The phenotypic spectrum of polymerase gamma (POLG) disease from birth to late adulthood

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Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2020

UNIVERSITY OF BERGEN

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Date of defense: 25.09.2020

Year: 2020

Title: The phenotypic spectrum of polymerase gamma (POLG) disease from birth to late adulthood

Name: Omar Hikmat

Print: Skipnes Kommunikasjon / University of Bergen

CONTENT

1. PREFACE

1.1 ACKNOWLEDGEMENTS

This thesis would not have been possible without the invaluable contributions of my supervisors, the national and international collaborators, and my colleagues. I wish to express my sincere gratitude to:

My supervisor Professor Laurence A. Bindoff for introducing me to the field of clinical research and to this fascinating world of neuro-genetics. You are a never-ending source of ideas, your stimulating discussion and constructive criticism and outstanding guidance throughout my PhD journey have helped me to stand gradually on my own and produce in this complex and difficult field of medicine. Your excellent supervision has helped me to acquire knowledge not only in the field of clinical research, but also in clinical neurology. Laurence, it is really a privilege to be one of your students. I am also grateful to know you as a person; you are very caring and supportive and not least have a very good sense of humour. I have really enjoyed our meetings, travels and the time we have spent together; thank you and I am looking forward to continuing our success.

My co-supervisor Professor Charalampos Tzoulis for your invaluable support particularly in the early stage of my PhD project, and for your help in establishing the National Norwegian POLG Registry.

Professor Shamima Rahman for your unending enthusiasm and support. Your scientific input and your problem-solving and positive attitude have been invaluable, particularly in the difficult phases of my PhD project. Thank you for your unlimited generosity during all of my visits to London and for including me in the scientific community of Great Ormond Street Hospital and considering me as a member of your research group. I owe you my deepest gratitude.

All the members of the Mitochondrial Clinical Research Network (MCRN) for their collaboration, discussion and scientific input. Special thanks to: Professor Niklas Darin, Dr. Karin Naess, Dr. Martin Engvall, Dr.Elsebet Ostergaard, Dr. I.F.M de Coo, Dr. Pirjo Isohanni, Professor Johanna Uusimaa, Dr. Leticia Pias-Peleteiro for their active

contribution to the POLG database and being actively involved in my PhD-related studies and publications.

The national collaborators of the POLG study; Professor Claus Klingenberg, Dr. Magnhild Rasmussen, Professor Chantal ME Tallaksen and Professor Eylert Brodtkorb for their ongoing contribution in recruiting patients to the National Norwegian POLG Registry and being actively involved in the studies and publications related to this thesis.

The Genetic Department, Haukeland University Hospital for recruiting patients to the National Norwegian POLG Registry.

Professor Geir Egil Eide for his help with some of the statistical analysis.

My colleagues Dr. Sura Aziz, Dr. Erling Tjora and Novin Balafkan for helping me in editing some of the figures and the tables related to my papers and this thesis, and in running some of the statistical analysis.

The former director of the paediatric department, Dr. Britt Skadberg for her kind support and for giving me the opportunity to do my research beside the clinical work.

The director of the paediatric department, Professor Ansgar Berg, and the head of the clinic, Dr. Karin Birgitta Tylleskär, for their positive attitude, being supportive, flexible, and for facilitating my research and creating such a good atmosphere for both research and clinical work.

Western Norway Health Authority (Helse-Vest) for the financial support.

My former tutor during my paediatric training, Dr. Hallvard Reigstad, for his never-ending support. Your knowledge covers every aspect of the paediatric medicine.

My tutor in paediatric neurology, Professor Kristian Sommerfelt, for introducing me to the amazing field of paediatric neurology. I really admire your clinical expertise and your compassion for the patients and their families.

My colleagues and friends in the paediatric neurology section, Dr. Ånen Aarli, Dr. Nanna Margrethe Mjellem, Dr. Katrine Leversen, Dr. Silja Griffithis, Dr.Lene Aase, Dr.

Gudrun Henriksen and Dr. Kathinka Aslaksen, for your flexibility, understanding and support.

Sincere thanks to my family, especially my parents, my great source of inspiration. You always encouraged me to work hard and never give up, I am forever grateful. It was my father's wish to see me receiving my PhD, and so I dedicate my thesis to his memory.

My beloved daughters, Lara and Leen, "the salt and the pepper of my life"; I feel so lucky to have such wonderful daughters like you. Thank you for filling our lives with joy and happiness and thank you for your kindness and understanding.

My beautiful wife Sura, you are the best thing that happened to me, your beautiful face is the remedy of all my stress and your love gives me the power to carry on. Thank you for being alone with Lara and Leen for considerably long periods during my PhD study, your ability to carry the extra weight on your shoulders while I am doing my PhD has been essential. Thank you for your understanding, patience, positive attitude, encouragement, and for being with me every step in my life, thank you for your love.

1.2 SCIENTIFIC ENVIRONMENT

The present PhD project was carried out at the Centre for Mitochondrial Medicine & Neurogenetics, Department of Clinical Medicine (K1), University of Bergen, Department of Neurology and the Department of Paediatrics and Adolescent Medicine, Haukeland University Hospital.

The project was conducted in collaboration with the members of the Mitochondrial Clinical Research Network (MCRN) and in close collaboration with the Mitochondrial Research Group, Genetics and Genomic Medicine Program at UCL Great Ormond Street Institute of Child Health, London, United Kingdom.

1.2.1 National collaborating centres include:

- Department of Paediatric and Adolescent Medicine, University Hospital of North Norway and Paediatric Research Group, Department of Clinical Medicine, UiT-The Arctic University of Norway, Tromsø, Norway.
- Women and Children's Division, Department of Clinical Neuroscience for Children and Department of Neurology at Oslo University Hospital, and Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway.
- Department of Neurology and Clinical Neurophysiology, St. Olav's University Hospital and Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway.

1.2.2. International collaborating centres include:

- Mitochondrial Research Group, UCL Great Ormond Street Institute of Child Health, and Metabolic Unit, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK.
- Centre for Inherited Metabolic Diseases, Department of Medical Biochemistry and Biophysics, and Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.
- Department of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.

- Department of Neurology, Medical Spectrum Twente, Enschede, and Department of Genetics and Cell Biology, University of Maastricht, Maastricht, The Netherlands.
- Department of Neurology, Sant Joan de Déu Children´s Hospital, Barcelona, Spain.
- Department of Paediatric Neurology, Children's Hospital, Helsinki University Hospital and Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland.
- PEDEGO Research Unit and the Department of Paediatric Neurology, Clinic for Children and Adolescents, Medical Research Centre, Oulu University Hospital, Oulu, Finland.
- Department of Paediatrics, The Queen Silvia Children's Hospital, University of Gothenburg, Gothenburg, Sweden.

1.3 SUMMARY OF THE THESIS

Variants in *POLG*, the gene encoding the catalytic subunit of DNA-polymerase gamma (polγ), the enzyme that replicates and repairs the mitochondrial genome, are among the most common causes of inherited mitochondrial disease. The clinical phenotypes of POLG disease are overlapping and extremely heterogeneous, making early clinical recognition challenging. The aim of my PhD project was to study the clinical spectrum and natural course of POLG disease in a large cohort of patients in order to provide a reliable clinical classification that was useful in both paediatric and adult populations, and to identify robust diagnostic and prognostic biomarkers which could facilitate early diagnosis and/or predict the prognosis.

Multinational, retrospective studies of individuals recruited from 13 centres in seven European countries (Norway, Sweden, Denmark, Finland, Netherlands, Spain and the United Kingdom) were performed. Clinical, laboratory, neurophysiological, neuro-imaging, and genetic data were systematically collected using a standardized electronic, web-based clinical record form.

The results of this project provide clear evidence that the clinical features of POLG disease are a continuum, i.e. the same spectrum of symptoms/features is found in all age groups. This allowed us to classify POLG disease more simply than the earlier attempts which only generated a plethora of syndromes with overlapping features.

The project provides also an extensive phenotypic characterisation of patients with early onset disease and demonstrated the breadth of clinical manifestations and natural history of the disease in this age group. Highlighting the existence of POLG disease without seizures will improve diagnosis of those with early onset disease.

The study cohort included individuals with disease onset from birth to late adulthood; this enabled us to study the clinical spectrum of the disease through all the ages. We could identify clear phenotypic and prognostic differences by grouping the patients simply using age of onset to: early onset, juvenile and adult, and late onset disease. We believe that our simplified classification will facilitate early clinical recognition, guide the investigation and predict the prognosis of the disease.

