multi-system involvement is however common, causing functional impairments secondary to dysfunction of (proximal) skeletal muscles, retina, cochlea, cerebrum, cerebellum and heart [2].

The frequency and nature of these impairments (body level) are well documented in previous studies, but their impact on daily life activities (individual level) or participation (society level) are unknown [3-6]. In our experience, there is often a discrepancy between the generally mild impairments and the severe limitations in activities and participation in CPEO patients. This discrepancy may lead to underestimation of disease impact by the treating physician, which in turn could result in inadequate symptomatic treatment at the level of activities and participation.

Here, we used a set of validated questionnaires to systematically determine disease impact on daily life activities and participation in a large cohort of CPEO patients from a single tertiary referral centre. In order to identify possible associations between disease impact and mutation type or clinical features we only included patients with a known causative mutation, and all participants underwent standardized neurological examinations. To estimate the clinical relevance of our results, we compared questionnaires outcomes of CPEO patients to those of matched myotonic dystrophy type I (DM1) patients. DM1 is suit-able for comparison with CPEO since it is a relatively common neuromuscular disorder and therefore well known to many neurologists. Moreover, like CPEO, DM1 also affects muscle, eye, brain, and heart.

METHODS

Patients

All CPEO patients known in Department of Neurology of the Radboud University Nij-megen Medical Centre were invited to participate. As there are no commonly accepted criteria for CPEO, we only included patients with both characteristic clinical features and a known causative mutation [2]. Consequently, all participants met the following three criteria:

(1) a phenotype including a slowly progressive bilateral external ophthalmoplegia,

(2) a proven pathogenic mutation or deletion in the mitochondrial DNA (mtDNA) or in the nuclear polymerase gamma 1 (*POLG1*), *Twinkle* or adenine nucleotide translocator 1 (*ANT1*) genes,

(3) exclusion of an alternative diagnosis.

These inclusion criteria also cover two more or less specific mitochondrial phenotypes with external ophthalmoplegia as a key symptom: the Kearns–Sayre syndrome (KSS: ophthalmoplegia, pigmented retinopathy, age of onset below 20 years, and at least one of the following symptoms: cardiac conduction block, cerebellar ataxia, elevated CSF protein content) and SANDO (Sensory Atactic Neuropathy, Dysarthria and Ophthalmoplegia) [7-9]. In the present study, we used the term CPEO to describe all patients meeting the inclusion criteria and the terms KSS and SANDO to describe the specific subtypes only.

Age and sex matched controls were selected from a previously published cohort of genetically confirmed, adult onset DM1 patients, all of whom attended normal education [10]. All participants gave informed consent. Local ethical committee approved this study and the patients' consent was obtained according to the Declaration of Helsinki.

Questionnaires

We used a set of validated questionnaires to evaluate several important determinants of disease impact:

Fatigue severity

Experienced fatigue is defined as an overwhelming sense of tiredness, lack of energy and feeling of exhaustion. We used the Checklist Individual Strength (CIS) to determine the severity of experienced fatigue [11]. CIS is a 20-item questionnaire, which measures the severity of fatigue in four different sub items: fatigue severity (8 items, range 8–56), reduced concentration (5 items, 5–35), reduced activity (3 items, range 3–21), and reduced motivation (4 items, range 4–28). Each item was scored on a seven-point scale. A CIS fatigue score equal to or higher than 35 indicates severe fatigue [11;12].

Pain

Analgesic use and intensity and location of pain were assessed with the McGill's Pain Questionnaire (MPQ) [13]. Pain intensity was scored on a 100 mm horizontal visual analogue scale (VAS) and was divided in minimal, actual and maximal VAS scores. Patients could allocate pain to one or more of 32 predefined body areas.

Depression

Depression was rated according to the Beck's Depression Inventory for Primary Care (BDI-PC) [14]. This is a 7 item self-report instrument composed of cognitive and affective symptoms. Each item is scored on a 4-point scale. Scores equal to or higher than 5 indicate depression [14]. BDI-PC was preferred over the complete BDI to avoid an overlap between physical aspects of fatigue and the somatic symptoms of depression.

Functional impairments and independency

Functional impairments were assessed with the Dutch version of the Sickness Impact Profile (SIP-136) [15]. The 136 items are divided into 12 categories: sleep and rest, emotional behavior, body care and movement, household management, mobility, social interaction, ambulation, alertness and intellectual functioning, communication, work, recreation and pastimes, and eating. The SIP-136 has no validated cut off value, as healthy controls are by definition free of disease related functional impairments.

The level of independency was assessed with the modified Rankin scale (mRS), a 7-point observer rated scale. The mRS scores 0–2 indicate independency in daily life activities [16]. In all questionnaires, higher scores indicate more disability.

Statistical analysis

Data analysis was performed using SPSS (version 17.0) for Windows. An independent Mann–Whitney U-test was used to compare means and a Fisher's exact test to compare frequencies. Correlations were calculated using a Spearman coefficient and odds ratios using a binary logistic regression. Significance level was set at $p < 0.05$ (two-tailed) in all cases.

RESULTS

Patients

We identified 30 patients meeting the inclusion criteria. All were invited to participate, two male patients (one with an m.12315G→A mutation and one with a mtDNA deletion) refused because of a lack of motivation. In general, their clinical features and disease severity were in the same range as the participants. Of 28 participants (12 men, 16 women), 16 patients had a single mtDNA deletion, 4 a pathogenic mtDNA point mutation and 8 a *POLG1* mutation (Table 1). *POLG1* mutations included a homozygous A467T in six patients and compound heterozygous A467T and W748S mutations in two (patients 27 and 28). None of the patients had *Twinkle* or *ANT1* mutations. Mean disease duration was 23.3 ± 10.6 years and mean age of onset was 23.6 ± 11.7 years. Three patients met the criteria for KSS and 7 for SANDO.

Questionnaires

Fatigue severity

Severe fatigue (CIS fatigue score ≥ 35) was the most frequent complaint, being reported by 19 patients (67.9%) with a mean CIS fatigue score for the whole group of 40.0 ± 12.6.

Table 1: Demographic, genetic and clinical data of 28 CPEO patients

no.	sex / age / age at onset	mutation	cerebellar ataxia	periph. neurop.	dysarthria	cognitive impairm.	proximal myopathy	retinal / cochlear involv.	other
1	F/41/32	deletion	-	-	-	-	-	-/+	HT
2	F/28/17	deletion	-	-	-	-	+	-/-	
3	F/33/14	deletion	-	-	-	-	-	-/-	M
4	M/30/8	deletion	+	-	-	+	+	+/+	
5	F/49/25	deletion	-	-	-	-	+	-/-	
6	F/57/34	deletion	-	-	-	-	+	-/-	CM, E
7	M/51/25	deletion	-	-	+	-	+	-/-	
8	M/43/14	deletion	+	-	+	+	+	+/+	CB
9	F/50/13	deletion	-	-	-	-	+	-/-	
10	F/56/17	deletion	-	-	-	-	-	-/-	
11	M/57/12	deletion	-	-	+	-	+	-/-	
12	F/45/5	deletion	-	-	-	-	-	-/-	
13	F/35/25	deletion	-	-	-	-	-	-/-	
14	F/42/26	deletion	-	-	-	-	+	-/-	
15	F/37/12	deletion	+	-	+	-	+	+/+	
16	F/54/29	deletion	+	-	+	+	+	+/+	AF
17	M/55/30	m.05709T>C	-	-	-	-	-	-/-	O, E, C
18	M/54/48	m.04267A>G	-	-	-	-	+	-/-	
19	F/50/12	m.03243A>G	+	-	+	-	+	-/+	DM, M
20	M/49/12	m.03243A>G	-	+	-	+	-	-/+	DM
21	M/75/54	POLG1	-	+	+	-	+	-/-	
22	F/42/30	POLG1	+	SAN	+	-	+	-/-	
23	M/54/35	POLG1	+	SAN	+	+	+	-/-	
24	F/48/25	POLG1	+	SAN	+	+	+	-/-	
25	M/46/39	POLG1	+	SAN	+	+	+	-/-	
26	M/39/23	POLG1	+	SAN	+	-	+	-/-	
27	F/48/24	POLG1	+	SAN	+	-	+	-/+	
28	M/46/21	POLG1	+	SAN	+	+	-	-/-	

Age and age at onset in years

C, cataract; CB, cardiac conduction block; CM, cardiomyopathy; DM, diabetes mellitus; E, epilepsy; HT, hypothyroidism; M, migraine; O, otosclerosis; POLG1, polymerase gamma 1; SAN, sensory atactic neuropathy.

+ presence of symptom; – absence of symptom.

Demographic characteristics, clinical features, mutation type, and outcomes on all other questionnaires did not differ between patients with or without severe fatigue.

Pain

CPEO patients mainly reported pain in the upper legs and head (Figure 1). Only one patient (3.6%) reported no pain (actual, minimal and maximal VAS scores = 0 mm). Twelve patients (42.9%) reported pain on waking up, of whom 5 had moderate to severe pain. Ten patients (28.6%) reported limitations in daily activities due to pain. Among the 27 patients with pain, sixteen (59.3%) used analgesics, mostly acetaminophen (13 patients, 48.1%), NSAIDs (7 patients, 25.9%) and opioids (2 patients, 7.4%). Patients using analgesics had higher CIS reduced concentration (21.1 ± 10.0 vs. 13.5 ± 8.3, p=0.036) and SIP sleep and rest scores (104.8 ± 64.1 vs. 47.6 ± 33.2, p=0.033) than patients without analgesic use, while there were no differences in pain severity.

Figure 1. Distribution of pain in CPEO patients showing the percentage of patients with pain in 32 predefined body areas
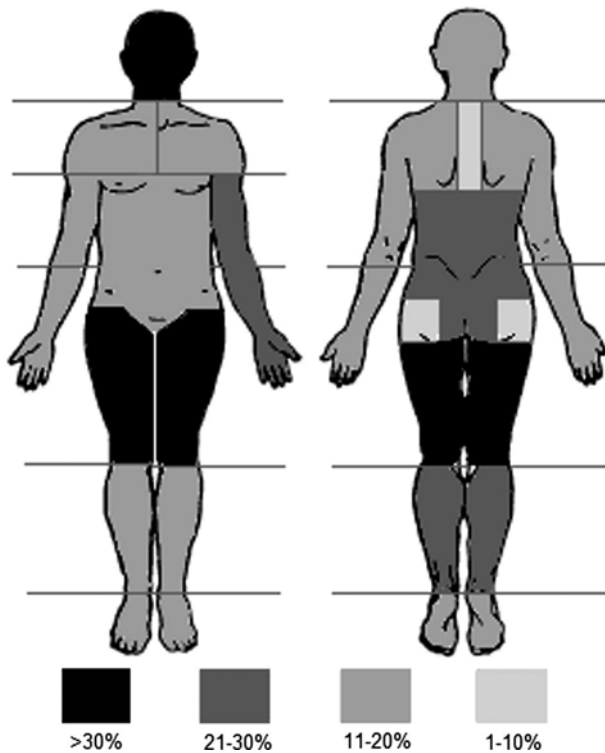
Depression

Depression (BDI-PC score ≥ 5) was found in 9 patients (32.1%) and mean BDI-PC score was 3.8 ± 3.5. Only 2 of the nine patients with a depression used antidepressants, together with 2 non-depressed patients. Depression was associated with various other determinants of disease impact: depressed patients had higher CIS reduced motivation (20.3 ± 4.6 vs.

14.5 ± 6.4, p=0.012), CIS reduced activity (16.7 ± 3.6 vs. 11.4 ± 6.1, p=0.030) and total CIS scores (101.2 ± 16.9 vs. 80.7 ± 26.1, p=0.049) compared to non-depressed patients. Moreover, depressed patients were less likely to be independent (mRS 2) in daily life activities (11% vs. 74%, p=0.004) and had more severe functional impairments (total SIP score 2993 ± 1380 vs. 1621 ± 1320, p=0.017). This difference in total SIP score mainly consisted of significantly higher subscores on emotional behavior (204.9 ± 149.8 vs. 74.0 ± 120.7, p=0.010), body care and movement (580.1 ± 428.7 vs. 267.2 ± 348.6, p=0.025), mobility (248.7 ± 158.4 vs. 81.6 ± 189.6, p=0.005), social interaction (463.6 ± 274.4 vs. 209.7 ± 243.1, p=0.018), and ambulation (257.6 ± 110.9 vs. 124.9 ± 116.8, p=0.005).

Functional impairments and independence
Fifteen patients (53.6%) were independent in daily life activities (mRS ≤ 2), whereas 13 (46.4%) were not (mRS >2). Patients who were independent in daily life activities had lower CIS reduced motivation (13.8 ± 5.3 vs. 19.2 ± 6.6, p=0.015), CIS reduced activity (10.5 ± 5.5 vs. 16.4 ± 5.1, p=0.011), and total CIS scores (75.4 ± 25.6 vs. 101.1 ± 16.8, p=0.009) compared to patients who were not independent. As expected, non-independent patients reported more severe functional impairments (total SIP score 2659 ± 1175 vs. 1584 ± 1616, p=0.018), with significantly higher SIP subscores on body care and move-ment (497.8 ± 392.9 vs. 255.1 ± 377.3, p=0.017), mobility (191.0 ± 164.2 vs. 87.1 ± 210.3, p=0.043), social interaction (377.7 ± 235.9 vs. 216.5 ± 294, p=0.021), ambulation (237.6 ± 119.4 vs. 106.8 ± 107.3, p=0.004), and communication (239.2 ± 191.2 vs. 94.7 ± 113.5, p=0.032).

CPEO vs. DM1 patients

To estimate the clinical relevance of our findings, we compared questionnaire results of CPEO patients to those of age and sex matched DM1 patients. CPEO patients were more often depressed than DM1 patients (32.1% vs. 7.1%, p=0.040). In addition, CPEO patients had higher CIS fatigue, CIS reduced activity and VAS scores and more severe functional impairments, indicated by a higher total SIP score (Table 2). The higher total SIP score mainly consisted of significantly higher subscores on emotional behavior, household management, social interaction and eating. Except for CIS reduced concentration, scores on all other questionnaires were non-significantly higher in CPEO than in DM1 patients.

Influence of clinical features or mutation type on disease impact

Several clinical features were associated with an increased risk of depression (Table 3). In contrast, none of the clinical features was associated with severe fatigue or non-inde-pendency in daily activities, though there was a strong tendency towards an association between non-independency and cerebellar ataxia, peripheral neuropathy and dysarthria.

Table 2: Aspects of disease impact in patients with CPEO and DM1

	CPEO (n=28)	DM1 (n=28)	
CIS			
Fatigue	40.0 ± 12.6	34.0 ± 10.5	p=0.035
Concentration	17.9 ± 10.0	18.1 ± 6.2	
Motivation	16.4 ± 6.4	14.1 ± 6.2	
Activity	13.1 ± 5.9	9.8 ± 5.4	p=0.044
BDI-PC	3.8 ± 3.5	1.3 ± 1.4	p=0.001
MPQ			
Actual VAS pain	22.5 ± 24.6	9.1 ± 12.1	p=0.028
Minimal VAS pain	12.0 ± 14.9	7.7 ± 15.4	p=0.045
Maximal VAS pain	63.3 ± 27.2	30.6 ± 33.8	p=0.001
SIP			
Sleep and rest	93.9 ± 78.6	68.2 ± 61.0	
Emotional behavior	116.1 ± 142.3	15.3 ± 29.9	p=0.001
Body care and movement	367.8 ± 397.0	201.0 ± 184.4	
Household management	228.2 ± 180.4	124.0 ± 86.6	p=0.026
Mobility	135.3 ± 194.2	48.8 ± 91.9	
Social interaction	291.3 ± 276.2	109.7 ± 155.4	p=0.002
Ambulation	167.5 ± 129.3	146.8 ± 125.5	
Alertness	224.4 ± 216.9	137.5 ± 132.0	
Communication	161.8 ± 168.3	103.9 ± 121.7	
Work	160.0 ± 159.4	130.3 ± 144.9	
Recreation and pastimes	114.7 ± 95.7	85.9 ± 76.3	
Eating	22.8 ± 45.1	4.8 ± 19.2	p=0.022
Total	2083 ± 1505	1176 ± 821	p=0.031

DM1, myotonic dystrophy type 1; CIS, Checklist Individual Strength; BDI-PC, Beck's Depression Inventory for Primary Care; MPQ, McGill's Pain Questionnaire; VAS, Visual Analogue Scale; SIP, Sickness Impact Profile.
Scores are expressed as mean ± SD.

Also, fatigue severity, depression, pain and level of independency were not influenced by sex, age or disease duration.

The SANDO phenotype was only present in patients with *POLG1* mutations, while only one of the patients with *POLG1* mutations had a clinical phenotype different from SANDO. Patients with *POLG1* mutations had more functional impairments (i.e. a higher total SIP score), with higher SIP subscores on body care and movement, household management, social interaction, ambulation, and communication (Table 4). The percentage of patients being independent in daily activities did not differ between patients with *POLG1* muta-

Table 3: Odds ratios indicating the relation between clinical features and three aspects of quality of life: fatigue, depression and functional impairments

	Severe fatigue (CIS fatigue ≥ 35)	Depression (BDI-PC ≥ 5)	Non-independent (mRS>2)
Cerebellar ataxia	0.5 (0.1-2.3)	9.8 (1.5-63.8)*	4.4 (0.9-21.8)
Peripheral neuropathy	1.2 (0.2-6.2)	7.5 (1.3-44.1)*	4.7 (0.9-24.8)
Dysarthria	0.7 (0.1-3.5)	2.8 (0.5-14.4)	4.5 (0.9-22.1)
SANDO phenotype	0.5 (0.1-3.1)	10.6 (1.5-76.1)*	4.1 (0.6-26.1)
Cognitive impairment	0.7 (0.1-4.0)	6.7 (1.1-40.4)*	2.5 (0.5-13.5)
Proximal myopathy	1.4 (0.3-7.8)	4.7 (0.5-45.5)	3.7 (0.6-22.8)
Retinal involvement	1.5 (0.1-16.8)	2.4 (0.3-20.8)	4.2 (0.4-46.5)
Cochlear involvement	1.6 (0.3-10.2)	3.0 (0.5-16.7)	2.5 (0.5-13.5)

CIS, Checklist Individual Strength; BDI-PC, Beck's Depression Inventory for Primary Care (BDI-PC); mRS, modified Rankin scale; SANDO, sensory atactic neuropathy, dysarthria, ophthalmoplegia.

Expressed as odds ratio (95% confidence interval).

* $p < 0.05$

tions and patients with mitochondrial DNA mutations or deletions (mRS 0–2: 75% vs. 35%, p=0.096). However, moderately severe disability (mRS = 4: unable to walk without assistance and unable to attend to own bodily needs without assistance) was more common in patients with *POLG1* mutations (50.0% vs. 11.1%, p=0.032). Fatigue severity, depression and pain did not differ between patients with or without *POLG1* mutations. However, depression was more common in patients with SANDO phenotype than in patients with non-SANDO phenotypes (71% vs. 19%, p=0.020).

DISCUSSION

We showed that severe fatigue, depression, pain and functional impairments contribute to disease impact in CPEO patients. We also found that disease impact was related to the mutation type, as patients with *POLG1* mutations had more severe functional impairments than patients with mtDNA mutations or deletions.

We demonstrated that severe fatigue, defined as an overwhelming sense of tiredness, lack of energy and feeling of exhaustion was present in the majority of CPEO patients. Moreover, we found that severe fatigue was more common in CPEO than in DM1 or in several other neuromuscular disorders [17]. The excessive fatigue reported by CPEO patients could in part be attributed to exercise intolerance, a key symptom in mitochondrial myopathies [18]. However, the CIS fatigue questionnaire which we used to rate fatigue severity covers multiple aspects of fatigue. CIS fatigue questionnaire scores were

Table 4: Aspects of disease impact in CPEO patients with mtDNA mutations or deletions (non-POLG1) versus patients with *POLG1* mutations (POLG1)

	CPEO Non-POLG1 (n=20)	CPEO POLG1 (n=8)	
Demographics			
Sex (M:F)	7 : 13	5 : 3	
Age	45.8 ± 9.3	49.8 ± 11.1	
Age at onset	20.5 ± 10.7	31.4 ± 11.1	p=0.041
Disease duration	25.3 ± 11.4	18.4 ± 6.3	
CIS			
Fatigue	40.7 ± 12.9	38.5 ± 12.4	
Concentration	18.1 ± 10.7	17.3 ± 8.3	
Motivation	15.1 ± 5.4	19.5 ± 7.9	
Activity	12.6 ± 6.0	14.4 ± 5.9	
BDI-PC	3.6 ± 3.7	4.5 ± 2.9	
MPQ			
Actual VAS pain	25.3 ± 23.5	15.0 ± 27.5	
Minimal VAS pain	12.8 ± 13.9	9.7 ± 18.6	
Maximal VAS pain	65.6 ± 23.8	57.1 ± 36.3	
SIP			
Sleep and rest	100.1 ± 82.1	78.6 ± 71.9	
Emotional behavior	120.1 ± 150.2	106.1 ± 129.1	
Body care and movement	204.3 ± 208.8	776.4 ± 471.7	p=0.001
Household management	172.6 ± 138.6	367.1 ± 206.1	p=0.019
Mobility	103.7 ± 195.4	214.4 ± 178.4	
Social interaction	206.6 ± 248.1	503.3 ± 235.0	p=0.003
Ambulation	122.4 ± 98.9	280.5 ± 132.4	p=0.007
Alertness	213.4 ± 229.1	251.9 ± 194.6	
Communication	113.0 ± 127.1	283.8 ± 203.8	p=0.032
Work	158.9 ± 158.3	162.8 ± 173.3	
Recreation and pastimes	103.8 ± 100.2	142.0 ± 82.8	
Eating	16.6 ± 40.2	38.5 ± 55.4	
Total	1635 ± 1317	3205 ± 1421	p=0.011
Modified Rankin scale	2.2 ± 1.0	3.3 ± 0.9	p=0.017

CIS, Checklist Individual Strength; BDI-PC, Beck's Depression Inventory for Primary Care; MPQ, McGill's Pain Questionnaire; mtDNA, mitochondrial DNA; POLG1, polymerase gamma 1; VAS, Visual Analogue Scale; SIP, Sickness Impact Profile.

Scores are expressed as mean ± SD

uniformly high on all aspects and we therefore think that fatigue in CPEO is more than exercise intolerance alone.

The high frequency of depression in this study is consistent with previous reports on CPEO as well as with reports on other mitochondrial disorders [19-25]. It is however in contrast to other chronic neuromuscular disorders such as DM1, hereditary motor and sensor neuropathy type I and facioscapulohumoral dystrophy, which are not associated with an increased risk of depression [17]. This discrepancy between mitochondrial disorders and other neuromuscular disorders suggests a causative relation between mitochondrial dysfunction and an increased risk of depression. This concept is supported by the previously reported association between mood disorders and decreased cerebral metabolic activity [26-28]. The frontal lobes may play a particularly important role in the development of depression in mitochondrial disorders since they have an important function in mood regulation, carry a relatively high percentage of mutant mitochondrial DNA and have a relatively high metabolic demand [29-31].

Patients with *POLG1* mutations had more functional impairments than patients with mtDNA point mutations or deletions, despite a (non-significantly) shorter disease duration. Since there were no differences in concentration, motivation, activity and depression, these aspects of disease impact cannot explain the increased functional impairments in patients with *POLG1* mutations. *POLG1* mutations were however strongly associated with a sensory atactic neuropathy, which is a debilitating syndrome and may therefore account for the increased functional impairments in patients with *POLG1* mutations [32].

To estimate the clinical relevance of our results, we compared questionnaires outcomes of CPEO patients to those of matched DM1 patients. Although disease impact in DM1 is commonly considered to be profound, CPEO patients more frequently and more seriously suffered from depression, pain and functional impairments in several domains (emotional behavior, household management, social interaction, and eating), while fatigue severity and limitations in almost all other domains were at least equal to DM1 [33;34].

Literature on disease impact in CPEO is limited. The present results confirm and extend previous findings of limitations in health related physical and common role activities, and perception of impaired general health and vitality in CPEO patients [35]. However, in contrast to our results, this previous study found normal subjective perception of mental health and normal social activities, and a low frequency of depression. The discrepancy between the former and the present study might be explained by the fact that we used a more extensive set of validated questionnaires to evaluate a wider range of quality of life aspects.

This study has several methodological limitations. First, the cross sectional design makes it impossible to determine the direction of associations. A longitudinal study design should be performed to differentiate between causes and consequences. Second, although our study population is one of the largest ever published in a prospective clinical study on

genetically confirmed CPEO patients, sample size is still small for extensive statistical analysis. As a consequence, possible relations and correlations might not be detected. Since CPEO is a rare disorder, inclusion of more genetically confirmed patients is very difficult. Last, this is a single centre study from a tertiary referral clinic. Our study population might therefore not be representative for the total population of CPEO patients. However, since the prevalence of CPEO is low and the diagnosis of CPEO requires specialized techniques, nearly all CPEO patients in the Netherlands are referred to a tertiary referral clinic. Moreover, since this study has a high participation rate, we think that the study population is indeed representative.

In conclusion, CPEO causes a profound impact on social and daily life activities, which is indeed more than meets the eye. From our data we conclude that management of CPEO patients should include screening for the main aspects of disease impact: fatigue, depression, pain, and functional impairments. As there is no cure for CPEO, symptomatic treatment of these aspects may improve quality of life. For this purpose we suggest a multidimensional approach aimed at the specific needs of the individual patient. This approach may include rehabilitation and medication for the treatment of depression or pain.

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Chapter 5

Sleep disturbances in chronic progressive external ophthalmoplegia

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ABSTRACT

Background: Chronic progressive external ophthalmoplegia (CPEO) is a relatively common mitochondrial disorder. In addition to extraocular muscle weakness, various other organs can typically be affected, including laryngeal and limb muscles, cerebrum, cerebellum, and peripheral nerves. Given this multi-organ involvement, patients are likely to be prone to sleep disturbances. Here, we determined the nature, prevalence, and determinants of sleep disturbances in CPEO.

Methods: We used validated questionnaires for various sleep disorders and possible determinants such as mood and anxiety, and we performed ambulant polysomnography (PSG) in 20 patients with genetically confirmed CPEO.

Results: Three quarters of patients reported nocturnal sleep dysfunction. Thirty-five percent of patients fulfilled the criteria for restless legs syndrome, 30% had excessive daytime sleepiness and 70% significant periodic limb movements. PSG recordings revealed several indicators of a disrupted sleep architecture. Obstructive sleep disordered breathing was present in only one patient. However, four patients had an increased central sleep apnea index, all of whom had a polymerase gamma 1 mutation and a SANDO phenotype (sensory atactic neuropathy, dysarthria, ophthalmoplegia). Physical examination and questionnaire outcomes were poor predictors of PSG results.

Conclusion: Several specific sleep disturbances are part of the phenotype of CPEO. Given that the disease is otherwise incurable, symptomatic treatment of sleep disturbances may be an important tool to improve quality of life. Therefore, patients with CPEO should be actively screened for sleep disorders, with a low threshold to perform PSG.

INTRODUCTION

Chronic progressive external ophthalmoplegia (CPEO) is a relatively common mitochondrial disorder. In addition to extraocular muscle weakness, various other organs can typically be affected, including laryngeal and limb muscles, cerebrum, cerebellum, and peripheral nerves.

Given this multi-organ involvement, patients are likely to be prone to sleep disturbances. Sleep disturbances are important to recognize, because they can have a profound negative effect on quality of life, but are often treatable. As there are little therapeutic options in CPEO, recognizing and treating a coexisting sleep disorder could provide an important therapeutic option to improve quality of life.

We therefore performed a detailed study on sleep disturbances in one of the largest cohorts of genetically confirmed CPEO patients.

PATIENTS AND METHODS

All 26 patients registered in the Dutch CRAMP database with a diagnosis of CPEO according to previously published criteria were invited to participate [1;2]. The study was approved by the local ethical committee. All participants gave written informed consent according to the Declaration of Helsinki.

Patients were physically examined and completed several questionnaires: the Pittsburgh Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS), the Checklist Individual Strength-fatigue (CIS-fatigue), and the Hospital Anxiety and Depression Scale (HADS). Restless legs syndrome (RLS) was diagnosed based on the presence of the four diagnostic criteria [3].

Polysomnography (PSG) was performed at the patients' homes using an ambulatory Embla A10 Recorder (Embla Systems, Broomfield, CO, USA). We recorded a respiratory polygraphy, bilateral central and occipital electroencephalography (EEG), eye movements, and submental and tibial electromyography (EMG). Sleep was scored according to the Rechtschaffen and Kales criteria [4].

We used the Fisher's exact test, the Mann–Whitney U test, and the Spearman's rank correlation coefficient for data analyses.

RESULTS

Twenty patients agreed to participate (Table 1). A majority of patients (75%) had subjective nocturnal sleep dysfunction (indicated by a PSQI >5). Six patients (30%) reported

Table 1: Characteristics, mutation type and clinical features of 20 patients with CPEO

	Sex / age / age at onset	Mutation	mRS	Cerebell. ataxia	PNP	Retinal / cochlear involv.	Prox. myop.	Dysart.	Cogn. impair.	Other
1	F/61/34	Deletion	3	–	–	-/-	+	–	+	CM, E
2	F/34/14	Deletion	2	–	–	-/-	–	–	–	
3	F/60/17	Deletion	1	–	–	-/-	–	–	–	
4	F/29/17	Deletion	2	–	–	-/-	+	–	–	
5	F/42/32	Deletion	2	–	–	-/+	–	–	–	HT
6	M/39/11	Deletion	2	–	+	-/-	–	–	–	
7	M/53/25	Deletion	2	–	+	-/-	+	B	–	
8	M/44/14	Deletion	2	+	–	+/+	+	B	+	CB, PM
9	F/54/12	m.03243A>G	4	+	–	+/+	+	C, B	–	DM, RA, M
10	M/55/12	m.03243A>G	4	–	+	-/+	–	–	+	DM
11	M/65/36	m.12315G>A	2	–	–	-/-	–	–	–	
12	M/56/30	m.05709T>C	2	–	–	-/-	–	–	–	O, E, CT
13	M/55/48	m.04267A>G	2	–	–	-/-	+	–	–	
14	M/47/21	POLG	4	+	+	+/+	–	C, B	+	
15	M/23/4	POLG	4	+	+	+/-	+	C	+	MC, CB
16	F/30/13	POLG	4	–	+	-/-	+	–	–	MC
17	M/43/23	POLG	4	+	+	+/+	+	C	+	
18	M/51/39	POLG	4	+	+	+/+	+	C, B	+	IF
19	M/63/53	POLG	1	–	–	-/-	–	–	–	
20	F/50/24	POLG	4	+	+	-/+	+	C,B	–	

PNP, polyneuropathy; CM, cardiomyopathy; CB, conduction block; PM, pacemaker; C, cerebellar dysarthria; B, bulbar dysarthria; DM, diabetes mellitus; E, epilepsy; RA, rheumatoid arthritis; M, migraine; MC, myoclonia; O, otosclerosis; C, cataract; HT, hyperthyroidism; IF, infertility.

excessive daytime sleepiness (ESS score >10) and 15 (75%) severe fatigue (CIS-fatigue score ≥35). Nine patients (45%) had symptoms of depression (HADS-depression >8) and 5 (25%) increased anxiety scores (HADS anxiety >8).