Further, the results of this project showed that POLG disease is associated with blood brain barrier dysfunction and that the presence of raised cerebrospinal fluid protein/albumin can be used as a biomarker both for early diagnosis and to predict those who will be at risk to develop epilepsy. Moreover, the project revealed, for the first time, that anaemia is a feature of POLG disease, and the presence of anaemia is associated with significantly worse survival and can be used as a predictor for poor prognosis.

1.4 LIST OF PUBLICATIONS

Paper I

Hikmat O, Tzoulis C, Chong WK, Chentouf L, Klingenberg C, Fratter C, Carr LJ, Prabhakar P, Kumaraguru N, Gissen P, Cross JH, Jacques TS, Taanman JW, Bindoff LA, Rahman S. The clinical spectrum and natural history of early-onset diseases due to DNA polymerase gamma mutations. Genet Med. 2017 Nov;19(11):1217-1225. doi: 10.1038/gim.2017.35. Epub 2017 Apr 27.

Paper II

Hikmat O, Naess K, Engvall M, Klingenberg C, Rasmussen M, Tallaksen CME, Brodtkorb E, Ostergaard E, de Coo I.F.M, Pias-Peleteiro L, Isohanni P, Uusimaa J, Darin N, Rahman S, Bindoff LA. Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset; studies using a cohort of 155 cases. J Inherit Metab Dis. 2020; 43:726-736. doi: 10.1002/jimd.12211. Epub 2020 May 12.

Paper III

Hikmat O, Naess K, Engvall M, Klingenberg C, Rasmussen M, Tallaksen CME, Brodtkorb E, Fiskerstrand T, Isohanni P, Uusimaa J, Darin N, Rahman S, Bindoff LA. Elevated cerebrospinal fluid protein in POLG-related epilepsy: Diagnostic and prognostic implications. Epilepsia. 2018 Jun 19. doi: 10.1111/epi.14459.

Paper IV

Hikmat O, Tzoulis C, Klingenberg C, Rasmussen M, Tallaksen CME, Brodtkorb E, Fiskerstrand T, McFarland R, Rahman S, Bindoff LA. The presence of anaemia negatively influences survival in patients with POLG disease. J Inherit Metab Dis. 2017 Nov; 40 (6):861-866. doi: 10.1007/s10545-017-0084-9. Epub 2017 Sep 1.

1.5 LIST OF ABBREVIATIONS

AD: Autosomal dominant.

ADP: Adenosine diphosphate.

adPEO: autosomal dominant chronic progressive external ophthalmoplegia.

AED: Antiepileptic drug.

ALAT: Alanine aminotransferase.

AR: Autosomal recessive.

ASAT: Aspartate aminotransferase.

ATP: Adenosine triphosphate.

BBB: Blood brain barrier.

CFL: Cortical focal lesion.

CNS: Central nervous system.

CSF: Cerebrospinal fluid.

DNA: Deoxyribonucleic acid.

dNTP: Deoxyribonucleotide triphosphate.

eCRF: electronic-Case Report Form.

EEG: Electroencephalogram.

EPC: Epilepsia partialis continua.

FAD: Flavin adenine dinucleotide.

$FADH_2$: Reduced flavin adenine dinucleotide.

FDG-PET: ^{18}F fluoro-deoxy-glucose positron emission tomography.

FGF21: Fibroblast growth factor 21.

GDF15: Growth differentiation factor 15.

MCHS: Myocerebrohepatopathy spectrum.

MELAS: Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes.

MEMSA: Myoclonic epilepsy, myopathy, sensory ataxia.

MNGIE: Mitochondrial neurogastro-intestinal encephalomyopathy.

MRI: Magnetic resonance imaging.

MRS: Magnetic resonance spectroscopy.

mtDNA: mitochondrial DNA.

mtSSB: mitochondrial single-strand binding protein.

NAD^+: Nicotinamide adenine dinucleotide.

NADH: Reduced nicotinamide adenine dinucleotide

nDNA: Nuclear DNA.

NF-L: Neurofilament light chain.

OXPHOS: Oxidative phosphorylation.

PEO: Progressive external ophthalmoplegia.

POLG: The catalytic subunit of Pol γ.

Pol γ: Polymerase gamma.

RBC: Red blood cell.

RC: Respiratory chain.

SE: Status epilepticus.

tRNA: Transfer RNA.

2. INTRODUCTION

2.1 HISTORICAL OVERVIEW

Mitochondria were first described as intracellular structures with vital cellular functions by Altmann in 1890. The name mitochondria was first introduced in 1898 and originates from The Greek “mitos” (threads) and “chondros” (granules)(1). The early recognition of the nature of cell respiration started in the 1910s and the reconstitution of the respiratory chain was first described early in the 1960s (1-3). Mitochondria were for the first time linked to human disease in 1962 by the Swedish endocrinologist Rolf Luft who described a young woman with hyper-metabolic syndrome and biochemical and histological findings suggesting mitochondrial dysfunction (4). In 1963 it was shown that mitochondria have their own genome, mitochondrial DNA (mtDNA) (5, 6). Initially, it was thought that variants in mtDNA were the most common cause of human mitochondrial disease (7, 8). However, it was subsequently shown that the respiratory chain is under dual genetic control, mtDNA and nuclear DNA (nDNA), and a new class of mitochondrial disease emerged: disorders of nuclear-mitochondrial intergenomic cross talk (9), which is becoming the most common form of mitochondrial disease (10).

Almost four decades have passed since the discovery that mtDNA is inherited exclusively from the maternal side (11) and mtDNA point mutations (12) or deletions (13) can cause human disease. Since then, a growing number of mtDNA defects and variants in nuclear genes encoding proteins essential for mitochondrial structure and function have been identified and associated with human disease both in paediatric and adult populations. Advances in the laboratory diagnostic methods, including in recent years whole-exome sequencing, have enhanced this process. Approximately 1500 genes are currently known to encode the mitochondria-related proteins (14) and approximately 300 of those are known to cause a disease (http://www.mitomap.org). It is now well established that mitochondrial disorders due to variants in either mtDNA or nDNA genes are the most common inborn error of metabolism with an estimated prevalence of >1:5000 (15).

Mitochondria have received increasing attention in recent decades as evidenced by the expanding number of mitochondrial related publications compared to other organelles such as the nucleus, Golgi apparatus and endoplasmic reticulum (16) . This reflects the increasing relevance of mitochondrial disease in modern medicine.

Pathogenic variants in any of the mtDNA genes encoding for the 13 subunits of the oxidative phosphorylation (OXPHOS) complexes, the 22 mitochondrial tRNAs, two rRNAs, or in any of the nuclear genes encoding the rest of the approximately 1500 proteins essential for mitochondrial structure and function, may result in mitochondrial dysfunction and disease. Clinically, affected individuals can present with a spectrum of heterogeneous phenotypes and disease onset at any time during their life span, and often with multi-organ involvement. However organs with high energy demand, such as the brain, heart and the skeletal muscles, are the most vulnerable (17).

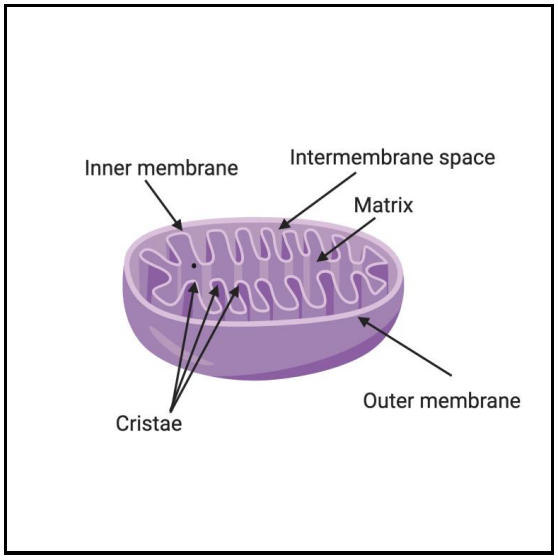
Despite the advances in diagnostic methods and better understanding of mitochondrial biology, early clinical recognition of patients with mitochondrial disorders is still challenging, demonstrating the translational gap between the advances in science and clinical practice.

2.2 MITOCHONDRIAL STRUCTURE AND FUNCTIONS

Mitochondria are complex organelles present in the cytoplasm of almost all human cells, apart from mature erythrocytes. Each mitochondrion is enclosed by two highly specialized, phospholipid membranes known as the outer and the inner membranes. These create two separate compartments; the inter-membrane space and the matrix. The outer mitochondrial membrane contains many porin molecules forming large aqueous channels which are freely permeable to all molecules of 5000 Daltons or less. These molecules can enter into the inter-membrane space, but most of them cannot proceed further into the matrix as the inner membrane is far less permeable and highly specialized, allowing only very small molecules to cross into the matrix. It is impermeable to most charged and hydrophilic substances such as ADP, ATP and pyruvate. The inner membrane is highly convoluted and forms specialised folds known as cristae, which provide an increased surface area available for chemical reactions and

higher capacity for ATP production. The inner membrane also contains transport proteins to allow molecules to cross into the matrix, however, short chain fatty acids appear able to permeate the inner mitochondrial membrane without specialized transport mechanisms (18). The matrix contains mtDNA, mitochondrial ribosomes, tRNA and proteins involved in many biochemical pathways, including the Krebs cycle (also known as the citric acid cycle, or tricarboxylic acid), and beta-oxidation of fatty acids (19) (Figure 1).

Figure 1. Mitochondrial structure.



Mitochondria are cytoplasmic organelles with an inner and outer membrane, between which is the intermembrane space. The mitochondrial matrix lies within the inner membrane which is highly convoluted to form cristae. (Created with Biorender.com).

Mitochondria are highly dynamic organelles and continuously change their size, shape and position, often forming networks through the opposing processes of fusion and fission (20). This machinery has an important quality control function as fusion contributes to mitochondrial maintenance, and fission allows the elimination of dysfunctional mitochondria. Dynamin-related GTPases on the outer mitochondrial membrane (mitofusins, MFN1 and MFN2) and inner mitochondrial membrane (OPA1)

are involved in the control of the fusion process, while the cytosolic soluble dynamin-related protein 1 (DRP1) is involved in the fission process. (21, 22).

The main function of the mitochondria is energy production in the form of ATP via the process of oxidative phosphorylation (OXPHOS) carried out by the respiratory chain, an enzyme pathway consisting of five multi-subunit protein-complexes located within the inner mitochondrial membrane. This pathway consists of the electron transport chain (complexes I -IV) and ATPase (complex V). Thirteen of the respiratory chain subunits are encoded by mtDNA while the remaining are encoded by nDNA (23).

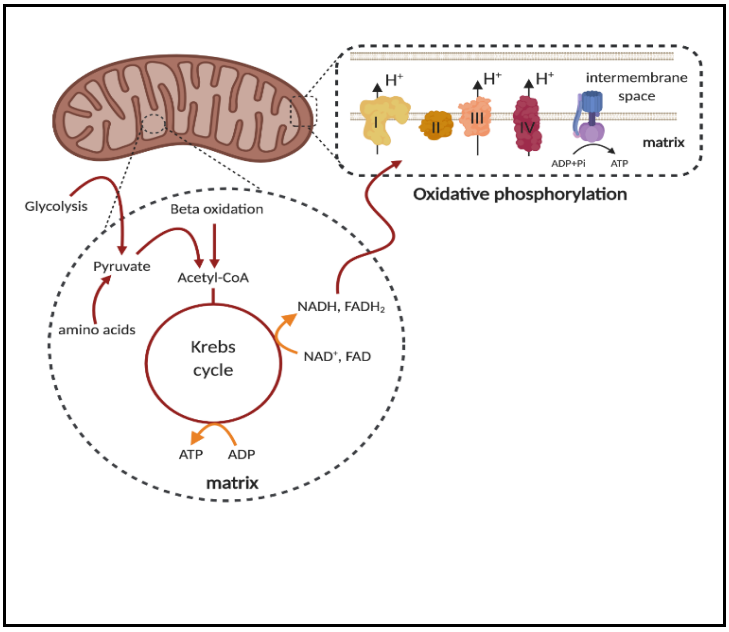
Mitochondria use both pyruvate and fatty acids as fuel. Glucose is metabolised to pyruvate by the process of glycolysis and then it is either converted to lactate or enters the mitochondrial matrix where it is oxidised by the pyruvate dehydrogenase (PDH) complex to form acetyl-CoA. This acetyl–CoA is then metabolized via the Krebs cycle, a process that generates NADH from NAD^+ and $FADH_2$ from FAD.

The metabolism of fatty acids, which includes fatty acid oxidation or beta (β)-oxidation, starts in the cytoplasm, where fatty acids are first converted into fatty acyl-CoA molecules. The fatty acyl-CoA combines with carnitine to form a fatty acyl carnitine molecule, which is an important step in the transport of the fatty acid across the mitochondrial membrane. Once inside the mitochondrial matrix, the fatty acyl carnitine molecule is converted back into fatty acyl-CoA and then into acetyl-CoA by repeated cycles of β-oxidation. The newly formed acetyl-CoA enters the Krebs cycle. In contrast to long chain fatty acids ($>C_8$) that are activated to acyl-CoA in the cytosol and transferred to the mitochondrial matrix by the carnitine shuttle, short and medium chain fatty acids, at least those of carbon atom number up to C_8, permeate the inner mitochondrial membrane in the non-esterified form and are activated to their CoA-derivatives in the mitochondrial matrix (18, 19).

Complex I (NADH ubiquinone oxidoreductase) reoxidises NADH and the electrons released by this process shuttle to coenzyme Q_{10} (CoQ_{10}, ubiquinone). Similarly, complex II (succinate ubiquinone oxidoreductase) oxides $FADH_2$ and provides electrons to CoQ_{10}. CoQ_{10} moves along the inner membrane, carrying the electrons from complex

I and II to complex III (ubiquinol cytochrome *c* oxidoreductase) which subsequently transfers the electrons from reduced CoQ_{10} (ubiquinol) to cytochrome *c*. These electrons will finally be donated to molecular oxygen (O_2) via complex IV (cytochrome *c* oxidase) with the formation of water (H_2O). During this process hydrogen cations (protons) are pumped from the mitochondrial matrix to the intermembranous space creating an electrochemical gradient across the mitochondrial inner membrane. The movement of these protons back into the matrix through complex V (ATP-synthase) provides the energy that generates ATP from ADP (24, 25) (figure 2).

Figure 2: Mitochondrial metabolism and ATP production through the process of OXPHOS (Created with Biorender.com).



Besides energy production, mitochondria play an important role in several processes essential for maintaining cellular homeostasis including; iron-sulphur biogenesis, porphyrin and pyrimidine biosynthesis, calcium buffering and phospholipid metabolism (26, 27). Moreover, mitochondria are considered to be the major producers of reactive oxygen species (ROS), which are directly involved in programmed cell death (apoptosis) (28-30).

2.3 THE GENETICS OF MITOCHONDRIAL DISEASE

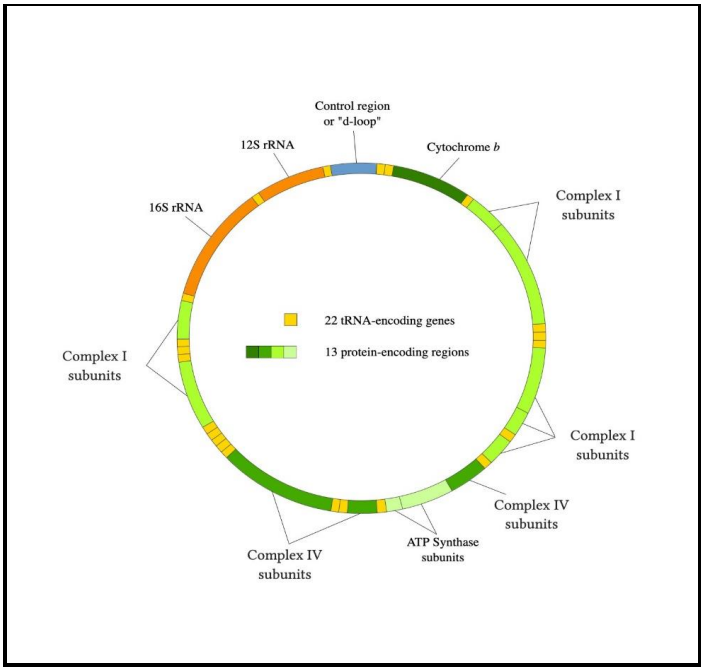
The mitochondrial respiratory chain is under dual genetic control. Components of four of the five complexes contain subunits encoded by mtDNA and nDNA. This means that the cross-talk between these two genomes is essential for the function of the respiratory chain (9). Primary mitochondrial disease can be caused by pathogenic variants in either mtDNA or nDNA, and manifests any mode of inheritance including maternal, autosomal recessive, autosomal dominant and X-linked (31).