There were several indicators of disrupted sleep architecture: sleep efficiency was decreased (<80%) in five patients (25%) and the duration of wake after sleep onset was high (74 ± 61 min) (Table 2). Other indicators for disturbed nocturnal sleep were a high percentage of stage 1 sleep (20 ± 8.5%) and an increased arousal index (24.8 ± 17.2/h). Nine patients (45%) had an increased periodic limb movement (PLM)-index (>15/h), eight of whom also reported subjective nocturnal sleep dysfunction. However, when comparing patients with and without increased PLM-index, there were no differences in sleep efficiency (83.5 ± 9.5 vs. 87.6 ± 12.0, p = 0.24) or arousal index (28.4 ± 23.7 vs. 21.8 ± 9.5, p = 0.68). Seven patients (35%) reported RLS, two patients with *POLG1* mutations and five patients with mtDNA mutations or deletions (29% vs. 38%, p = 1.00). Four of the seven patients

Table 2: Outcomes of sleep monitoring

Sleep architecture	
Total sleep time (min.)	429 ± 61 (320-535)
Sleep latency (min.)	16 ± 13 (0-43)
REM latency (min.)	146 ± 81 (2-327)
WASO (min.)	74 ± 61 (8-235)
Sleep efficiency (%)	86 ± 11 (60-98)
Sleep efficiency < 80%	5 (25%)
Sleep stages (%)	
S1	20 ± 8.5
S2	34 ± 10.4
SWS	25 ± 9.6
REM	19 ± 6.4
Sleep stage changes (/h)	12.1 ± 3.0 (7.7-17.5)
Arousal index (/h)	24.8 ± 17.2 (5.0-88.2)
Arousal index > 15/h	15 (75%)
Limb movements	
PLM index (/h)	25.5 ± 30.5 (0-115.2)
PLM index >15/h	9 (45%)
Sleep related breathing	
AH-index (/h)	9.8 ± 11.8 (0-47.2)
AH-index > 15/h	4 (20%)
Obstructive apnea index (/h)	1.2 ± 1.8 (0-7.0)
Obstructive apnea index > 5/h	1 (5%)
Central apnea index (/h)	5.0 ± 10.1 (0-36.1)
Central apnea index > 5 /h	4 (20%)
Desaturation index (/h)	4.2 ± 4.6 (0-13.2)
Desaturation index > 10/h	4 (20%)

WASO, wake after sleep onset; SWS, slow wave sleep; PLM, periodic limb movement; AH-index, apnea-hypopnea index.
Values are expressed as mean ± SD (range) or number (percentage).

with RLS had an increased PLM-index, whereas three did not (p = 0.642). Patients with RLS had comparable sleep latency (11.1 ± 11.3 vs. 18.1 ± 13.2 min, p = 0.19) and PSQI score (8.3 ± 3.0 vs. 7.2 ± 3.9, p = 0.316) as patients without RLS.

Four patients (20%) had an increased central apnea index (>5/h), all with a moderately severe disability (mRS score of 4), cerebellar ataxia, severe polyneuropathy, dysarthria, and a *POLG1* mutation. Patients with an increased central apnea index had decreased total sleep time (365 ± 15 vs. 445 ± 57 min, p = 0.014), sleep efficiency (75.2 ± 13.1%

vs. 88.4 ± 8.8%, p = 0.047), arousal index (13.6 ± 8.0 vs. 27.6 ± 17.9, p = 0.023), and percentage of Rapid Eye Movement (REM) sleep (12.2 ± 5.1% vs. 20.1 ± 5.9%, p = 0.033). Obstructive apneas were very rare. Only patient had a mildly increased obstructive apnea index of 7.0/h which coincided with a severely increased central apnea index (36.1/h). Subjective nocturnal sleep dysfunction did not predict any of the PSG results, while ESS and CIS-fatigue scores did not correlate with arousal index, sleep efficiency, or total sleep time.

DISCUSSION

Using validated questionnaires and ambulant polysomnography, we showed that various sleep disturbances are part of the CPEO phenotype. Most notably, three quarters of patients reported subjective nocturnal sleep dysfunction. In addition, 35% of patients fulfilled the diagnostic criteria for RLS, and 30% had excessive daytime sleepiness. In contrast to what could be expected in a neuromuscular disorder that commonly affects laryngeal muscles, sleep recordings showed that obstructive sleep apneas were rare in CPEO. However, in CPEO patients with *POLG1* mutations and a SANDO phenotype (Sensory Atactic Neuropathy, Dysarthria and Ophthalmoplegia), central apneas were common.

Nine patients had an increased PLM-index, associated with elevated PSQI scores in eight. This combination suggests the presence of clinically relevant periodic limb movement disorder (PLMD). The overall prevalence of PLMD in our cohort was therefore 40% and RLS was found in 35%. These prevalences are clearly increased compared to the healthy population, but are also higher than in patients with neurodegenerative disorders including Parkinson's disease [5].

The coexistence of RLS and CPEO was described recently in a single patient with a *POLG1* mutation [6]. In contrast to the conclusions of this article, our data suggests that RLS is a clinical feature of CPEO in general, rather than a specific feature of *POLG1* mutations.

In conclusion, CPEO is commonly associated with a number of sleep disorders. Routine screening may be warranted, as treatment of sleep disturbances may be one of the few therapeutic options to increase quality of life in patients with CPEO. As both subjective complaints and physical examination were poor predictors of polysomnography results, there should be a low threshold to perform sleep recordings in patients with CPEO.

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Chapter 6

Nature and frequency of respiratory involvement in chronic progressive external ophthalmoplegia

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ABSTRACT

Chronic progressive external ophthalmoplegia (CPEO) is a relatively common mitochondrial disorder. Weakness of the extra-ocular, limb girdle and laryngeal muscles are established clinical features. Respiratory muscle involvement however has never been studied systematically, even though respiratory complications are one of the main causes of death. We therefore determined the prevalence and nature of respiratory muscle involvement in 23 patients with genetically confirmed CPEO. The main finding was decreased respiratory muscle strength, both expiratory (76.8% of predicted, p=0.002) and inspiratory (79.5% of predicted, p=0.004). Although the inspiratory vital capacity (92.5% of predicted, p=0.021) and the forced expiratory volume in 1 second (89.3% of predicted, p=0.002) were below predicted values, both were still within the normal range in the majority of patients. Expiratory weakness was associated with a decreased vital capacity (rho 0.502, p=0.015) and decreased peak expiratory flow (rho 0.422, p=0.045). Moreover, expiratory muscle strength was lower in patients with limb girdle weakness (62.6 ± 26.1% of predicted vs. 98.9 ± 22.5% in patients with normal limb girdle strength, p=0.003), but was not associated with other clinical features, subjective respiratory complaints, disease severity or disease duration. Since respiratory involvement in CPEO is associated with severe morbidity and mortality, the present data justify periodic assessment of respiratory functions in all CPEO patients.

INTRODUCTION

Chronic progressive external ophthalmoplegia (CPEO) is one of the most common mitochondrial disorders in adults [1]. The characteristic clinical feature is a slowly progressive weakness of the extraocular muscles, resulting in ptosis and restriction of eye movements [2]. CPEO is however often a multi-system disorder, affecting limb girdle and laryngeal muscles, retina, cochlea, cerebrum, cerebellum or heart. The prevalence and nature of respiratory involvement has never been systematically studied.

Two cohort studies have shown that respiratory complications are one of the main causes of death in CPEO [3;4]. Conversely, studies that specifically investigated respiratory function in CPEO are limited to case reports and studies on small series of patients without a genetically confirmed diagnosis [5-10]. Most consistent findings were a decreased ventilatory drive to hypoxia and hypercapnia and respiratory muscle weakness. However, the prevalence, nature and determinants of respiratory involvement in CPEO cannot be reliably determined from these uncontrolled studies. More insight in the nature of respiratory involvement is nevertheless desirable, since it may help to prevent respiratory complications and respiration related deaths [4].

In a large cohort of genetically confirmed CPEO patients, we performed an extensive standardized assessment of respiratory muscle function, including spirometry, measurement of respiratory muscle strength and of diffusion capacity. In order to identify possible determinants of respiratory muscle involvement, patients also completed questionnaires on subjective respiratory symptoms and underwent a standardized neurological examination.

METHODS

Patients

All CPEO patients known in the Department of Neurology of the Radboud University Nijmegen Medical Centre were invited to participate. As there are no commonly accepted criteria for CPEO, we only included patients with both characteristic clinical features and a known causative mutation. Consequently, all participants met the following three criteria:

1) a phenotype including a slowly progressive bilateral external ophthalmoplegia
2) a proven pathogenic mutation or deletion in the mitochondrial DNA (mtDNA) or in the nuclear polymerase gamma 1 *(POLG1)*, *Twinkle* or adenine nucleotide translocator 1 *(ANT1)* genes
3) exclusion of an alternative diagnosis

These inclusion criteria also cover two more or less specific mitochondrial phenotypes with external ophthalmoplegia as a key symptom: the Kearns-Sayre syndrome (KSS: external ophthalmoplegia, pigmented retinopathy, age of onset below 20 years, and at least one of the following symptoms: cardiac conduction block, cerebellar ataxia, elevated CSF protein content) and SANDO (Sensory Atactic Neuropathy, Dysarthria and Ophthalmoplegia) [11-13]. In this article, we use the term CPEO to describe all patients meeting the inclusion criteria and the terms KSS and SANDO to describe the specific phenotypes only.

All participants gave informed consent. Local ethical committee approved this study and the patients' consent was obtained according to the Declaration of Helsinki.

Patients' characteristics

We performed a standardized neurological examination, including an assessment of muscle strength (ocular, bulbar and extremities), sensory functions, tendon reflexes and coordination. We also measured length and weight and calculated body mass index. Results of ancillary investigations and of genetic investigations were derived from the patients' files.

Subjective complaints

All participants completed a questionnaire with items on history of smoking and pulmonary disease, dyspnea, swallowing, coughing, and quality of sleep. We assessed the level of independency in daily activities using the modified Rankin score (mRS), with an mRS ≤ 2 indicating independency [14].

Pulmonary function tests

All eligible patients were invited to undergo respiratory function tests in our pulmonary function department. To exclude a possible selection bias (patients who were unable to visit our hospital might have more advanced disease), we offered to perform respiratory function tests at home to those patients who were willing to participate but could not visit our hospital.

Respiratory function tests were performed according the American Thoracic Society / European Respiratory Society standards [15]. First, we measured respiratory muscle strength, including the maximal expiratory mouth pressure (PE-Max), and the maximal inspiratory mouth pressure (PI-Max). Next, to evaluate the effect of respiratory muscle weakness on the inspiratory vital capacity (IVC) we performed spirometry, including the forced expiratory volume in 1 second (FEV1), the FEV1/IVC ratio, the peak expiratory flow (PEF) and the forced inspiratory volume in 1 second (FIV1). We also measured the total lung capacity (TLC), the diffusion capacity for carbon monoxide (TLCO) and the diffusion capacity for carbon monoxide corrected for alveolar volume (KCO).

All tests were performed by a trained pulmonary function technician. The TLC, FIV1, TLCO and KCO could not be measured at the patients' homes and were therefore measured in patients visiting our hospital only.

Statistical analysis

Data analysis was performed using SPSS (version 17.0). To assess the extent of pulmonary involvement in CPEO we compared the test results of the individual participants to their own predicted values using a paired Wilcoxon signed rank test. For comparison between various subgroups, we performed an independent Mann-Whitney U-test on the test results relative to the predicted values, which are noted as "value % pred": for example, "IVC" indicates the inspiratory vital capacity (in liters), whereas "IVC% pred" indicates the inspiratory vital capacity relative to the predicted value (in %). A Fisher's exact test was used to compare frequencies and a Spearman coefficient to calculate correlations. Significance level was set at $p < 0.05$ (two-tailed) in all cases.

RESULTS

Patients' characteristics

We identified 26 eligible patients. All were invited to participate, two patients refused (one male patient with an m.12315G→A mutation and one female patient with a mtDNA deletion). In general, their clinical features and disease severity were in the same range as the participants. During the tests, one of the participants appeared to be unable to follow the instructions due to severe vision and hearing impairments. Her test results were unreliable and therefore excluded from all analyses. Of the remaining 23 patients (11 female, 12 male), 14 patients (60.9%) were tested in the pulmonary function department and nine (39.1%) at home. Mean age of the participants was 49.1 ± 11.0 years and mean age at onset was 22.9 ± 12.2 years (Table 1). Mutation type included a single mtDNA deletion in 14 patients (60.9%), a mtDNA point mutation in four (17.4%) and a *POLG1* mutation in five (21.7%). *POLG1* mutations included compound heterozygous T251I, P587L and A957S mutations in one patient (patient 19), a homozygous A467T in two patients (patients 20 and 21) and compound heterozygous A467T and W748S mutations in two (patients 22 and 23). Three patients (19, 20 and 23) also had multiple mtDNA deletions in their skeletal muscle mtDNA. None of the patients had *Twinkle* or *ANT1* mutations. Three patients (two with a mtDNA deletion and one with an m.03243A→G point mutation) met the criteria for KSS and four (all with *POLG1* mutations) for SANDO. Fifteen patients (65.2%) were independent in daily life activities, whereas eight (34.7%) were not.

Questionnaires

Five patients (21.7%) were current smokers and six of the remaining 18 patients (33.3%) were former smokers (Table 1). Three patients reported a history of asthma, of whom only one used pulmonary medication (budesonide). Three patients (13.0%) had a history of pneumonia, including two out of the three patients with asthma. Shortly after participating in this study, one additional patient (no. 3) developed a severe pneumonia, requiring mechanical ventilation and long term rehabilitation. We included this patient in the further analyses on patients with a history of pneumonia. Fourteen patients (60.9%) reported exertional dyspnea. Three patients (13.0%), two current smokers and one patient with

Table 1: Demographical, genetic and clinical results of 23 CPEO patients

no.	sex / age / age at onset	mutation	smoking history	pulmon. history	CNS involv.	limb girdle	periph. neurop.	dysarthria / dysphagia	other
1	F/28/21	deletion	C	-	-	-	-	-/-	
2	F/43/32	deletion	-	-	-	-	-	-/-	PHL, HT
3	F/29/17	deletion	C	P	-	+	-	-/+	
4	F/35/14	deletion	-	-	M	-	-	-/-	
5	M/32/8	deletion	-	-	CA, CI	+	-	-/+	PHL, RI
6	F/52/25	deletion	-	-	-	+	-	-/-	
7	F/62/34	deletion	F	-	CA, CI, E	+	-	-/-	CM
8	F/43/15	deletion	C	-	-	+	-	+/+	
9	M/54/25	deletion	F	-	-	+	-	+/+	
10	M/40/11	deletion	C	-	-	-	-	-/-	
11	M/45/14	deletion	F	-	CA, CI	+	-	+/+	CB, PHL, RI
12	F/54/13	deletion	F	-	-	+	-	-/-	
13	F/61/17	deletion	F	-	-	-	-	-/-	
14	M/63/12	deletion	-	P	-	+	-	+/+	
15	M/55/48	m.4267A>G	-	-	-	+	-	-/-	
16	M/66/36	m.12315G>A	-	-	-	-	-	-/-	
17	F/54/12	m.3243A>G	F	A, P	CA, M	+	-	+/-	PHL, RI, DM
18	M/57/12	m.3243A>G	C	A	CI	-	+	-/-	PHL, DM
19	M/63/53	POLG1	-	-	-	-	+	-/-	
20	M/52/39	POLG1	-	-	CA, CI	+	SAN	+/-	PHL, RI
21	M/43/23	POLG1	-	-	CA, CI	+	SAN	+/+	PHL, RI
22	F/51/24	POLG1	-	A, P	CA	+	SAN	+/+	PHL
23	M/48/21	POLG1	-	-	CA, CI	-	SAN	+/+	PHL, RI

CNS, central nervous system; POLG1, polymerase gamma 1; C, current smoker; F, former smoker; P, pneumonia; A, asthma; M, migraine; CA, cerebellar ataxia; CI, cognitive impairment; E, epilepsy; SAN, sensory atactic neuropathy; CM, cardiomyopathy; CB, cardiac conduction block; PHL, peripheral hearing loss; RI, retinal involvement; DM, diabetes mellitus; HT, hypothyroidism

asthma, reported dyspnea in rest. Eleven patients (47.8%) reported dysphagia, of whom three (13.0%) also reported difficulty coughing.