2.3.1 Mitochondrial DNA

A. What is mitochondrial DNA (mtDNA)?

Mitochondria are the only organelles, other than the nucleus, that contain genetic information in the form of the mtDNA. Human mtDNA is a 16.5 kb, double stranded, circular molecule encoding 37 genes (Figure 3). Thirteen of these genes encode OXPHOS subunits, the remaining 24 encode RNAs, two ribosomal RNAs (rRNA) and 22 transfer RNAs (tRNA), that are needed for synthesis of mtDNA encoded proteins (32). Purine and pyrimidine content are unequally distributed between the two mtDNA-strands resulting in a purine rich (heavy strand) and purine poor (light strand). MtDNA is a compact genome and contains little non-coding sequence apart from the displacement loop (D-Loop), which contains the promoters for transcription of both heavy and light strands (33, 34).

Figure 3: Human mtDNA.



Human mtDNA, double stranded, circular and consists of 16 569base pair. (Adapted and modified from https://en.wikipedia.org/wiki/Mitochondrial_DNA, created with biorender.com).

B. Replication of mtDNA

Replication of mtDNA is independent of the cell cycle (35, 36). The exact mechanism is still unclear and this has led to different theories: the asynchronous strand displacement and the strand coupled models (33, 34, 37). The *POLG* gene encodes the catalytic subunit of the mitochondrial DNA polymerase gamma (pol γ) which, together with two other proteins (a helicase called Twinkle and the mitochondrial single stranded binding protein (mtSSB), is required for mtDNA replication (38, 39). POLG also contains an exonuclease function that proofreads newly synthesised DNA and which is important for mtDNA repair.

C. Inheritance of mtDNA

Mitochondrial DNA is thought to be inherited exclusively from the mother (11). One case of paternal transmission of mtDNA mutation has been reported in a patient with myopathy (40) and paternal mtDNA sequences have been identified in next generation sequencing studies of healthy individuals (41).

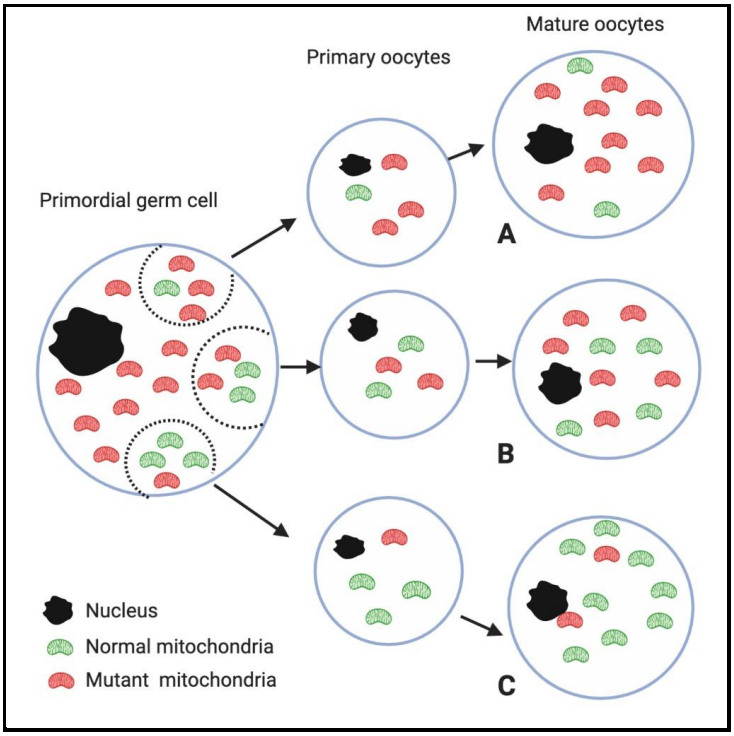
*D. MtDNA is a multicopy genom*e

Each cell contains multiple copies of mtDNA varying from approximately 100 in a sperm to more than 100,000 in a mature oocyte (42). Normally, all mtDNA copies within a cell have the same sequence, a situation called *homoplasmy*. Pathogenic variants of mtDNA can affect some or all mtDNA copies. The situation in which there are two populations of mtDNA, one mutated and one wild-type is called *heteroplasmy* (43). Whether a phenotype manifests or not depends on there being a critical proportion of mutant mtDNA present in the cell; this is called the threshold, and this varies depending on the mutation and is usually between levels of 60-90 % mutated mtDNA (44).

During the formation of the female germline, which occurs during early fetal development, the number of copies of mtDNA in the female primordial germ cell falls. The exact number is unclear but estimated to be in the order of 100-200 copies. Expansion of the copy number occurs during oocyte maturation such that the mature oocyte contains 100,000 copies. This contraction of the number of mtDNA copies with subsequent expansion is the basis of the bottleneck and the reason why mtDNA mutation heteroplasmy level can change dramatically from one generation to the next (45) (Figure 4).

Following fertilisation, varying amounts of wild-type and mutant mtDNA are randomly segregated to each of the daughter cells and since there is initially no replication, there is another decrease in mtDNA copy number per cell. Lastly, not all the cells in the blastocyst are destined to go into the fetus. Thus, if there is any cell to cell variation in the level of heteroplasmy, this can lead to different levels in the tissues that develop from the different germ layers. (45).

Figure 4: The mitochondrial genetic bottleneck.



The mitochondrial genetic bottleneck. Reduction followed by rapid replication of mtDNA copy number occurs during the process of oocyte maturation. This restriction and then amplification leads to variable level of mutant mtDNA being passed from one generation to the next. A: mature oocyte with high level of mutation (affected), B: mature oocyte with a medium level of mutation (mildly affected), C: mature oocyte with low level of mutation (probably not affected) (created with Biorender.com).

E. MtDNA and disease

The majority (80%) of primary mitochondrial disorders in adults and about 25% of mitochondrial disorders in the paediatric population are caused by pathogenic variants in mtDNA (46, 47) and more than 300 pathogenic mtDNA variants have been described (http://www.mitomap.org). Mutations in mtDNA can affect specific OXPHOS proteins or tRNA/rRNA leading to disruption of the synthesis of the mitochondrial proteins (48). Mutations can be divided to large-scale rearrangement (duplications/deletions) or point

mutations (49). The genotype-phenotype correlation is generally poor and this reflects several factors not least the different level of heteroplasmy.

Large-scale mtDNA rearrangement syndromes: While the size of the deletions can vary from small to several kilobases, duplications are usually large. Rearrangements usually affect several genes including protein coding and tRNA genes. Syndromes associated with the rearrangement of mtDNA include: Pearson syndrome (PS), which is an early onset and often life-threatening condition associated with transfusion-dependent sideroblastic anaemia and exocrine pancreatic dysfunction; Kearns-Sayre syndrome (KSS), a childhood or juvenile onset multi-systemic syndrome characterized by PEO, ptosis, mitochondrial myopathy with ragged red fibres, ataxia and life-threatening abnormalities of cardiac rhythm. Children surviving the pancytopenic phase of PS show evolution of clinical features into early onset KSS; Chronic Progressive External Ophthalmoplegia (CPEO) which may be an isolated paralysis of eye muscles and ptosis or associated with other extra-ocular manifestations such as myopathy, hearing loss, cataract (49-53).

Point mutations of mtDNA. These can be maternally inherited or sporadic (54) and have been found in all mtDNA-encoding genes. Common syndromes include: Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS) often caused by m.3243A>G in *MT-TL1*; Myoclonus Epilepsy with Ragged Red Fibre (MERRF) caused mainly by m.8344A>G in *MT-TK;* and Leber Heredity Optic Neuropathy (LHON) due to m.3460G>A in *MT-ND1*, m.11778G>A in *MT-ND4* or m.14484T>C in *MT-ND6*. Mutations in the *MT-ATP6* encoding subunit 6 of the ATP synthase (complex V) typically give rise to maternally inherited Leigh syndrome or a milder phenotype characterized by neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP), depending on the level of heteroplasmy. Several other clinical manifestations are seen with mutations in cytochrome b of complex III and the mtDNA encoded protein subunits of cytochrome oxidase (49, 53).

2.3.2 Nuclear DNA

Pathogenic variants in the nuclear genome account for the majority of mitochondrial disorders. Approximately 1500 nuclear genes are necessary for proper mitochondrial function and maintenance (14). Only a small proportion of these encode structural subunits of the OXPHOS complexes (Table 1). The majority of nuclear genes encode factors involved in mtDNA maintenance, transcription and translation, or proteins involved in biosynthesis of lipids and cofactors, proteins involved in mitochondrial protein import and dynamics, assembly factors of OXPHOS complexes, enzymes involved in detoxification pathways or factors involved in mitochondrial dynamics, apoptosis, ion transport and protein import (23, 49, 55) .