Pulmonary function tests

The main finding was decreased expiratory and inspiratory muscle strength: PE-Max was 76.8% of the predicted value (p=0.002) and PI-Max 79.5% (p=0.004) (Table 2). Lower PE-Max% pred was associated with lower PI-Max% pred (rho 0.625, p=0.001) but also with lower IVC% pred (rho 0.502, p=0.015) and lower PEF% pred (rho 0.422, p=0.045). IVC was also significantly decreased (92.5% of predicted, p=0.021), as were FEV1 (89.3% of predicted, p=0.003) and TLCO (81.9% of predicted, p=0.002). The decreased TLCO is unlikely due to intrinsic lung disease, since the diffusion capacity corrected for alveolar volume (i.e. the KCO) was normal (97.9% of predicted, p=0.26). Three patients had obstructive pulmonary disease (FEV1/IVC < 70%), of whom two were former smokers and one a non-smoker without a history of pulmonary disease. In these three patients, all other test results were within the range as the rest of the cohort.

Determinants of respiratory function

Patients with limb girdle weakness had a lower PE-Max% pred than patients with normal limb girdle strength (62.6 ± 26.1% vs. 98.9 ± 22.5%, p=0.005). Limb girdle weakness was not associated with any of the other outcomes. PEF% pred was lower in patients who reported difficulty coughing (74.3 ± 9.5% vs. 98.8 ±18.1%, p=0.028), as well as in patients with a history of pneumonia (76.6 ± 9.0% vs. 99.7 vs. 18.3%, p=0.019). None of the other subjective complaints, demographic or clinical features were associated with any of the respiratory test results.

As to a possible genotype-phenotype relation, there were no differences in any of the respiratory test results between patients with mtDNA mutations or deletions and patients with *POLG1* mutations, though there was a strong tendency toward a lower PE-Max% pred in patients with mtDNA deletions (66.4 ± 31.0% vs. 100.5 ± 26.0%, p=0.064). In addition, all eight patients with a PE-Max below 65% of the predicted value had a mtDNA deletion.

DISCUSSION

We found that both expiratory and inspiratory muscle strength was decreased in CPEO patients and that decreased respiratory muscle strength resulted in a decreased vital capacity, FEV1 and PEF. Respiratory muscle weakness was associated with limb girdle weakness, which is in concordance with studies in other myopathies [16;17]. In contrast, there was no association between respiratory muscle function and other clinical features, disease duration, disease severity, subjective respiratory complaints, or mutation type.

Table 2: Results of respiratory function assessment

no.	BMI	PE-Max	PI-Max	IVC	TLC	FEV1	FEV1/IVC	PEF	FIV1	TLCO	KCO
1	24.7	9.4 (101)	8.0 (109)	3.8 (100)	4.7 (91)	3.3 (101)	87	9.0 (125)	3.3 (94)	8.9 (94)	2.0 (109)
2	34.7	11.6 (118)	8.3 (104)	4.7 (121)	6.2 (108)	3.7 (115)	80	8.0 (110)	4.5 (128)	8.2 (86)	1.4 (87)
3	16.8	4.0 (45)	5.1 (74)	2.1 (61)	4.3 (89)	2.0 (68)	95	5.2 (76)	2.0 (60)	6.9 (77)	1.8 (94)
4	23.5	9.5 (95)	6.6 (79)	3.8 (90)	5.1 (83)	3.3 (92)	87	8.0 (103)	3.7 (93)	7.0 (68)	1.6 (94)
5	17.9	7.7 (52)	7.2 (67)	4.0 (92)	4.9 (84)	3.1 (87)	78	7.8 (89)	3.8 (94)	7.2 (73)	1.5 (90)
6	34.4	4.3 (46)	5.4 (73)	2.5 (77)		2.1 (79)	85	5.6 (85)			
7	30.0	7.3 (74)	5.4 (66)	3.9 (111)	6.9 (116)	2.6 (87)	67	8.3 (121)	3.8 (118)	7.8 (89)	1.3 (86)
8	19.0	2.8 (30)	3.7 (50)	2.9 (84)	4.5 (86)	2.6 (90)	95	6.2 (91)	2.4 (77)	6.5 (74)	1.6 (95)
9	27.8	2.4 (19)	1.7 (20)	4.4 (86)		3.5 (91)	81	6.8 (74)			
10	27.3	10.7 (75)	4.4 (44)	4.8 (82)	6.1 (75)	3.7 (81)	77	10.0 (99)	4.3 (81)	10.0 (80)	1.7 (112)
11	18.9	6.9 (51)	10.2 (109)	4.8 (100)		3.8 (103)	81	9.0 (100)			
12	21.8	5.1 (52)	5.0 (63)	3.7 (105)	5.3 (94)	2.9 (98)	77	6.8 (100)	3.6 (113)	7.3 (74)	1.5 (97)
13	30.1	10.6 (116)	8.0 (112)	3.2 (109)	4.4 (89)	2.3 (97)	78	7.1 (117)	2.9 (112)	5.6 (73)	1.4 (89)
14	25.9	7.2 (60)	2.7 (35)	3.7 (79)		2.7 (76)	72	5.5 (64)			
15	25.3	12.7 (99)	8.2 (97)	5.1 (120)	6.8 (103)	4.5 (135)	87	11.9 (142)	5.0 (130)	9.5 (101)	1.5 (104)
16	24.2	10.1 (86)	5.0 (68)	2.8 (80)		2.3 (84)	80	7.6 (103)			
17	23.0	7.7 (80)	8.1 (105)	3.8 (109)	5.3 (95)	2.3 (80)	61	5.6 (83)	3.4 (111)	7.6 (79)	1.5 (97)
18	16.9	8.4 (66)	8.2 (99)	3.5 (72)		2.6 (70)	75	6.2 (70)			
19	29.1	16.5 (138)	8.2 (110)	3.7 (92)	6.4 (97)	2.1 (68)	56	5.9 (75)	3.5 (98)	9.4 (107)	1.8 (132)
20	24.4	9.0 (69)	8.0 (91)	3.6 (81)		2.8 (81)	80	8.1 (94)			
21	18.0	12.3 (89)	6.4 (67)	5.3 (94)	7.9 (99)	4.1 (94)	78	9.7 (99)	5.1 (98)	8.6 (71)	1.3 (83)
22	21.5	10.7 (111)	8.9 (116)	3.5 (101)		2.6 (90)	74	5.6 (83)			
23	27.2	12.7 (95)	6.6 (73)	4.4 (82)		3.6 (87)	82	9.1 (95)			
mean		8.68	6.49	3.82	5.63	2.97	79	7.52	3.66	7.89	1.56
SD		3.49	2.13	0.80	1.11	0.70	9.2	1.73	0.88	1.27	0.20
%pred.		76.8**	79.5**	92.5*	93.6	89.3**	99.5	95.6	100.5	81.9**	97.9

In value (percentage of predicted)

BMI, body mass index (in kg/m^2); PE-Max, maximal expiratory mouth pressure (in kPa); PI-Max, maximal inspiratory mouth pressure (in kPa); IVC, inspiratory vital capacity (in litres); TLC, total lung capacity (in litres); FEV1, forced expiratory volume in 1 second (in litres); PEF, peak expiratory flow (in litres/sec); FIV1, forced inspiratory volume in 1 second (in litres); TLCO, diffusion capacity for carbon monoxide (in mmol/min/kPa); KCO, diffusion capacity for carbon monoxide corrected for alveolar volume (mmol/min/kPa/litre)

Blank fields indicate that the patient was tested at home and as a result the specific test was not performed

* paired Wilcoxon p< 0.05

** paired Wilcoxon p< 0.01

In the present study, we took several measures to include a representative cohort: our study was prospective and used strict clinical and genetic inclusion criteria. We also offered to perform the tests at the patients' homes, which resulted in a high participation rate. This not only enabled us to provide a representative overview of the prevalence and nature of respiratory involvement in CPEO, but also to identify possible determinants of respiratory involvement. The present results therefore not only confirm but also extend the conclusions of the previous smaller case studies on respiratory involvement in CPEO [5; 6;9].

A major consequence of decreased respiratory muscle weakness in neuromuscular disorders is an increased risk of pneumonia [18]. CPEO patients may however be particularly prone to pneumonias because of concurrent laryngeal muscle weakness. Laryngeal muscle weakness can lead to aspiration of foods and to diminish glottic closure during coughing [4;19;20]. Inadequate glottic closure in turn, especially when combined with decreased inspiratory and expiratory strength, results in insufficient generation of intrathoracic pressure and thus in impaired expectoration.

In addition to an increased risk of pneumonia in CPEO, the course of pneumonia may theoretically also be more severe: a decreased ventilatory drive to hypoxia or hypercapnia predisposes to respiratory failure and prolonged weaning after mechanical ventilation [5;6;10]. In addition, CPEO patients may have trouble meeting the increased demands associated with infections because of limited metabolic and hemodynamic reserves [21]. Despite the fact that pneumonia is one of the main causes of death in CPEO, little is known about its prevention [3;4]. As in other neuromuscular disorders, conservative treatment aimed at minimizing the risk of pneumonia seems rational [22]. This includes speech therapy and physiotherapy aimed at optimizing cough effectiveness and swallowing function, while a cricopharyngeal myotomy may be performed in selected cases of severe dysphagia [19]. Moreover, any sign of pneumonia or respiratory failure in a CPEO patient should prompt rapid diagnosis and treatment to prevent further complications.

The present study does not quantify the relative contributions of neurogenic or myogenic weakness of the respiratory muscles, nor the relative contribution of the various muscles involved in respiration. For instance we did not directly measure diaphragm strength, which ideally requires transdiaphragmic oesophageal and gastric manometry. This is however an invasive procedure and carries a risk of aspiration. Alternative indicators for diaphragm strength are the nasal sniff pressure and the supine vital capacity. The use of supine vital capacity is however limited by the lack of normal values. Sniff pressure is mainly validated in amyotrophic lateral sclerosis and depends partly on the nasal airway conductance, while in patients with myopathies, PI-Max is possibly a more reliable indicator of inspiratory weakness than nasal pressure [23]. Therefore in this study, we used the PI-Max as an indicator of diaphragm strength, since it is also correlated to transdiaphragmic pressure [24].

CONCLUSIONS

Respiratory muscle weakness is common in CPEO patients. The presence of respiratory muscle weakness has important consequences for patient management, since it is associated with an increased risk of pneumonia and possibly of increased mortality. As there are no clinical predictors of respiratory muscle weakness and the rate of progression of respiratory muscle weakness has yet to be determined in a longitudinal study, we propose that periodic assessment of pulmonary function tests including respiratory muscle strength measurements should be included in evaluation of CPEO patients.

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Chapter 7

Renal involvement in adult chronic progressive external ophthalmoplegia, a mitochondrial myopathy

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ABSTRACT

Background: Renal involvement is a common feature in mitochondrial disorders. Children with mitochondrial disorders are especially at risk, whereas the extent of renal involvement in adults with mitochondrial disorders is less established. We therefore performed a prospective study to determine the nature and frequency of renal involvement in a cohort of patients with genetically confirmed chronic progressive external ophthalmoplegia (CPEO), one of the commonest mitochondrial disorders in adulthood.

Methods: All 24 participants completed a questionnaire on urinary tract symptoms. We measured blood pressure, fasting glucose, assessed eGFR and micro-albuminuria as markers of glomerular damage and measured serum potassium, uric acid, phosphate and bicarbonate, and urinary alpha1-microglobulin as markers of tubular dysfunction.

Results: Five patients (21%) had evidence of tubular dysfunction (i.e. an increased urinary alpha1-microglobulin/creatinine ratio). However, four of these five patients had hypertension or diabetes mellitus, while the alpha1-microglobulin/creatinine ratio was correlated with the urinary albumin/creatinine ratio. Two patients had micro-albuminuria, associated with hypertension in both. Seven patients (29%) had hypertension and five (21%) diabetes mellitus, a known comorbidity in CPEO. Fourteen patients (58%) reported incontinence, mainly urge incontinence (10 patients, 42%)

Conclusions: Although we failed to detect clinically relevant renal involvement in our cohort, we did find a high prevalence of hypertension and diabetes mellitus. We therefore recommend routine evaluation of blood pressure and glucose, while screening for micro-albuminuria and tubular dysfunction should be restricted to selected cases. Moreover, the cause of incontinence in CPEO is unknown and should be the focus of future study.

INTRODUCTION

Mitochondrial disorders are the most common group of diseases among the inborn errors of metabolism, with an estimated overall incidence of approximately 1:5000 [1;2]. They comprise a genetically and clinically heterogeneous group of diseases, with manifestations ranging from adult-onset single organ dysfunction to severe neonatal multi-system failure.

One of the commonest mitochondrial disorders in adulthood is chronic progressive external ophthalmoplegia (CPEO). The defining clinical feature of CPEO is bilateral slowly progressive weakness of the extra-ocular muscles, resulting in drooping of the eyelids and restriction of movement of the eyeballs. In addition, various other organ systems can also be affected to various degrees, mainly the central nervous system, skeletal muscles, heart, peripheral nerves, retina, cochlea, and pancreatic beta-cells.

Renal tubuli have a very high ATP demand and tubular involvement is therefore likely in mitochondrial disorders. In CPEO patients there are indeed several reports of tubular dysfunction (the Toni-Debre-Fanconi syndrome [3], a Bartter-like syndrome [4;5], hypomagnesemia due to renal losses [6] or renal tubular acidosis [7]). Interestingly, all but one of the previously published CPEO patients with renal involvement had a phenotype compatible with the Kearns Sayre syndrome (KSS) [8], a CPEO subtype defined as a combination of ophthalmoplegia, pigmented retinopathy, age of onset below 20 years, and at least one of the following symptoms: cardiac conduction block, cerebellar ataxia or elevated CSF protein content [9].

Despite these various case reports, the prevalence of clinically relevant kidney involvement appears low since in the two largest published CPEO cohorts tubulopathy was reported in only 2 out of 91 patients [10;11]. This is in contrast with studies on other mitochondrial disorders, which found a prevalence of up to 50% in a pediatric population [12]. This discrepancy might be explained by the fact that renal involvement has never been systematically and prospectively studied in CPEO, resulting in an underestimation of subclinical or mild renal involvement. The aim of this study was therefore to prospectively investigate the prevalence and nature of renal involvement in an adult population of genetically confirmed CPEO patients.

PATIENTS AND METHODS

Patients

All CPEO patients known in the Department of Neurology of the Radboud University Nijmegen Medical Centre were invited to participate. All had a phenotype including a slowly progressive bilateral external ophthalmoplegia and a proven pathogenic mutation

or deletion in the mitochondrial DNA or in one of the nuclear genes associated with CPEO. We considered KSS a CPEO subtype, not a distinct clinical entity [13]. Local ethical committee approved this study and all patients gave written informed consent according to the Declaration of Helsinki.

Methods

Patients' characteristics
We measured blood pressure three times using a calibrated mercury manometer and calculated means. We performed routine neurological examination and systematically inquired about prior medical history and use of medication. Hypertension was defined as a mean systolic blood pressure > 140 mmHg and/or a mean diastolic blood pressure > 90 mmHg and/or the use of antihypertensive medication. In addition, we measured fasting glucose and glycosylated haemoglobin (HbA1c) in venous plasma. Diabetes was defined as a fasting glucose ≥ 7.0 mmol/l and/or an HbA1c ≥ 6.5%.

Renal function assessment
We measured sodium, potassium, chloride, bicarbonate, phosphate, creatinine, albumin and uric acid in venous blood drawn from the antecubital vein. Albumin, alpha1-micro-globulin, glucose, creatinine and pH were measured in fresh midstream urine samples. The glomerular filtration rate (eGFR) was estimated using the simplified MDRD formula [14].
In addition, we calculated the urine albumin/creatinine ratio (ACR) and the alpha1-microglobulin/creatinine ratio (α1CR). Microalbuminuria was defined by an ACR ≥ 2.5 mg/mmol in males and an ACR ≥ 3.5 mg/mmol in females.

Questionnaire
All participants completed a questionnaire on family history, voiding frequency, urinary incontinence and symptoms of urinary tract calculi. Results of ancillary investigations and of genetic investigations were derived from the patients' files.

Statistical analysis
Data analysis was performed using SPSS (version 17.0). A Spearman coefficient was used to calculate correlations. A Mann Whitney test was used to compare means, a Fisher's exact test to compare frequencies. Significance level was set at $p < 0.05$ (two-tailed) in all cases.

RESULTS

Patients' characteristics

We identified 27 eligible patients. All were invited to participate. After agreeing to participate a 54-year-old female patient with a mtDNA deletion died from sepsis before she was included in the study. Venapuncture was repeatedly unsuccessful in a 28-year-old female patient with a mtDNA deletion, while an 80-year-old male patient with compound heterozygous c.752C>T, c.1760C>T and c.2243G>C mutations in the *POLG1* gene was unable to collect a fresh urine sample due to severe incontinence. These three patients were excluded from further analyses.

Of the remaining 24 patients (13 female, 11 male), mean age was 47.5 years (range 28-64) and mean age at onset was 21.0 years (range 5-48) (Table 1). Mutation type included a single mtDNA deletion in 16 patients (67%), a mtDNA point mutation in four (17%) and a *POLG1* mutation in four (17%). *POLG1* mutations included a homozygous A467T in two patients (21 and 22) and compound heterozygous A467T and W748S mutations in two others (patients 23 and 24). None of the patients had *Twinkle* or *ANT1* mutations. Four patients met the criteria for KSS, three with a mtDNA deletion (patients 5, 15 and 16), and one with an m.03243A→G mutation (patient 19). The brother of patient 19 also carried the m.03243A→G mutation (patient 18).

Seven patients had hypertension, for which only one patient received treatment. Three patients were known diabetics, while two additional patients were newly diagnosed with diabetes: patient 13 had an increased fasting glucose (9.6 mmol/l) and patient 17 had an increased HbA1c (6.6%).

Renal function

Laboratory test results are listed in Table 1. Although there were minor electrolyte disturbances in some patients, there was no evidence of clinically relevant renal involvement. Renal function as defined by eGFR was normal in all patients. Two male patients (1 and 17) had microalbuminuria (Table 1), both also had hypertension and a history of urinary tract calculi. Five patients had an increased α1CR (>1.5 mg /mmol), of whom three had hypertension, one had diabetes and one used NSAIDs daily. There was a significant correlation between α1CR and ACR (rho=0.623, p=0.001).

Questionnaire

Incontinence was reported by 11 female (85%; stress incontinence in 4, urge in 4 and mixed incontinence in 3) and 3 male patients (27%, all urge). There was no relation between incontinence and age, age of onset or duration of disease. Neither urge nor stress incontinence was associated with any of the clinical features included in Table 1.

Table 1: Demographic and clinical features of 24 adult CPEO patients and results of blood and urine analysis

Patient	Sex / age/ age onset	Mutation	Mean BP*	DM	Medication	Organ involvement	eGFR	Potassium	Bicarbonate	Phosphate	Uric acid	α1M/ creat	Alb/ creat
1	M/61/12	deletion	189/121*	-	-	PM	161	4.0	25.5	0.62	0.38	4.1	4.7
2	F/60/17	deletion	135/85	-	-	-	87	5.2	26.8	1.14	0.22	0.5	0.8
3	M/39/11	deletion	128/81	-	-	-	177	3.6	28.2	1.09	0.38	0.0	1.0
4	F/43/26	deletion	110/70	-	-	PM	107	3.8	29.1	1.12	0.21	0.6	1.7
5	M/31/8	deletion	123/85	-	NSAID	CA, CI, PM, PHL, RI	112	3.7	28.4	1.05	0.40	0.6	2.3
6	F/54/13	deletion	147/83*	-	-	PM	168	3.7	30.7	1.08	0.16	0.0	0.0
7	F/42/32	deletion	125/83	-	-	PHL, HT	84	3.3	23.4	0.83	0.37	0.0	0.6
8	F/36/25	deletion	143/89*	-	-	-	134	4.0	24.8	1.15	0.20	2.9	3.4
9	F/46/5	deletion	131/83	-	-	-	88	3.4	26.1	0.98	0.26	0.8	3.0
10	F/50/25	deletion	143/92*	NIDDM	-	PM	177	3.8	26.2	1.17	0.20	1.0	1.2
11	F/28/21	deletion	116/73	-	NSAID	-	144	3.7	25.0	1.15	0.17	1.8	1.3
12	F/34/14	deletion	102/80	-	-	M	122	3.8	na	0.90	0.20	0.6	0.6
13	F/61/34	deletion	131/76	-	NSAID	CA, CI, PM, CM, E	137	3.8	22.1	0.99	0.25	1.0	0.7
14	M/52/25	deletion	124/83	-	NSAID	PM	101	3.0	30.7	1.02	0.43	1.2	1.0

15	F/38/12	deletion	121/82	-	-	CA, PM, PHL, RI	93	3.2	24.4	0.95	0.21	0.9	0.6
16	M/44/14	deletion	122/79	-	-	CA, CI, PM, PHL, RI, CB	114	4.4	30.4	1.12	0.35	0.7	2.0
17	M/64/36	m.12315G>A	163/96*	-	-	-	168	4.2	26.7	1.19	0.25	2.6	3.2
18	M/55/12	m.03243A>G	129/89	IDDM	-	CI, PN, PHL	137	3.1	27.5	1.09	0.31	2.3	1.3
19	F/54/12	m.03243A>G	129/61	IDDM	-	CA, M, PM, PHL, RI	137	4.3	28.4	1.50	0.34	0.0	0.0
20	M/55/48	m.04267A>G	133/91*	-	-	PM	140	3.9	30.4	1.07	0.24	0.0	0.6
21	M/55/35	POLG1	131/93*	-	Ca-I, ACE-I	CA, CI, PM, PN	113	4.3	29.0	1.10	0.36	0.0	0.9
22	M/43/23	POLG1	111/75	-	-	CA, CI, PM, PN, PHL, RI	178	4.2	25.4	1.40	0.24	0.8	0.8
23	M/47/21	POLG1	121/86	-	-	CA, CI, PN, PHL, RI	123	3.9	na	1.17	0.26	0.6	0.6
24	F/49/24	POLG1	128/81	-	-	CA, PM, PN, PHL	87	3.5	28.0	1.23	0.21	0.8	1.2

(N)IDDM, (non) insulin dependent diabetes mellitus; NSAID, non-steroid anti-inflammatory drug; Ca-I, calcium inhibitor; ACE-I, ACE inhibitor; CA, cerebellar ataxia; CI, cognitive impairment; PM, proximal myopathy; PN, peripheral neuropathy; PHL, peripheral hearing loss; RI, retinal involvement; CB, cardiac conduction block; CM, cardiomyopathy; E, epilepsy; HT, hypothyroidism; M, migraine; na, not available; eGFR = estimated GFR (MDRD formula, in ml/min/1.73m^2); α1M/creat, urine alpha1-microglobulin/creatinine ratio (mg/mmol); Alb/creat, urine albumin/creatinine ratio (mg/mmol)

* hypertension (mean systolic blood pressure > 140 mmHg, mean diastolic blood pressure > 90 mmHg or the use of antihypertensive medication)

Potassium, bicarbonate, phosphate and uric acid in mmol/ml

Laboratory test indicating renal dysfunction are underlined: potassium < 3.6, bicarbonate < 22, phosphate < 0.7, uric acid < 0.15, α1CR > 1.5 and Alb/creat ≥ 2.5 (males) or ≥ 3.5 (females).

Four patients (17%) reported a voiding frequency ≥ 10/day, including three patients (number 6, 20 and 24) with urge incontinence and one (19) with diabetes mellitus. Two patients (1 and 17) reported a history of urinary tract calculi. Two patients had a family history of renal disease: the mother of patient 13 had nephrotic syndrome, while the mother of patient 7 had diabetic nephropathy.

DISCUSSION

In the present study, we systematically investigated potential renal involvement in an adult population of CPEO patients. We estimated GFR using serum creatinine, assessed micro-albuminuria as marker of glomerular (podocyte) damage and serum potassium, uric acid, phosphate and bicarbonate and urinary alpha1-microglobulin as markers of tubular dysfunction. In this cohort of 24 patients, we observed hypertension in seven patients and diabetes mellitus in five. Evidence of tubular dysfunction, as reflected by the increased α1CR ratio was seen in five patients. Four of these five patients had hypertension or diabetes mellitus. Two patients had micro-albuminuria, which could be attributed to hypertension in both cases. Moreover, there was a correlation between the α1CR and the ACR. Overall, this suggests that the observed abnormalities are most likely secondary to abnormal blood pressure and glucose regulation. Clearly, we failed to detect any kind of clinically relevant tubular involvement or decreased eGFR in our cohort. Even the patients with a KSS phenotype or with the m.03243A→G mutation had normal kidney function, despite the fact that both conditions have previously been associated with renal involvement. Admittedly, serum creatinine based formulas are not validated in patients with mitochondrial disorders, and it is likely that in such patients eGFR overestimates real GFR.

In mitochondrial disorders in general, the clinical features, age of onset, the progression and extent of organ involvement vary considerably. Organs most commonly affected in mitochondrial disorders include the central and peripheral nervous system, skeletal muscles, heart, cochlea, and retina [15]. Although there seems to be no apparent relation between these commonly affected organs, as a rule of the thumb, the susceptibility of tissues and cells to mitochondrial dysfunction largely depends on two factors [16]. First, since the main function of mitochondria is ATP production through oxidative phosphorylation, tissues with a high degree of oxidative phosphorylation are more susceptible to mitochondrial dysfunction. Second, tissues with a low mitotic activity are also more susceptible, especially in mtDNA mutations. The relation between mitotic activity and susceptibility to mitochondrial dysfunction is related on the fact that both normal and mutated mtDNA can coexist in one cell. During mitosis, normal and mutated mtDNA copies are randomly distributed over the two daughter cells. If by chance a daughter cell

receives only few mutated mtDNA copies, it is likely to survive whereas a daughter cell with a high percentage of mutated DNA will not. As a result, after several mitotic cycles, the percentage of mutated mtDNA decreases.

Based on these two factors kidneys are likely to be affected in mitochondrial disorders. First, renal oxidative phosphorylation activity is very high: kidneys make up only 1% of the body weight, but account for about 10% of the total body ATP turnover [17]. The proximal tubule in particular has very high oxidative activity. Second, the podocyte is post-mitotic, implying it has no mitotic activity at all [18]. Moreover, in addition to these theoretical considerations, the interest of nephrologists has been fostered by publications concerning the involvement of the kidneys in mitochondrial disorders [19;20].

There is a striking difference between the pediatric and adult population regarding renal involvement in mitochondrial disorders [21]. The most common presentation in childhood is dysfunction of the proximal tubule or the thick ascending limb, presenting as Fanconi or Bartter-like syndromes [3-5;12;22;23]. The pathophysiology of these syndromes in mitochondrial disorders is not established, but it is thought to result from deficient ATP production in the tubule cells. Proximal tubule dysfunction in mitochondrial disorders is often associated with multi-system involvement and has a poor prognosis.

In contrast, tubular dysfunction is uncommon in adults with mitochondrial disorders. Possibly, due to the regenerative capacity of the tubule epithelial cells, the percentage of mutated mtDNA decreases over time. As such, adults with mitochondrial disorders often present with signs of podocyte injury, mainly steroid resistant focal segmental glomerulosclerosis (FSGS) [24-26]. Adults often have limited multi-organ involvement and a better prognosis. Only one gene mutation, the m.03243A→G mutation, has been associated with FSGS in adults and this particular mutation is by far the most common cause of renal disease in adults with mitochondrial disorders. The m.03243A→G mutation was first discovered in patients with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), but later also in patients with CPEO or MIDD (maternally inherited diabetes and deafness). At present, it has become evident that the m.03243A→G mutation can also result in isolated renal disease in patients without signs of MELAS or MIDD, even after prolonged follow-up. The relation between the m.03243A→G mutation and renal disease is further illustrated by the fact that MIDD patients have a much higher rate of renal involvement than other diabetes patients and by a much higher prevalence of the m.03243A→G mutation in patients on dialysis than in the general population [27-29].

The m.03243A→G mutation alters the A14 nucleotide that is highly conserved in the tRNA[Leu(UUR)]. This mutation affects multiple pathways that can be traced to the destabilization of structural features that destroy the tRNA conformation required for protein synthesis efficiency, aminoacylation, post-transcriptional modification and processing. Besides mutations in the mtDNA, nuclear gene defects may also cause renal disease. Over the last decade several novel gene defects resulting in kidney disease have been described and it

Table 2: Mutations associated with renal involvement in mitochondrial disorders

	Associated genes	Gene function
Childhood presentation		
Tubulopathy	mtDNA deletion [22;23;32]	various genes
	COX10 [33]	Complex IV
	BCS1L [34]	Complex III
	RRM2B [35]	P53R2
Tubular acidosis	mtDNA deletion [7]	various genes
Focal segmental glomerulosclerosis	m.03243A>G [36;37]	tRNA[Leu(UUR)]
	m.05843A>G [38]	Complex I
	COQ2 [39]	Coenzyme Q10
	COQ6 [40]	Coenzyme Q10
	mtDNA deletion [41]	various genes
Tubulointerstitial nephropathy	mtDNA deletion [42]	various genes
	m.00608A>G [43]	Complex I
Cystic renal disease	m.12425A-del [44]	Complex I
Adult presentation		
Focal segmental glomerulosclerosis	m.03243A>G [24-26]	tRNA[Leu(UUR)]
Hypomagnesemia	m.04291T>C [45]	tRNA[Ile]
Tubulointerstitial nephropathy	m.03243A>G [46]	tRNA[Leu(UUR)]
Cystic renal disease	m.03243A>G [46]	tRNA[Leu(UUR)]

has become more evident that defects in several proteins involved in the mitochondrial energy generating system may cause various renal disorders (Table 2).