Table 1: Encoding of OXPHOS complexes

Complex	I	II	III	IV	V	Total
mtDNA encoding subunits	7	0	1	3	2	13
nDNA encoding subunits	37	4	10	11	17	79
Total	44	4	11	14	19	92

Encoding of OXPHOS complexes. Mitochondrial OXPHOS complexes comprise more than 90 proteins, 13 of those encoded by mtDNA while the remaining subunits are encoded by nDNA.

The most frequently affected nuclear gene causing mitochondrial disease is *POLG*, the main focus of this thesis and discussed in detail in the following sections. Other nuclear genes that are known to be associated with disorders of mtDNA stability, and which may mimic POLG disease clinically, are summarized in table 2.

Table 2: Summary of the genes, other than *POLG*, associated with disorders of mtDNA maintenance and stability.

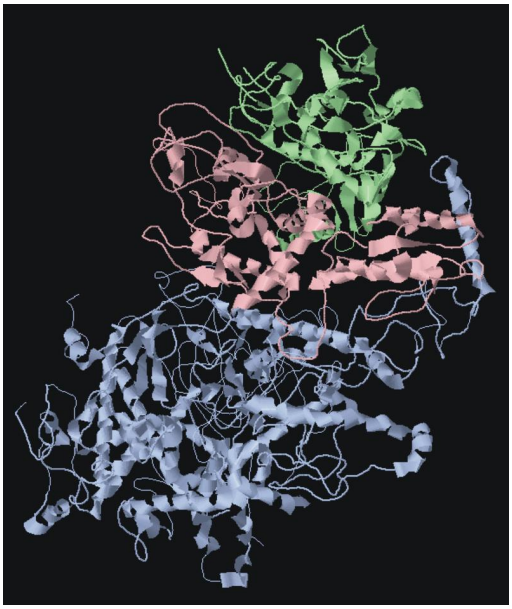
Age of onset	Gene	Pathway	Clinical features
Neonatal, infancy, early childhood	*TWNK*	mtDNA replication	Ataxia, encephalopathy, neuropathy, hepatopathy[56]
	TFAM	mtDNA replication	Hepatopathy[57]
	TK2	dNTP metabolism	Myopathy[58]
	DGUOK	dNTP metabolism	Encephalopathy, hepatopathy[59]
	SUCLA2	dNTP metabolism	Encephalopathy, myopathy, ↑MMA[60]
	SUCLG1	dNTP metabolism	Encephalopathy, myopathy, ↑MMA[61]
	ABAT	dNTP metabolism	Encephalopathy, myopathy, ↑MMA[62]
	RRM2B	dNTP metabolism	Encephalopathy, myopathy[63]
	AGK	dNTP metabolism	Cardiac and skeletal myopathy, cataract[64]
	MPV17	dNTP metabolism	Encephalopathy, hepatopathy, myopathy, neuropathy[65]
	OPA1	Mitochondrial dynamics	Optic atrophy, neuropathy, spinocerebellar degeneration[66]
	GFER	Mitochondrial dynamics	Myopathy, developmental delay, cataract, hearing loss[67]
	FBXL4	Mitochondrial dynamics	Encephalopathy, myopathy[68]
	MGME1	mtDNA repair	Progressive ataxia[69]
Adolescence, early adulthood	*RNASEH1*	mtDNA replication	Encephalopathy, myopathy[70]
	MGME1	mtDNA repair	Myopathy, PEO[71]
	DNA2	mtDNA repair	Myopathy[72]
	TK2	dNTP metabolism	Ophthalmoplegia, myopathy[58]
	DGUOK	dNTP metabolism	Myopathy[59]
	TYMP	dNTP metabolism	Gastrointestinal dysmotility, encephalopathy, myopathy[73]
	RRM2B	dNTP metabolism	Gastrointestinal dysmotility, encephalopathy, myopathy[74]
	SLC25A4	dNTP metabolism	Myopathy[75]
	MPV17	dNTP metabolism	Neuropathy, myopathy[76]
	OPA1	Mitochondrial dynamics	Optic atrophy[77]
	AFG3L2	Mitochondrial dynamics	Spinocerebellar ataxia, progressive spasticity, dystonia[78]
	SPG7	Mitochondrial dynamics	Progressive spastic paraplegia[79]
	MFN2	Mitochondrial dynamics	Optic atrophy and neuropathy[80]
Adulthood	TWNK	mtDNA replication	AD ophthalmoplegia, myopathy[56]
	TOP3A	mtDNA replication	AR ophthalmoplegia[81]
	RRM2B	dNTP metabolism	AD/AR ophthalmoplegia[74]
	SLC25A4	dNTP metabolism	AD ophthalmoplegia[82]

AD: autosomal dominant, AR: autosomal recessive, MMA: methylmalonic acid, PEO: progressive external ophthalmoplegia. The table is adapted and updated from REF(83).

2.4 MITOCHONDRIAL DNA POLYMERASE GAMMA (pol γ)

Polymerase gamma (pol γ) is the only DNA polymerase within the mitochondrion of animal cells that replicates and repairs the mitochondrial genome (84). The enzyme is a heterotrimer (Figure 5) composed of one catalytic subunit (polγA) of 139kDa, encoded by *POLG* on chromosome 15q25, and a dimer of two accessory subunits (polγ B) of 53kDa, encoded by *POLG2* on chromosome 17. While the catalytic subunit is responsible for DNA synthesis and proof-reading the accessory subunit promotes DNA binding and processivity (85).

Figure 5: The structure of the pol γ enzyme

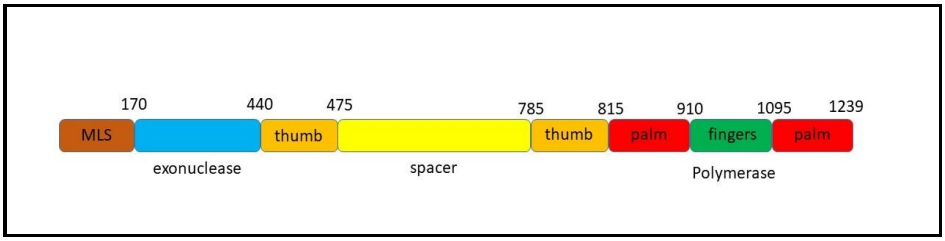


The pol γ enzyme is comprised of catalytic subunit, polγA (blue) and two accessory subunits (green and pink). Protein data bank ID:3iKM (85).

The catalytic core of pol γ comprises: A) a mitochondrial leader sequence (MLS); B) an N-terminal exonuclease domain; C) a C-terminal polymerase domain that contains the

polymerase active site; and D) the spacer (linker region) which separates the exonuclease and polymerase domains (Figure 6).

Figure 6: The linearized structure of the catalytic pol γ subunit



Linear schematic diagram. The catalytic subunit comprises MLS, exonuclease, palm, thumb, fingers and linker domains.

The polymerase domain consists of three sub-domains: A) the palm sub-domain (residues 816-910 and 1096-1239), a positively charged domain stabilizing the negatively charged DNA backbone and containing the polymerase catalytic site and two Mg^{2+} ions, which are vital for formation of the phosphodiester bond between the 3'OH end and the phosphate group of the incoming nucleotide (dNTP); B) The fingers sub-domain (residues 911-1095), which is involved in binding the incoming dNTP substrate; C) the thumb sub-domain (residues 441-475 and 785-815), which forms the major surface of the DNA binding channel. The linker domain (residues 476-785) comprises two sub-domains: the accessory interacting sub-domain, which forms a major hydrophobic contact with the proximal accessory subunits and the intrinsic processivity domain which forms a region for the upstream DNA binding. The exonuclease domain repairs replication errors by 3'-5' excision and is important for fidelity (39, 85, 86).

2.5 POLG DISEASE

2.5.1 General overview

The first pathogenic variant in the *POLG* gene was identified in families with autosomal dominant Progressive External Ophthalmoplegia (adPEO) (OMIN 157640) in 2001 (87). Since then, an increasing number of overlapping phenotypes with wide variation in the age of disease onset have been linked to pathogenic variants in the *POLG* gene.