Despite the lack of specific renal involvement in our cohort, we did observe a high incidence of hypertension. This may reflect subtle renal injury. In addition, more than one fifth of the patients had increased blood glucose levels. The high prevalence of hypertension and diabetes may justify regular evaluation for these cardiovascular risk factors in CPEO patients.

We also found a very high prevalence of both urge and stress incontinence. Although urge incontinence has previously been described in mitochondrial disorders, the underlying pathophysiological mechanism is unclear [30]. One might suggest, that urge incontinence results from impaired voiding control by the central nervous system. However, we did not find a relation between central nervous system involvement and urge incontinence in the present cohort. Stress incontinence on the other hand might result from pelvic muscles

weakness, though incontinence due to myopathic weakness of the pelvic floor is uncommon even in severe myopathies such as Duchenne muscular dystrophy [31]. Moreover, no association was found between stress incontinence and proximal muscle weakness or peripheral neuropathy in this study. Unfortunately, the present study was not designed to determine the pathophysiology of incontinence in CPEO and future studies should focus on this issue.

In conclusion, clinically relevant renal involvement, though common in mitochondrial disorders in general, is rare in adult CPEO. In contrast, hypertension and diabetes were common. We therefore recommend routine evaluation of blood pressure and glucose, while screening for microalbuminuria and tubular dysfunction should be restricted to selected cases.

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Part IV

Summary and discussion

Chapter 8

Summary and discussion

GENERAL CONSIDERATIONS

Our understanding of mitochondrial genetics and function has greatly increased over the last two to three decades. The care of patients with mitochondrial disorders has also improved due to increased knowledge of the natural course of various mitochondrial disorders, the development of diagnostic guidelines and by more advanced genetic assays [1;2]. Moreover, several therapeutic strategies have been developed, including l-arginine in stroke-like episodes associated with MELAS, co-enzyme Q10 in primary co-enzyme Q10 deficiency and bone marrow transplant or dialysis in MNGIE patients [3-5]. Unfortunately, causative treatment is not available for most mitochondrial disorders, nor is it expected in the near future [6]. At present, the management of patients with mitochondrial disorders is therefore largely symptomatic, attempting to minimize the impact of the disease on patients' functioning.

Symptomatic management aimed at the specific needs of the individual patients however requires awareness of the signs and symptoms, the natural course and the disease impact. In this thesis we therefore systematically studied the phenotype and impact of disease in one of the commonest mitochondrial disorders, chronic progressive external ophthalmoplegia (CPEO). In addition, we evaluated the effectiveness of the diagnostic workup in patients with a suspected mitochondrial disorder, as patients' management obviously requires the confirmation of a correct diagnosis. Ultimately, the conclusions of this thesis could help physicians to optimize the diagnostic process and to provide more effective symptomatic management to patients with a mitochondrial disorder. Below, a short summary of previous chapters is provided in boxes and the results are discussed in more detail.

SUMMARIES

After the Introduction **(Part I)**, we provide several considerations for the workup of a suspected mitochondrial disorder in **Part II** (**Chapters 2 and 3**). **Chapter 2** discusses the extent of mitochondrial involvement in chronic fatigue syndrome (CFS), as well as the value of measurement of respiratory chain complex (RCC) activities in the discrimination between CFS and mitochondrial disorders. Identifying procedures to discriminate between CFS and mitochondrial disorders can improve the diagnostic process in patients presenting with non-specific features such as fatigue, exercise intolerance and myalgia, which are common in both disorders [7;8].

Summary Chapter 2

We determined the extent of mitochondrial dysfunction in CFS patients and found that CFS patients had a significantly lower skeletal muscle mitochondrial content than controls. When corrected for mitochondrial content, the mitochondrial RCC activities did not differ between the two groups.

Next, we investigated whether measurement of RCC activities discriminated between patients with CFS and patients with mitochondrial disorders. Mitochondrial content did not discriminate between CFS and mitochondrial disorders, but respiratory chain complexes I, III and IV and the ATP production did, all with lower activities in the patients with mitochondrial disorders.

The interpretation of the decreased mitochondrial content in skeletal muscle of CFS is difficult. One could argue that decreased mitochondrial content is the cause of CFS and therefore of symptoms such as fatigue, exercise intolerance and exercise induced myalgia. However, one could also argue that decreased mitochondrial content is the consequence of physical inactivity, which is a common feature in CFS. The latter concept is supported by studies that found a decreased mitochondrial content in healthy but physically inactive subjects, and an increase in mitochondrial content in response to training [9-12]. Additional studies are required to elucidate whether decreased mitochondrial content is cause or consequence of CFS. For this purpose, it would be worthwhile to determine whether the relation between physical activity and mitochondrial content is continuous or mitochondrial content decreases only when the degree of physical inactivity reaches a certain threshold. It would be interesting to investigate the effect of cognitive behavioral therapy (CBT) on mitochondrial content in CFS patients. CBT is an established treatment in CFS [13]. It results in an increase in physical functioning and ameliorates fatigue [14]. Should mitochondrial content increase in response to a successful treatment with CBT, it is reasonable to assume that decreased mitochondrial content in CFS is the consequence of physical inactivity rather than the cause.

In the second part of the study we discuss the use of RCC measurement in the diagnostic workup of patients with nonspecific neuromuscular complaints such as fatigue, exercise intolerance and exercise induced myalgia. Although these non-specific complaints may be the sole manifestations of a mitochondrial disorder, often no underlying somatic disorder is found and patients are consequentially considered to have CFS [7]. Differentiation between a mitochondrial disorder and CFS is often troublesome, especially when skeletal muscle histology or evaluation of mtDNA in leukocytes fails to confirm the diagnosis of a mitochondrial disorder. RCC measurement in skeletal muscle may then be performed for further differentiation, though the discriminative value has never been established [15]. In **Chapter 2**, we demonstrated that measurement of RCC activities can reliably be used to discriminate between CFS and a mitochondrial disorder. The ATP production rate, which

can only be measured in a fresh muscle biopsy, was shown to have the highest discriminative value.

Although **Chapter 2** underlines the use of RCC measurement in the workup of a suspected mitochondrial disorder, the most definite proof of a mitochondrial disorder is the detection of a known pathogenic DNA mutation. In CPEO, a large variety of pathogenic mtDNA point mutations and large scale rearrangements have previously been reported (see **Chapter 1**, Table 3). The CPEO patient in **Chapter 3** was found to have a pathogenic mtDNA point mutation, which had not previously been associated with CPEO [16].

Summary Chapter 3

We found a heteroplastic m.04267A→G mutation in the mitochondrial tRNA^{Ile} in a 52-year-old patient with a five year history of progressive external ophthalmoplegia and exercise induced muscle cramps. This specific mutation had been described only once before in another phenotype: a patient with deafness, cerebellar dysarthria, muscle ache, fatigue and mental slowing, but no signs of external ophthalmoplegia.

The discovery of a new mtDNA point mutation is often associated with some degree of uncertainty whether the mutation is the actual cause of the phenotype or a coincidental non-pathogenic polymorphism. The fact that we found the m.04267A→G mutation in a patient with CPEO, which is an established mitochondrial phenotype, provides additional evidence for its pathogenicity. Moreover, our findings support the approach of total mtDNA sequencing in cases of CPEO that are not due to large scale mtDNA rearrangements.

In **Part III** (**Chapters 4 to 7**), we describe the results of four studies on various aspects of the CPEO phenotype. The results of a study on the disease impact associated with CPEO are summarized in **Chapter 4**.

Summary Chapter 4

We determined the disease impact in 28 patients with genetically confirmed CPEO and compared the outcomes to those of matched myotonic dystrophy type I (DM1) patients. CPEO patients reported a high frequency of severe fatigue (68%), pain (96%), depression (32%) and dependence in daily life (46%). All of these aspects of disease impact were significantly more severe than in the DM1 cohort. CPEO patients with polymerase gamma 1 *(POLG1)* mutations reported more functional impairments than those with mitochondrial DNA mutations, whereas most of the clinical features did not influence disease impact.

This study identifies fatigue, depression, pain, and functional impairments as the main aspects of disease impact in CPEO and thus helps to provide symptomatic management of the specific needs of the individual patient. As such, the treatment of depression may be of particular interest, since it was associated with functional impairments and decreased independence. Treatment of depression might therefore ideally result in an increase of functional capacities. However, the cross sectional design of our study does not allow to determine whether depression is the cause or consequence of functional impairments. Moreover, depression in mitochondrial disorders most likely results from mitochondrial dysfunction in the frontal lobes [17-19]. The pathogenesis of depression is therefore possibly different in patients with mitochondrial disorders than in the general population. Consequently, the beneficial effect of antidepressants is uncertain in patients with mitochondrial disorders.

A greater emphasis on the treatment of pain may also be warranted. Pain was both common and severe in our cohort, despite the use of analgesics by a majority of patients. This might indicate that CPEO patients are either refractory to analgesics or that they are often undertreated. The latter is supported by the fact that despite the severity of pain only two patients used opioids.

Treatment of fatigue will also likely improve well-being. To date, aerobic training is the only therapy with an evidence based beneficial effect on fatigue in mitochondrial patients [20]. However, the three to four times weekly cycle schedule is demanding and therefore not feasible for patients with advanced disease. Since fatigue is such a major symptom in CPEO, it is desirable to study the effect of other treatments, such as cognitive behavioral therapy and/or pharmacological interventions such as amantadine.

In **Chapter 5**, we describe the prevalence and determinants of sleep disturbances in CPEO.

Summary Chapter 5

We assessed the prevalence and determinants of sleep disturbances in 20 genetically confirmed CPEO patients. Using questionnaires, we found that 75% reported nocturnal sleep dysfunction, partly associated with anxiety. Thirty-five percent fulfilled the criteria for restless legs syndrome and 30% had excessive daytime sleepiness. Polysomnography revealed several indicators of a disrupted sleep architecture: 70% had significant periodic limb movements, while 4 patients, all with *POLG1*-mutations, had an increased central sleep apnea index. Obstructive sleep disordered breathing was present in only one patient. Physical examination and questionnaire outcomes were poor predictors of polysomnography results.

This study demonstrates that several specific sleep disturbances are part of the CPEO phenotype. Sleep disturbances are important to recognize, as they have a profound nega-

tive impact on the quality of life. Identifying CPEO patients at risk for a sleep disorder may however be complicated, since subjective sleep quality and clinical features were poor predictors of polysomnography outcomes. From a practical point of view, it seems reasonable to perform a polysomnography only in patients with symptoms of impaired sleep, as they are the ones that might benefit from treatment of the underlying sleep disorder. The common therapeutic options for sleep disorders, e.g. positive airway pressure for sleep apnea and dopamine agonists or gabapentin for restless legs and periodic limb movements, are safe and non-invasive and can therefore be used empirically in individual patients. However, the effectiveness of these treatments in patients with mitochondrial disorders should preferably be determined in a clinical trial. The end points in such a trial should not only be the improvement of the apnea and limb movement index or the quality of sleep, but also the perception of health and quality of life in general.

Chapter 6 describes the nature and frequency of respiratory involvement in CPEO.

> **Summary Chapter 6**
>
> We determined the prevalence, nature and determinants of respiratory muscle involvement in 23 patients with genetically confirmed CPEO. The main finding was decreased respiratory muscle strength, both expiratory (76.8% of predicted) and inspiratory (79.5% of predicted). Inspiratory vital capacity (92.5% of predicted) and the forced expiratory volume in 1 second (89.3% of predicted) were also decreased. Expiratory weakness was associated with a decreased vital capacity and a decreased peak expiratory flow. Moreover, expiratory muscle strength was lower in patients with limb girdle weakness than in patients with normal limb girdle strength. Other clinical features such as subjective respiratory complaints, disease severity or disease duration were not associated with respiratory muscle weakness.

This study indicates that respiratory muscle weakness is common in CPEO. Since respiratory weakness is associated with an increased chance of pneumonia in other myopathies, the risk of pneumonia should theoretically also be increased in CPEO [21]. However, the majority of patients in our cohort had no history of pneumonia. This might be explained by a high mortality rate of pneumonias in CPEO. To test this hypothesis, we reviewed our files of all deceased CPEO patients and found that two out of the three patients with a confirmed cause of death indeed died as a direct result of their first pneumonia. Nevertheless, it would be desirable to study the relation between respiratory weakness and the risk of pneumonia in a longitudinal study.

It is tempting to speculate about the role of respiratory muscle weakness on exercise intolerance, which is one of the key symptoms in mitochondrial disorders. Exercise intolerance in mitochondrial myopathies is generally assumed to result from insufficient ATP produc-

tion in skeletal muscles, causing premature fatigue, weakness and myalgia [22]. Previous studies on exercise capacity in patients with mitochondrial disorders however found an association between respiratory muscle weakness and decreased maximal minute ventilation with rapid shallow breathing [23]. Respiratory muscle weakness may therefore be an independent factor in the pathogenesis of exercise intolerance in mitochondrial disorders. Should future research confirm this hypothesis, it would be worthwhile to investigate the effect of respiratory muscle training on both respiratory muscle strength and on exercise capacity. Lastly, respiratory muscle strength may prove to be a useful outcome measure in future intervention trials, since it is cheap, reproducible and easily obtainable.

In **Chapter 7**, we describe the results of our study on renal involvement in CPEO.

Summary Chapter 7

We determined the nature and frequency of renal involvement in 24 patients with genetically confirmed CPEO. Five patients (21%) had evidence of tubular dysfunction, though this could be attributed to hypertension or diabetes mellitus in four. Two patients (8%) had micro-albuminuria, associated with hypertension in both. Seven patients (29%) had hypertension and five (21%) diabetes mellitus. Fourteen patients (58%) reported incontinence, mainly urge incontinence (10 patients, 42%).

This study failed to detect any kind of clinically relevant renal involvement in our cohort. Nevertheless, we did find a high prevalence of hypertension and diabetes mellitus. We therefore recommend routine evaluation of blood pressure and glucose. Screening for microalbuminuria and tubular dysfunction should be restricted to selected cases.

Incontinence was very common in CPEO. Unfortunately, the present study was not designed to determine the pathophysiology of the incontinence in CPEO and future studies should focus on this issue.

The absence of renal involvement in our cohort provides an interesting view on the CPEO phenotype. Based on theoretical considerations and on the fact that renal involvement is common in other mitochondrial disorders one would expect to have found renal involvement in the present CPEO cohort. The absence of renal involvement in our cohort therefore indicates that despite the large overlap in clinical features between various mitochondrial disorders, the CPEO phenotype has a specific phenotypical "fingerprint".

Combining the results of all previous chapters, some clinical features appear to be related to a particular genotype: sensory atactic neuropathy and central sleep apneas were exclusively found in patients with *POLG1*-mutations, while clinically relevant expiratory muscle weakness was found in patients with mtDNA deletions only. Moreover, patients with *POLG1*-mutations had a higher rate of disease progression (i.e. more functional impairment), despite a higher age at disease onset.
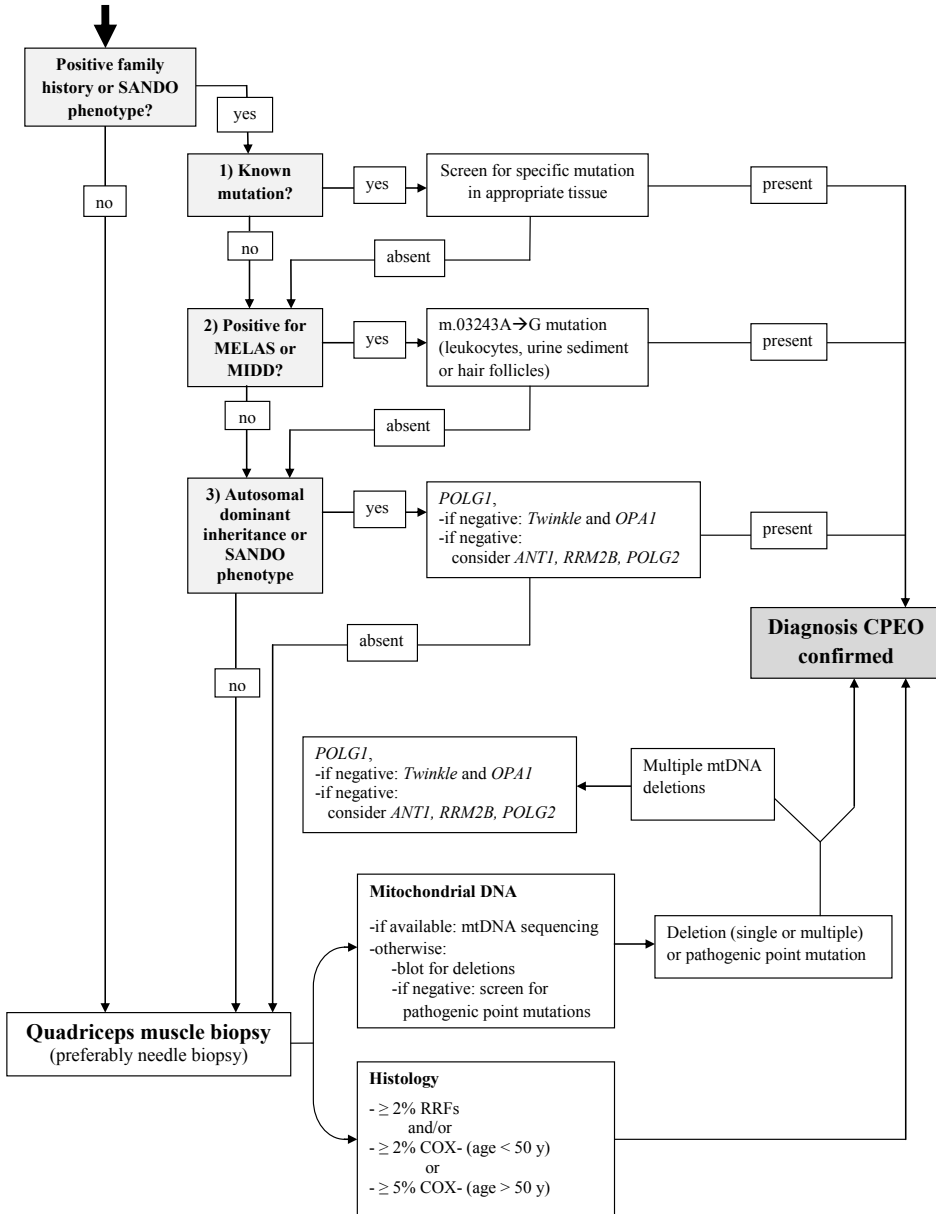
OVERVIEW

This thesis addresses two aspects of the care for mitochondrial patients. First, it provides important clues to improve the diagnostic workup of patients with a suspected mitochondrial disorder. Second, it draws attention to the various symptoms in these multi-system disorders. This may allow for a more effective diagnostic workup, better symptomatic management and more efficient (multidisciplinary) follow-up of patients with CPEO.

To facilitate the care for a patient with (suspected) CPEO, several elements of this thesis have been summarized in a diagnostic algorithm and a guideline for patient management. The diagnostic algorithm and guideline are not only based on the data from the studies in this thesis, but also on our personal experience with CPEO patients, as well as on previous literature [24;25].

Diagnostic algorithm for (suspected) CPEO

As stated in **Chapter 1**, a slowly progressive bilateral external ophthalmoplegia is a very specific clinical feature, with a limited differential diagnosis. In addition to CPEO, a slowly progressive bilateral external ophthalmoplegia can be caused by oculopharyngeal muscle dystrophy (OPMD), myasthenia gravis (MG) and congenital myasthenic syndromes (CM). Most cases of OPMD can likely be differentiated from CPEO by family history (autosomal dominant inheritance and late onset in OPMD) and most cases of MG and CM by course and history (marked fluctuations in muscle weakness, no extra-muscular symptoms, early age of onset in CM) and by repetitive nerve stimulation. When a fair degree of suspicion of CPEO remains, the following algorithm can help to confirm the diagnosis without unnecessary (and invasive) ancillary investigations.

Positive family history or SANDO phenotype?

yes

no

1) Known mutation?

yes → Screen for specific mutation in appropriate tissue → present

no

absent

2) Positive for MELAS or MIDD?

yes → m.03243A→G mutation (leukocytes, urine sediment or hair follicles) → present

no

absent

3) Autosomal dominant inheritance or SANDO phenotype

yes → *POLG1*, -if negative: *Twinkle* and *OPA1* -if negative: consider *ANT1, RRM2B, POLG2* → present

no

absent

Diagnosis CPEO confirmed

POLG1, -if negative: *Twinkle* and *OPA1* -if negative: consider *ANT1, RRM2B, POLG2* ← Multiple mtDNA deletions

Mitochondrial DNA

-if available: mtDNA sequencing
-otherwise:
    -blot for deletions
    -if negative: screen for
      pathogenic point mutations

Deletion (single or multiple) or pathogenic point mutation

Quadriceps muscle biopsy (preferably needle biopsy)

Histology

- ≥ 2% RRFs
    and/or
- ≥ 2% COX- (age < 50 y)
    or
- ≥ 5% COX- (age > 50 y)

COX-, cytochrome c oxidase negative fibers; MELAS, mitochondrial encephalomyopathy - lactic acidosis - and stroke-like episodes; MIDD, maternally inherited diabetes and deafness; mtDNA, mitochondrial DNA; RRF, ragged red fibers, SANDO, sensory atactic neuropathy - dysarthria and ophthalmoplegia

Suggested workup after confirmation of CPEO diagnosis

- Inquire about complaints of dysphagia, hearing loss, depression, periodic leg movements, sleep apnea, restless legs, incontinence, palpitations and (near)collaps
- Blood pressure measurement
- Assessment of body mass index
- Blood tests
  - o Full blood count, fasting glucose, potassium, creatinine, calcium, phosphorus, magnesium, uric acid, bicarbonate, albumin, PTH, TSH
  - o LH, FSH and oestrogen in premenopausal female patients
  - o LH, FSH and testosterone in male patients
  - o Growth hormone in patients with short stature or growth retardation
- Urine albumin/creatinine ratio
- Spirometry including measurement of respiratory muscle strength
- Evaluation by a cardiologist, including an echocardiography and EKG*
- Evaluation by a rehabilitation physician**

* Holter monitoring in patients with palpitations, (near)collaps or an abnormal EKG

** Consider an aerobic training program for patients in good clinical condition, consultation of a physiotherapist for optimizing balance or cough technique, speech therapy in case of dysarthria or dysphagia and occupational therapy to increase independence

In selected cases, consider consulting

- Ear-nose-throat physician: hearing loss, dysphagia
- Ophthalmologist: ptosis surgery, evaluation of retinal involvement
- Genetic counseling
- Psychiatrist: depression, anxiety
- Nephrologist: renal involvement
- Urologist: incontinence
- Endocrinologist: diabetes mellitus, other endocrine disorders
- Dietitian: overweight or underweight
- Polysomnography: suspected sleep disorder

Follow up

Annually
- Evaluation by a neurologist
- Inquire about complaints of dysphagia, hearing loss, depression, periodic leg movements, sleep apnea, restless legs, incontinence, palpitations and (near) collaps
- Blood pressure
- Assessment of body mass index
- Fasting glucose
- In patients with previous evidence of renal involvement: serum potassium, creatinine, calcium, phosphorus, magnesium, uric acid, bicarbonate, albumin and urine albumin/creatinine ratio
- EKG*
- Evaluation by rehabilitation physician**

Biennially
- Blood tests
  - o Full blood count, PTH, TSH
  - o LH, FSH and oestrogen in premenopausal female patients
  - o LH, FSH and testosterone in male patients
  - o Growth hormone in patients with short stature or growth retardation
- Urine tests: albumin/creatinine ratio
- Spirometry including measurement of respiratory muscle strength
- Echocardiography

*   Holter monitoring in patients with palpitations, (near)collaps or an abnormal EKG

**   Consider an aerobic training program for patients in good clinical condition, consultation of a physiotherapist for optimizing balance or cough technique, speech therapy in case of dysarthria or dysphagia and occupational therapy to increase independence

FUTURE PERSPECTIVES

Mitochondria in the chronic fatigue syndrome

In **Chapter 2,** we demonstrated that mitochondrial content in skeletal muscle is less in patients with chronic fatigue syndrome than in healthy controls, whereas mitochondrial function remains unaffected. Our results are in concordance with previous studies that found decreased mitochondrial content in healthy but physically inactive subjects, and an increase in mitochondrial content in response to training.

More insight into the cellular and molecular pathways underlying the relation between mitochondrial content and physical activity may lead to a better understanding of the pathogenesis of CFS. Moreover, manipulation of the affected pathways may be a key to a pharmacological treatment not only for CFS, but also for mitochondrial disorders or sarcopenia associated with aging or with long term immobility (e.g. in the intensive care unit). Regarding the relation between physical activity and mitochondrial content or function, CFS is highly suitable as a model of inactivity. By definition, any underlying medical condition must be excluded in CFS. It is therefore reasonable to assume, that all effects on mitochondrial content and function result from alterations in the level of physical activity. Moreover, physical inactivity in CFS is reversible, as both cognitive behavioral therapy and graded exercise have a proven beneficial effect on physical activity in CFS patients.

It would therefore be worthwhile to investigate whether an increase in physical activity in CFS patients successfully treated with behavioral therapy coincides with an increase in mitochondrial content and function and/or upregulation of the pathways involved in mitochondrial biogenesis, such as PGC-1alpha (peroxisome proliferator-activated receptor γ coactivator α) or *TFAM* (transcription factor A, mitochondrial).

The CPEO phenotype

This thesis illustrates the impact of disease and the extent of involvement of various organ systems. All data are however cross-sectional. The evolution of existing symptoms and the rate of occurrence of new symptoms over time are therefore unknown. A follow-up study on the same cohort could provide this information and thus allow for better patient counseling and management.

Therapy

To date, apart from aerobic training, there are no therapeutic interventions with a proven beneficial effect on the symptoms and course of CPEO. Management is therefore largely symptomatic, together with interventions aimed at limiting the detrimental effects of organ dysfunction. Several symptoms identified by the present studies have evidence based effective therapies in the general population. These include optimal analgesia in pain, antidepressants in depression, dopamine agonists for restless legs and periodic leg movement disorder, positive airway pressure therapy for central apnea during sleep and amantadine for fatigue. Whether these treatments are also effective in CPEO is unclear and this should preferably be established in a research setting.

Lastly, we found a high frequency of incontinence in our cohort. Incontinence is considered to have a profound negative impact on the quality of life. There are however several effective conservative, medical and surgical therapies for both stress and urge incontinence. The optimal approach towards incontinence in CPEO requires additional research on its pathophysiology.

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Part V

Nederlandstalige samenvatting
voor niet-medici

Chapter 9

Nederlandstalige samenvatting
voor niet-medici

ACHTERGROND

Dit proefschrift gaat over de ziekte chronische progressieve externe oftalmoplegie, kortweg CPEO. De naam verraadt enkele belangrijke kenmerken van de ziekte. *Extern* betekent buitenzijde en *oftalmoplegie* betekent verlamming van de ogen. Bij een externe oftalmo- plegie raken de spieren aan de buitenkant van de oogbol dus verlamd. Hierdoor kunnen patiënten niet meer goed zijwaards, naar boven en naar beneden kijken. Ook de spieren, die de oogleden naar boven ophouden worden zwakker. De oogleden hangen hierdoor over de oogbollen (Figuur 1). Verder is CPEO *chronisch*, wat betekent dat de verschijnselen blijvend zijn en *progressief*, wat inhoudt, dat de ziekte in de loop der jaren steeds erger wordt. Vaak hebben CPEO patiënten meer klachten dan alleen een oogspierverlamming. Andere spieren kunnen ook zwak zijn, vooral in bovenarmen en –benen, de hartspier en de spieren in de keel, die zorgen voor het slikken. Blindheid en doofheid kunnen ook voorkomen. Verder hebben sommige patiënten een slechte coördinatie, vanwege niet goed werkende kleine hersenen, of een aantasting van de zenuwen, wat leidt tot minder gevoel in de voeten en handen. Tot slot komen problemen met de hormoonhuishouding vaak voor, zoals suikerziekte of een te traag werkende (bij)schildklier.

CPEO is een zeldzame ziekte. Het komt bij ongeveer 1 op de 40.000 mensen voor, zowel bij mannen als vrouwen. De leeftijd waarop de eerste klachten beginnen is heel wis- selend. Sommige mensen hebben al vanaf de vroege jeugd klachten, terwijl anderen pas na hun vijftigste klachten krijgen.

Figuur 1
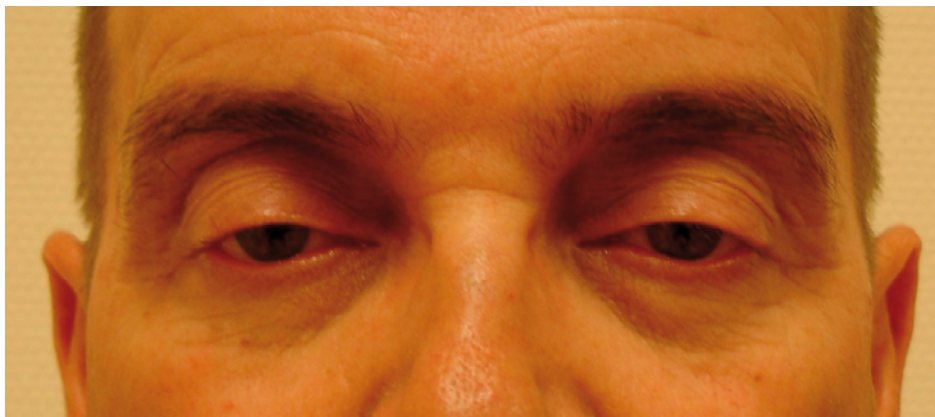
CPEO is een energiestofwisselingsziekte. Alle cellen in ons lichaam verbruiken energie: een spiercel heeft energie nodig om samen te trekken, een schildkliercel om hormoon te kunnen uitscheiden en een zenuwcel om elektrische stroompjes op te kunnen wek- ken. De energie, die hier voor nodig is, wordt geleverd in de vorm van ATP. ATP is een

klein molecuul, dat wordt gemaakt van suikers of vetten. De cel heeft hiervoor aparte "orgaantjes", mitochondriën genaamd. Cellen die veel energie gebruiken hebben meer mitochondriën dan cellen met een laag energieverbruik. Bij CPEO werken de mitochondriën niet goed. Het wordt daarom gerekend tot de groep van mitochondriële ziekten.

Door wetenschappelijk onderzoek is er in de laatste 20 tot 30 jaar veel meer duidelijk geworden over mitochondriën en mitochondriële ziekten. De code van het erfelijk materiaal (DNA) van mitochondriën is helemaal ontrafeld. Ook weten we, dat bepaalde foutjes in dit DNA, mutaties genoemd, kunnen leiden tot verschillende mitochondriële ziektes. Door nieuwe technieken is het de laatste jaren mogelijk geworden het hele mitochondriële DNA in een keer op mutaties te onderzoeken. Dit maakt het mogelijk om steeds sneller en nauwkeuriger de juiste diagnose te stellen. Ook is er uitgebreid onderzoek gedaan naar behandelingen. Helaas is er voor de meeste mitochondriële ziekten nog geen genezing mogelijk. De behandeling van patiënten met mitochondriële ziekten is tot nu toe dan ook vooral "symptomatisch". Dit betekent dat er naar wordt gestreefd de beperkingen en klachten die de ziekte veroorzaakt zo veel mogelijk terug te brengen.