The true prevalence of POLG disease is unknown, however, it has been estimated to be about 10-25 % of all adult patients with mitochondrial disorders (10, 83). The most common reported variants causing human diseases are c.2243G>C (p.Trp748Ser), c.1399G>A (p.Ala467Thr) and c.2542G>A (p.Gly848Ser) (88-91). The carrier frequency of p.Trp748Ser is estimated to be 1:125 in Finland (92), while for p.Ala467Thr it is estimated at 0.6% in Belgium and 1% in Norway (93, 94). Thus, the combined frequency of p.Trp748Ser and p.Ala467Thr maybe is as high as 1:50 in the Norwegian population (88). Variants in *POLG* have also been described in other non-European ethic groups (95).

The majority of POLG-related phenotypes are inherited as autosomal recessive traits. Pathogenic variants within the *POLG* gene can be homozygous or compound heterozygous. These variants can either decrease the processivity of polymerase gamma, its affinity for native DNA or the speed at which it incorporates nucleotides. Autosomal recessive phenotypes usually present early in life, however, onset late in adulthood has also been reported (96).

Almost all the variants which are associated with adPEO are in the polymerase domain (97). Variants in this domain usually interfere with the translocation and binding affinity to incoming nucleotide, which may result in increased mtDNA replication errors and decreased catalytic function. AdPEO patients usually present in adulthood (90). One patient with the p.Tyr955His mutation and early onset disease with bilateral sensorineural hearing loss, cataract, myopathy, and liver failure has however been previously reported (98).

As would be expected from the disruption of mtDNA caused by *POLG* mutation, almost all organ systems can be affected (table 3), but tissues with high energy demand and organs such as the brain, muscle and liver are particularly susceptible (84, 87, 89, 91, 92, 99-104).

Table 3: Major organ systems which are affected and related clinical manifestations in individuals with POLG disease

Organ system	**Clinical features**
Central nervous system	Seizures / status epilepticus
	Ataxia
	Hypotonia*
	Stroke-like episodes
	Migraine-like headache
	Encephalopathy
	Parkinsonism
Gastro-intestinal	Failure to thrive
	Liver dysfunction/ failure
	Gastro-intestinal dysfunction
Musculoskeletal	Ptosis
	PEO
	Myopathy
	Exercise intolerance
Psychiatric	Psychosis
	Depression
	Hallucination
Peripheral neuropathy	Axonal sensory neuropathy
Vision	Cortical blindness
	Cataract
	Retinopathy
Hearing	Sensorineural hearing loss
Endocrine	Primary ovarian failure
	Primary testicular failure
	Diabetes
Cardiac	Cardiomyopathy

*hypotonia can be central or peripheral.

2.5.2 Major clinical phenotypes

A. MyoCerebroHepatopathy Spectrum (MCHS):

MCHS is a severe and fatal phenotype that usually presents very early in the neonatal period with a triad of myopathy/hypotonia, encephalopathy/developmental delay and liver failure. Other findings include failure to thrive, renal tubular acidosis, cataract, and hearing loss. Diagnostic criteria for MCHS include: absence of hepatic histopathological features of classical Alpers-Hunttenlocher syndrome and at least two of the following: neuropathy, seizures, elevated blood or cerebrospinal fluid (CSF) lactate, dicarboxylic aciduria, renal tubular dysfunction with aminoaciduria, glycosuria or bicarbonaturia, hearing loss, abnormal MRI with either cerebral volume loss, delayed myelination or white matter disease, and either isolated deficiency of complex IV or a combined defect of two or more OXPHOS complexes in skeletal muscle or liver biopsy (84, 90, 100, 102, 105).

B. Alpers-Huttenlocher syndrome (AHS):

Alpers-Huttenlocher syndrome (AHS*)* (OMIM # 203700) is the most frequently reported phenotype in infancy and early childhood (106), although disease onset can occur at any time during childhood or early adulthood (107, 108). AHS was first recognised by Bernard Alpers in 1931, long before its genetic basis was identified, and diagnosis was based on typical neuropathological findings. Subsequently, when the association with liver involvement was described by Petter Huttenlocher, it was called Alpers-Huttenlocher syndrome (109, 110). It was not until the 1980s that the link to mitochondrial dysfunction was made (111) and the link to polymerase gamma was made first in 1999 (99). Pathogenic *POLG* variants causing AHS were first reported in 2004 (112).

AHS is characterized clinically by a triad of progressive encephalopathy with psychomotor regression, refractory epilepsy and liver disease (103). A prodromal phase with mild developmental delay, hypotonia and failure to thrive may occur and an infectious illness may precede the disease onset (113). Focal seizures, commonly evolving into bilateral convulsive seizures, are the most common seizure types with

epileptiform discharges predominantly seen over the occipital regions, at least initially (89, 114). The clinical features of occipital lobe involvement such as visual hallucination, vomiting and headache are less clearly manifested in young children than older children and adults. The majority of the patients develop myoclonic seizures and episodes of epilepsia partialis continua (EPC) and/or generalized status epilepticus (SE). Patients with AHS may also present with refractory SE from which they might never recover (84, 89, 115).

Patients with AHS may develop episodes of acute exacerbation that previously have been called stroke-like episodes (SLEs). These episodes are characterized by acute or subacute neurological dysfunction and are often associated with EPC. The aetiology of these episodes is neuronal dysfunction leading to damage, not vascular occlusion (116). In the older age group, prodromal symptoms such as migraine-like headaches, visual disturbance, and mental changes may occur. Clinically, such episodes are less often reported in children compared with adults, but radiological evidence of cortical lesions is common in both (117).

Hepatic involvement is a major feature of early onset POLG disease and may progress rapidly to end stage liver failure. Affected children with normal, mild, and transient abnormalities of liver function are, however, well recognized (91, 102). Liver failure can occur spontaneously or be triggered by sodium valproate (88, 118). Recovery after transient liver failure and after the discontinuation of sodium valproate has been reported (114). Nevertheless, sodium valproate clearly accelerates the development of liver failure in patients with pathogenic variants in the *POLG* gene and its use is absolutely contraindicated.

C. Myoclonic epilepsy myopathy sensory ataxia (MEMSA):

MEMSA (also referred to as mitochondrial spinocerebellar ataxia with epilepsy - MSCAE) includes a spectrum of manifestations: mainly epilepsy, ataxia and myopathy. Seizures, including SE, can be the first manifestation. Ataxia, which is often present at onset, is usually due to a combined central and evolving sensory polyneuropathy.

Myopathy and ophthalmoplegia develop later, if the patient survives the consequences of the epilepsy (89, 94, 100).

Seizure semiology is similar to AHS with focal seizures, commonly evolving into bilateral convulsive seizures, being the most common seizure type. However, myoclonic seizures, epilepsia partialis continua and generalized SE are frequently reported (119). Occipital lobe features, including visual hallucinations, scotomata, hemianopia and amaurosis, are common (88, 94). Episodes of encephalopathy, previously called SLEs, are more frequently reported than with AHS (117). Vomiting and migraine-like headache with aura occur and may precede the acute episodes. Hepatic dysfunction including liver failure is also a feature of MEMSA and may occur spontaneously or can be triggered by usage of sodium valproate as in AHS (88, 120).

D: Ataxia Neuropathy Spectrum (ANS):

Ataxia Neuropathy Spectrum is characterized by ataxia, neuropathy and encephalopathy that usually presents in adulthood and which can be associated with prolonged survival. The neuropathy may be sensory, motor or mixed and is usually severe enough to contribute to ataxia. The encephalopathy is slowly progressive (100). Individuals with ANS may also develop cognitive decline and psychiatric symptoms including depression. Ophthalmoplegia is more often a late feature and predominant myopathy is rare (91, 92, 101). Liver dysfunction may also occur, ranging from mildly elevated liver enzymes to liver failure. Seizures have also been reported, however, these are not a major feature of ANS (88, 90, 100). Other terms for this phenotype include mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO).

E: Progressive external ophthalmoplegia (PEO):

Pathogenic variants in the *POLG* gene can cause both autosomal dominant and autosomal recessive PEO. Affected individuals suffer from progressive weakness of the extraocular muscles resulting in unilateral or bilateral symmetrical ptosis and loss of eye movements both in the vertical and horizontal directions (87). Other system involvement

such as ataxia, peripheral neuropathy and generalized myopathy occur more frequently in the recessive form (arPEO) than in the dominant form (adPEO), and usually develop later during the disease course (87, 90, 100, 121-123). Other features which can be seen in individuals with adPEO may include sensorineural hearing loss (91), parkinsonism (101, 124), premature menopause (101, 121), male infertility (125), cataract (101) and depression (101).

In addition to the above described major clinical syndromes, variants in *POLG* have been associated with a spectrum of overlapping clinical phenotypes. The terminology used to describe these phenotypes has evolved haphazardly, becoming more complicated and difficult to use in an everyday clinical setting. A summary of the reported POLG related phenotypes with major clinical features and age of onset is summarized in table 4.

Table 4: Summary of the major syndromes associated with pathogenic variants in *POLG* gene reported in literature (126), reprinted with permission.

Phenotype Nomenclatures[reference]	Major clinical features	Age of onset
Myocerebrohepatopathy (MCHS)[90, 105, 106]	Myopathy, hypotonia, developmental delay, encephalopathy, and liver failure.	Neonate, early infancy
Alpers-Huttenlocher Syndrome (AHS)[103, 105, 112]	Encephalopathy, psychomotor regression, refractory epilepsy, liver dysfunction	Infancy, childhood, adolescence
Alpers syndrome [84, 127]	Synonym of AHS	As in AHS
Alpers-Huttenlocher like[128]	Synonym of AHS	As in AHS
Infantile hepatocerebral syndrome[105]	Includes AHS and MCHS	Neonate, infancy, childhood
Infantile mitochondrial DNA depletion syndrome[129]	Includes both AHS and MCHS	Infancy, childhood
Leigh like[129]	Psychomotor retardation, hypotonia, extrapyramidal dysfunction, symmetrical hyperintensities on T2 weighted images in basal ganglia, brain stem, thalamus	Infancy
Mitochondrial Neuro-Gastro-Intestinal Encephalopathy (MNGIE) like[127, 130]	Severe gastrointestinal dysmotility, encephalopathy, ptosis ophthalmoplegia, peripheral neuropathy	Childhood, adolescence Adulthood
Myoclonus, Epilepsy, Myopathy and Sensory Ataxia (MEMSA) [84]	Epilepsy, myopathy, ataxia, liver dysfunction, headache and stroke-like episodes	Adolescence, adulthood
Spinocerebellar ataxia with Epilepsy (SCAE)[84, 100]	Now incorporated under MEMSA umbrella	As in MEMSA
Mitochondrial Spinocerebellar ataxia with Epilepsy (MSCAE)[115]	Now incorporated under MEMSA umbrella	As in MEMSA
Ataxia Neuropathy Spectrum (ANS)[84, 100]	Ataxia, neuropathy, psychiatric symptoms, epilepsy and ophthalmoplegia	Adolescent and adult
Mitochondrial recessive ataxia syndrome (MIRAS)[92]	Now incorporated under ANS umbrella	As in ANS
Sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO)[84]	Now incorporated under ANS umbrella	As in ANS
MELAS like phenotype[131]	Headache, seizures, stroke-like episodes as in MEMSA	Adult
Recessive Charcot-Marie Tooth like[132]	Axonal polyneuropathy, muscle weakness, wasting, tremor, nystagmus, dysarthria and dysdiadochokinesis	Adult
Parkinsonism[101, 124]	Tremor, rigidity, hypo/bradykinesia, balance disturbance	Adult
Autosomal recessive progressive external ophthalmoplegia (arPEO)[87, 100]	Ptosis, ophthalmoparesis, may be associated with ataxia and myopathy	Adult, elderly
Autosomal dominant progressive external ophthalmoplegia (adPEO)[87, 100]	Ptosis, ophthalmoparesis, myopathy, neuropathy, ataxia	Adult-elderly
Chronic progressive external ophthalmoplegia plus (CPEO+)[100]	Synonym of adPEO	Adult-elderly

2.5.3 Diagnosis

A. Clinical awareness:

As with most mitochondrial disorders, the diagnosis of POLG disease is challenging owing to the extreme clinical heterogeneity of presentation, particularly in the paediatric population. There is no single clinical feature that is diagnostic for POLG disease, however, certain groups of symptoms and signs may provide a clue: for instance, disease onset soon after birth with hypotonia, failure to thrive and liver failure, but no seizures, would suggest MCHS. Presentation with acute-onset status epilepticus preceded by headache and visual disturbances and MRI changes suggestive of ischaemia in an adolescent or adults may raise the suspicion of MEMSA. EEG, biochemical and neuro-imaging findings, as described below, may also provide clues to the diagnosis. However none of those are diagnostic for POLG disease.

A detailed family and medical history and a thorough physical examination of central and peripheral nervous systems, as well as evaluation of the possible involvement of other organ systems and assessment of vision, hearing, growth and psycho-motor development are still essential initial steps to achieve the diagnosis.

The diagnosis of POLG disease should not only be considered in individuals presenting with one of the classic POLG phenotypes, since a large proportion of patients with POLG disease do not present with a discrete clinical syndrome (83). POLG disease should also be considered in patients with therapy resistant epilepsy, unexplained encephalopathy or ataxia. The difficulties associated with making the diagnosis of POLG disease are not helped by the unwieldy classification.

B. Biochemical analysis:

There are no specific blood or urine biomarkers for POLG disease. Peripheral blood lactate is a marker for mitochondrial disease generally, however, normal blood lactate does not exclude the diagnosis of POLG disease as elevation can be mild, transient or even absent. Further, inappropriate collection may result in a falsely pathologic high value. Pyruvate/lactate ratio can be used to indicate OXPHOS impairment (127, 133). Both lactate and pyruvate can be measured in the CSF, however, lack of specificity limits their diagnostic value since elevated CSF lactate can occur in other conditions such as seizures of other cause, and CNS inflammation and infection (134).

Fibroblast growth factor 21 (FGF21) and growth differentiation factor 15 (GDF15) are both potential biomarkers for mitochondrial disorders (135, 136). FGF21 has been reported to be elevated mainly in individuals with manifestations in skeletal muscle and appears rarely elevated in POLG disease. Values ranging from 25pg/ml to > 4000 pg/ml have however been reported in patients with AHS and in a single patient with ANS with terminal SE (135). FGF21 and GDF15 can be used as additional biomarkers in the initial diagnostic process and might have prognostic implications as FGF21 and GDF15 values appear to correlate with disease severity (135, 136). Nevertheless, negative results should not exclude the diagnosis. Owing to the observation of normal values in many patients with POLG disease, FGF21 and GDF15 are not considered useful markers to establish the diagnosis. Other findings such as low CSF folate, the presence of CSF oligoclonal bands, and raised CSF NF-L have also been reported, however, none is specific for POLG disease (137-139).

General blood and urine investigations including full blood count, glucose, creatine kinase (CK), liver transaminases, liver and renal function tests and urine analysis should be performed to evaluate the systemic involvement of the disease. Metabolic screening with measurement of plasma amino acid and acylcarnitine profiles and urinary organic acids are helpful to exclude other metabolic/mitochondrial disorders that may mimic POLG disease.

C. Histopathology and respiratory chain enzymology:

Classical mitochondrial muscle pathology findings such as ragged-red and cytochrome oxidase (COX) negative fibres can be seen in patients with POLG disease, however, these can be absent in patients with early onset disease (140). Adolescent patients may have less than 1% of COX-negative fibres, emphasizing that the major manifestations are in the CNS. Further, infants with normal muscle biopsy may have severe pathological liver changes (141). The characteristic hepatic histopathological changes of AHS which are required for the diagnosis are namely the presence of at least two of the following: microvesicular steatosis, bile ductular proliferation, hepatocyte dropout, bridging fibrosis or cirrhosis, collapse of liver cell plates, parenchymal lobular architecture, regenerative nodules and oncocytic changes in scattered hepatocytes not affected by steatosis (106).

In addition to morphological examination, mitochondrial RC analysis in muscle may provide a diagnostic clue. RC enzyme analysis may show isolated enzyme deficiency or combined deficiencies of multiple enzymes, especially in patients with primary muscle involvement, but the results may also be normal. Pathogenic variants in the *POLG* gene demonstrate tissue specific predilections and thus RC enzyme deficiencies may only be identified in clinically affected tissues such as liver or brain (83, 84).

D. Neurophysiological findings:

EEG findings may give a clue to the diagnosis of POLG disease in individuals with seizures. Ictal and inter-ictal occipital epileptic activity are highly suggestive of POLG disease. Focal epileptic discharges over the temporal and frontal regions can however also be observed and multifocal or generalized epileptic activity may occur during seizure evolution and SE (89, 119). Other EEG changes such as rhythmic high amplitude delta (RHADs) and focal slowing are frequently observed (114, 119).