De optimale symptomatische behandeling voor een bepaalde klacht kan per ziekte verschillen. Het is dan ook belangrijk dat eerst de juiste diagnose wordt gesteld. Verder moet het duidelijk zijn welke klachten het meest hinderlijk zijn in het dagelijks functioneren en welke organen precies betrokken kunnen zijn.

In het geval van CPEO weten we bijvoorbeeld dat spierzwakte vaak voorkomt, en dan vooral rond de ogen, in het hart, de keel en in de bovenarmen en -benen. Of de ademhalingsspieren ook zwak zijn is daarentegen niet bekend. Ook weten we niet hoe vaak problemen met de nieren voorkomen en of CPEO leidt tot een slechtere slaap. Tot slot is nog nooit onderzocht hoe CPEO patiënten hun ziekte ervaren en wat hun belangrijkste klachten zijn. Voor een goede symptomatische behandeling is het natuurlijk wel van belang om dit te weten. De behandelend arts kan immers alleen die klachten verlichten, waarvan hij weet dat ze er zijn.

Met dit proefschrift wilden we twee hoofdvragen beantwoorden. Eerst hebben we gekeken hoe bij een patiënt met verdenking op een mitochondriële ziekte het stellen van de juiste diagnose nog beter kan. Ten tweede hebben we onderzocht, welke klachten CPEO patiënten eigenlijk hebben in het dagelijk functioneren en welke organen er precies aangedaan zijn.

HET STELLEN VAN DE JUISTE DIAGNOSE

In de mitochondriën loopt de omzetting van suikers en vetten in ATP via vier enzymen, complex I, II, III en IV genoemd. In het laboratorium kunnen deze enzymcomplexen worden doorgemeten. Dit kan het beste in een klein stukje spier, wat uit het bovenbeen wordt

gehaald. Bij veel mitochondriële ziekten werken één of meerdere van deze complexen niet goed. Het meten van de enzymcomplexen is dan ook een belangrijk onderzoek, vooral als er getwijfeld wordt tussen een echte mitochondriële ziekte en een andere ziekte, die er qua klachten op lijkt. Eén van de ziektes die soms moeilijk te onderscheiden is van een mitochondriële ziekte is het chronisch vermoeidheidssyndroom (CVS). Zowel bij mitochondriële ziekten als bij CVS komen klachten van vermoeidheid en spierpijn na inspanning vaak voor. In hoofdstuk 2 hebben we onderzocht of het doormeten van de mitochondriële enzymcomplexen in een stukje spier gebruikt kan worden om mitochondriële ziekten te onderscheiden van CVS. Deze studie liet zien, dat bij patiënten met mitochondriële ziekten de enzymcomplexen inderdaad slechter werken dan bij patiënten met CVS. Ook hebben we de enzymcomplexen tussen CVS patiënten en gezonde proefpersonen vergeleken. Hiertussen waren geen verschillen. Wel bleken de spieren van CVS patiënten veel minder mitochondriën te bevatten dan die van gezonde proefpersonen.

Het onderzoek toont dus aan dat het meten van de mitochondriële enzymcomplexen bijdraagt aan het stellen van de diagnose van een mitochondriële ziekte. Verder blijken CVS patiënten op zich goed functionerende mitochondriën te hebben, maar hebben ze er wel minder dan gezonde personen. Het is niet helemaal duidelijk of de lagere hoeveelheid mitochondriën de oorzaak van CVS is, of het gevolg van verminderde lichamelijke activiteit.

Door het meten van de mitochondriële enzymcomplexen is dus het mogelijk een mitochondriële ziekte meer of minder waarschijnlijk te maken, maar het meest definitieve bewijs is het aantonen van een mutatie in het mitochondriële DNA. Er zijn verschillende mutaties in het mitochondrieël DNA die CPEO kunnen veroorzaken. In hoofdstuk 3 beschrijven we een patiënt met een mutatie die nog nooit eerder gevonden was bij CPEO.

KLACHTEN EN AANGEDANE ORGANEN BIJ CPEO

In hoofdstuk 4 hebben we de belangrijkste klachten in een groep van 28 CPEO patiënten in kaart gebracht. Het bleek dat zij vooral last hadden van vermoeidheid, pijn, depressie en beperkingen in de zelfredzaamheid. Om de ernst van deze klachten precies in te schatten hebben we de resultaten van deze groep CPEO patiënten vergeleken met een voor neurologen bekendere spierziekte, myotone dystrofie. Myotone dystrofie tast net als CPEO ook de spieren rondom de ogen en het hart aan en wordt over het algemeen gezien als een ernstige aandoening. Het bleek dat alle bovengenoemde klachten bij CPEO vaker voorkwamen en erger waren dan bij myotone dystrofie. Conclusie van deze studie is, dat CPEO een ernstige ziekte is, waarbij patiënten vaak meer klachten hebben dan je op het eerste oog zou verwachten.

In hoofdstuk 5 hebben we onderzocht of CPEO patiënten problemen hebben met slapen. Slaapproblemen komen vaak voor bij patiënten met spierziekten en kunnen veel klachten geven in het functioneren overdag. We hebben 20 CPEO patiënten vragenlijsten over slapen voorgelegd. Driekwart vond dat zij slecht sliepen. Rusteloze benen en slaperigheid overdag waren andere veel voorkomende klachten. Alle deelnemers hebben ook een nacht geslapen aan een "slaapmonitor". Hieruit bleek dat schoppende benen opvallend vaak voorkwamen. Enkele patiënten hadden 's nachts opvallend lange adempauzes. Deze slaapproblemen zijn doorgaans goed te behandelen, waardoor de kwaliteit van leven aanzienlijk kan verbeteren. Of deze behandelingen net zo effectief zijn bij CPEO patiënten als bij verder gezonde personen, moet nog verder onderzocht worden.

In hoofdstuk 6 hebben we gekeken of de longen ook zijn aangedaan bij CPEO. Hiervoor hebben we bij 23 patiënten longfunctieonderzoek gedaan. CPEO bleek geen invloed te hebben op de longen zelf. Wel vonden we dat de spieren, die zorgen voor in- en uitademen vaak zwak waren, vooral bij patiënten die ook zwakte van de bovenarmen en –benen hadden. Zwakte van de ademhalingsspieren kan belangrijk zijn, aangezien dit ook kan leiden tot minder krachtig hoesten. Minder krachtig hoesten kan weer leiden tot longontstekingen, wat een belangrijke doodsoorzaak is onder CPEO patiënten. Het lijkt dus belangrijk om bij CPEO patiënten regelmatig de kracht van de ademhalingsspieren te controleren.

In hoofdstuk 7 hebben we onderzocht hoe vaak de nieren zijn aangetast bij CPEO. Bij andere mitochondriële ziekten komen nierfunctiestoornissen namelijk vaak voor. In onze studie was de nierfunctie bij alle 24 deelnemende CPEO patiënten normaal. Wel kwamen hoge bloeddruk en suikerziekte opvallend vaak voor. Ook bleek meer dan de helft van de patiënten last te hebben van incontintentie. De precieze oorzaak van incontinentie bij CPEO is onduidelijk. Dit moet nog verder onderzocht worden.

Part VI

Appendices

DANKWOORD

Zorg voor patiënten met een mitochondriële ziekte is bij uitstek een multidisciplinaire aangelegenheid. Ook een proefschrift over mitochondriële ziekten schrijf je niet alleen. Vele personen hebben direct of indirect bijgedragen aan de totstandkoming hiervan. In dit dankwoord beperkt ik me tot enkele personen in het bijzonder.

Allereerst natuurlijk de patiënten, die ondanks hun ziekte toch altijd bereid waren bij te dragen aan de totstandkoming van dit proefschrift. Ik hoop dat dit proefschrift eraan zal bijdragen dat CPEO in de toekomst beter behandeld kan worden.

Beste Baziel (als AGNIO mocht ik je al geen prof. van Engelen meer noemen). Het is jouw opvatting dat promoveren meer is dan alleen het schrijven van een proefschrift. Promoveren is ook een persoonlijke ontwikkeling, een jeugdzonde. Ik waardeer het enorm dat je me veel vrijheid hebt gegeven om er mijn "eigen" boekje van te maken en me geduldig de kans hebt gegeven mijn eigen fouten te maken, zodat ik er nog meer van kon leren. Ik zal onze overlegmomenten missen, die net zo vaak niet als wel over mitochondriële zaken gingen.

Best Bert. Met je enorme moleculair-genetische kennis van mitochondriële ziekten bekeek je onze artikelen van een andere invalshoek, wat de kwaliteit altijd ten goede kwam. Ook heb je het publiceren van onze artikelen veel makkelijker gemaakt door de hele populatie genetisch in kaart te brengen. Bovendien, jij maakt het Brabants trio compleet, waardoor elk overleg vanzelfsprekend gemoedelijk en gezellig werd.

Het grootste deel van dit proefschrift gaat helemaal niet over neurologie. Zonder de kennis en het engelengeduld, waarmee Richard Rodenburg, Gijs Bleijenberg, Sebastiaan Overeem, Yvonne Heijdra en Jack Wetzels me wegwijs hebben gemaakt in hun vakgebied, zou er weinig van dit proefschrift terecht zijn gekomen. Vanzelfsprekend wil ik ook de andere medeauteurs bedanken voor hun aandeel in dit proefschrift.

Dank aan Femke Cuppen, longfunctielaborante, en aan de studenten die met hun wetenschappelijke stages hebben bijgedragen aan dit proefschrift: Marnix Kuindersma, Cathérine Delnooz en Henk-Jan Westeneng. Jullie hebben samen heel wat kilometers gemaakt om bij patiënten thuis metingen te verrichten. En natuurlijk aan ook Jiske Fermont, ongetwijfeld de meest getalenteerde AIOS neurologie aller tijden. Je was namelijk al afgestudeerd neuroloog, nog voordat je wetenschappelijke stage van de doctoraalfase geneeskunde (hoofdstuk 4) gepubliceerd was.

Alle collega's met wie ik de onderzoekerskamer heb gedeeld wil ik bedanken voor de gezelligheid en voor het aanhoren van mijn onuitputtelijke gezeur over….. nou, over vanalles eigenlijk. Ook wil ik me excuseren voor mijn beperkte aandachtsspanne, waardoor ik jullie zo vaak van het werk gehouden heb.

Mijn neef Leon van Loon. Na ons eerste gesprek over het ontwerp van de kaft had ik eerlijk gezegd helemaal geen vertrouwen in een goede afloop, maar ik was meteen verkocht toen ik je ontwerp zag. *Dè ge bedankt bènt, dè witte.*

Mijn Maasstadse collega, Ritu Saxena, voor het vertalen van mijn steenkolen-engels naar keurig Oxford English.

Dank aan de leden van manuscriptcommissie, prof. J. Smeitink, prof. S. Geurts en dr. R. de Coo, voor hun kritische en snelle beoordeling van het manuscript.

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Bram, in het licht van de arbeidsproductiviteit was het een kapitale fout van onze opleiders om ons tijdens onze eerste onderzoekstage samen met mede-musketier Ruben op één kamer te plaatsen. Ik vond dit desalniettemin een erg leuke en leerzame tijd, waaruit ik enkele essentiële vaardigheden voor de rest van mijn leven meeneem. Hierbij denk ik niet alleen aan kennis van methodologie en statistiek, maar ook aan de diverese technieken voor het torpederen van een stapel plastic bekertjes met een elastiekje. Willem, jij hebt een zeer grote bijdrage aan de totstandkoming van dit proefschrift geleverd. Geen idee waar ik het over heb? Een hint: het was in Nürnberg en het had iets maken met een slaapgedepriveerde Bart, een aanstormende bus en een perfect getimede ruk aan de kraag van mijn jas.

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Bedankt voor jullie interesse, voor de ontspanning tijdens diverse vakanties, en voor de hulp, als weer eens bleek dat een gezin bestaande uit twee promoverende artsen en twee jonge kinderen toch wel erg druk kan zijn.

Mijn broers, Edwin, Sander en Peter,
Edwin, als je op de lagere school zit, is een oudere broer die geneeskunde studeert echt heel stoer. Jij hebt me dan ook geïnspireerd om arts te worden. Ik ben blij dat je dat gedaan hebt, want het is echt een schitterend beroep. Sander, nadat jij gepromoveerd was, kon ik natuurlijk niet achterblijven. Het resultaat heb je nu voor je. Je hebt me in het begin ook enorm geholpen met tips voor "database-management". De rest van de promotie

werd daardoor eigenlijk gewoon een kwestie van een paar drukken op de knop. Peter, bedankt voor je relativerende kijk op de zaken.

Mijn ouders,
Zonder jullie steun was er nooit een proefschrift gekomen. Jullie vonden een goede opleiding altijd erg belangrijk. Als scholier of als student is dit belang niet altijd even duidelijk. Op die momenten wisten jullie me altijd weer te motiveren, altijd ondersteunend en nooit dwingend. Als je op de universiteit eenmaal weet wat ze van je verwachten, dan wordt afstuderen een trucje. Drie zonen op de universiteit laten afstuderen is daarentegen een prestatie van formaat.

Lonneke, Tom en Sofie,
Lonneke, meestal wordt hier tegen de partner gezegd, dat het hele proefschift zonder hem of haar niet mogelijk was geweest. Ik geef toe dat jouw motivatie en werklust zeker een voorbeeld voor mij waren, maar het zijn toch vooral de ijsjes in de "Kruidtuin", het racen over een Boliviaanse zoutvlakte, de helikoptervlucht over de Himalaya en natuurlijk onze geweldige kindjes, die zonder jou niet mogelijk waren geweest. Heel veel dank hiervoor. Tom en Sofie, jullie zijn echt heerlijke kinderen, daar hoeft je niet voor gepromoveerd te zijn om dat te zien.

CURRICULUM VITAE

Bart Smits is geboren op 14 mei 1976 in Deurne. In 1994 behaalde hij zijn VWO diploma aan het Sint Willibrord gymnasium te Deurne. In hetzelfde jaar begon hij aan de geneeskundeopleiding aan de Katholieke Universiteit Leuven, België, om in 1995 over te stappen naar de geneeskundeopleiding aan de Katholieke Universiteit Nijmegen. Tijdens deze studie deed hij wetenschappelijk onderzoek naar de immuundeficiëntie agamma-globulinemie (o.l.v. prof. J. van der Meer) en later in het kader van zijn wetenschappelijke stage naar de invloed van aangeboren stressgevoeligheid op vaattonusregulatie (o.l.v. prof. P. Smits). In 2001 behaalde hij zijn artsexamen en in 2002 begon hij bij de afdeling Neurologie in het UMC St. Radboud te Nijmegen, aanvankelijk als assistent geneeskunde niet in opleiding en later dat jaar als assistent geneeskunde in opleiding (opleider prof. G. Padberg). In 2003 startte hij onder leiding van prof. B. van Engelen en prof. L. van den Heuvel het onderzoek wat resulteerde in dit proefschrift. Na het voltooien van zijn opleiding was hij als neuroloog werkzaam in het Sint Franciscus Gasthuis, het Havenziekenhuis en vanaf medio 2011 in het Maasstadziekenhuis, allen te Rotterdam. Bart woont samen met Lonneke Staals. Ze hebben twee kinderen, Tom en Sofie.

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