Nerve conduction studies will confirm the presence of peripheral neuropathy particularly in juvenile and adult onset disease (88). POLG disease is mostly associated with axonal changes and a predominantly sensory and some motor component neuropathy. Demyelinating motor neuropathy has also been reported (142, 143).

E. Neuro-imaging:

Brain MRI is the modality of choice and the recommended sequences are T2 fluid-attenuated inversion recovery (FLAIR-T2) and diffusion weighted imaging (DWI). The most prevalent abnormalities are T2 / T2-FLAIR hyperintensities in the cerebral cortex, also known as cortical focal lesions (CFLs), which occur in patients with epilepsy and mainly affect the occipital regions although involvement of other regions such as parietal, temporal and frontal lobes may also occur (119). These changes may evolve over days or weeks and subsequent partial or complete regression may occur (88, 117, 119). Neuroimaging studies may, however, be normal early in the disease course and should not exclude the possibility of POLG disease.

Other neuroimaging abnormalities include lesions in the thalamus, olivary nucleus, and cerebellar white matter which may remain stable throughout the disease course. Generalized brain atrophy develops later during the disease course and is progressive, reflecting the clinical progression of the disease (117, 119, 120).

MRS of CFLs often shows a prominent lactate peak due to impaired aerobic respiration and decreased N-acetyl aspartate concentration which reflects neuronal loss (117). Ictal Cerebral FDG-PET imaging in the acute phase shows increased glucose uptake over these lesions (120).

F. Molecular genetic:

When the diagnosis of POLG disease is suspected clinically, direct sequencing of the *POLG* gene is the most appropriate first-line investigation. The inclusion of *POLG* in next generation sequencing (NGS) gene panels for epilepsy, ataxia and mitochondrial disorders should facilitate early diagnosis. In some regions, screening for the common founder mutations; p.Ala467Thr, p.Trp784Ser, and p.Gly848Ser may still be appropriate (87, 144). The finding of pathological variants in *POLG,* biallelic in recessive disease and heterozygous in dominant disease, establishes the diagnosis.

New variants in the *POLG* gene continue to be identified. Determining the pathogenicity of such variants as ‘pathogenic’, ‘likely pathogenic’ or of ‘uncertain significance’ can be challenging. Useful resources for judging pathogenicity are: the Human DNA

Polymerase Gamma Mutation Database (https://tools.niehs.nih.gov/polg/), and the POLG Pathogenicity Prediction Server (http://polg.bmb.msu.edu/) (145). Further, the identification of new variants either requires extensive laboratory research to prove its pathogenicity or verification by finding more families. Ideally, all pathological variants should be reported in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), however many laboratories are either slow or forget to submit variants. These challenges highlight the difficulties in establishing the definite genetic diagnosis in some individuals with POLG-related phenotypes and new variants in *POLG* gene.

2.5.4 Management

Currently, there are no cures for POLG disease. Clinical management is mainly symptomatic and based on conventional approaches to treat the clinical manifestations and associated complications. As in other rare diseases with high mortality, randomized controlled clinical trials are still lacking and may be challenging to perform as designing a trial is likely to be extremely difficult in the view of clinical heterogeneity, unpredictable clinical course, and spontaneous resolution of features such as SLE or cerebral ischaemia. Understanding the natural history of the disease may help to establish a clinical baseline which can be used in comparison in single-arm clinical trials for potential therapies. Unfortunately, however, earlier descriptions of the natural history of POLG disease were often based on case reports or cohorts with limited numbers of patients, making large cohorts such as ours even more valuable.

A. Management of epilepsy:

The presence of epilepsy is associated with increased mortality and morbidity in patients with POLG disease (88, 146). Early recognition and immediate, aggressive seizure treatment are crucial to improve patient survival. The majority develop therapy resistant seizures (88, 119) and this is particularly true for infants and children. Treatment with a single AED is usually not effective and high dose, multiple AED treatment is often required (119). There are currently no consensus recommendations for epilepsy

management in POLG disease and several AEDs have been used in various dosages and combinations.

No specific single or combined AEDs have been shown to be particularly effective in treating the seizures in POLG disease. AEDs known to be effective in treatment of focal seizures such as oxcarbazepine, carbamazepine, lacosamide and perampanel are appropriate, as focal and focal evolving to bilateral convulsive seizures are among the most common types. Lamotrigine, topiramate and levetiracetam have also been used, alone and in combination with a benzodiazepine such as clobazam or clonazepam. Lamotrigine can worsen mycolonic seizures and should be used with caution (89, 115, 146).

Sodium valproate is absolutely contraindicated due to the risk of acute and progressive hepatic necrosis (88). Transient liver failure with recovery after discontinuation of sodium valproate has been reported (114). If the clinical presentation raises the suspicion of POLG disease, sequencing of the *POLG* gene should be considered before prescribing sodium valproate, particularly in those with status epilepticus of unknown aetiology. (118).

Management of SE is challenging; benzodiazepines, phenytoin and levetiracetam can be used as first line treatment, however, failure to control the seizures is common. In a case of refractory SE, anaesthetic agents as propofol or a barbiturate (pentothal) should be instituted promptly. Propofol should be used with caution due to the risk of propofol infusion syndrome particularly in the paediatric population. Other agents as ketamine (147), magnesium infusion (148) and corticosteroids (114) have been reported to be effective in terminating SE in single cases, however, data available regarding the effectiveness are currently insufficient. Epilepsia partialis continua is generally resistant to pharmacotherapy.

Other non-pharmacological alternatives including ketogenic diet and vagus nerve stimulation have been used (149), but currently, there are insufficient data confirming the benefit of either of these entities in patients with POLG disease. Transcranial direct current stimulation gave promising results in one previously published case (150),

however a recent publication showed it was not effective (151). Palliative functional hemispherectomy can be an option when the short-term benefits outweigh the risk of surgery (152).

There is no clear evidence showing any significant clinical effect of nutritional supplements such as co-enzyme Q_{10}, folic acid, carnitine, L-arginine, EPI-734 or other vitamins, although these are widely used. Further studies need to be performed to investigate the effectiveness of these agents. (153, 154).

B: Gastrointestinal and nutritional:

Feeding difficulties and failure to thrive, mainly in the very young, and vomiting/gastric dysmotility regardless of the age of onset are common gastro-intestinal features of POLG disease (84, 127, 155). Evaluation by a gastroenterologist and dietician and the use of enteral nutrition via gastric tube/gastrostomy should be considered early during the disease course, particularly in young individuals.

C. Liver dysfunction:

Liver dysfunction is a common feature of POLG disease regardless of the age of onset and can range from acute and progressive liver failure to mild/transient elevation of liver enzymes. Liver dysfunction can also occur spontaneously or as a consequence of sodium valproate exposure. Spontaneous resolution of liver failure after exposure to sodium valproate has been reported (156). Close monitoring of the liver function by measuring liver enzymes (AST, ALT, GGT) and other liver function parameters such as ammonia, albumin, bilirubin, prothrombin time, is highly recommended and should be performed routinely at least every 3 to 4 months, especially in infants, children and adolescents.

Liver transplantation remains an option and it has been performed in more 40 patients with POLG disease (83, 157-159). However, there is some controversy around the use of liver transplantation, particularly in early onset disease, due to the aggressive nature of the disease and early death due to neurological decline occurring within one year of the liver transplantation. Survival after liver transplantation in adult onset disease is

better than with early onset disease (88, 90, 160). Thorough evaluation of the ethical aspects and an individualized risk-benefit analysis in all cases, regardless of age, are needed before proceeding to transplantation (160-162).

D. Movement disorders:

Parkinsonism is a feature of late-onset disease (101) and usually occurs together with PEO and peripheral neuropathy. However, early onset parkinsonism was reported in two sisters who also had neuropathy, but not PEO (124). Treatment with L-Dopa (163) appeared to be effective in some cases. Benzodiazepines may reduce the severity of other non-epileptic movement disorders including myoclonus and tremor. In individuals with dystonia, local injections with botulinum toxin and oral/intrathecal baclofen can be useful.

E. Ophthalmological manifestations:

Individuals with POLG disease may develop cortical blindness, nystagmus, ptosis and ophthalmoplegia and should be referred for ophthalmological evaluation. Surgery for ptosis may be considered and may provide some symptomatic relief, although post-operative relapse is frequent.

3. AIMS OF THE THESIS

3.1 GENERAL AIMS

The overarching aim of the work in this thesis was to bridge the translational gap between research and clinical practice in order to facilitate faster and more accurate diagnosis, and impact the management of patients with POLG disease.

3.2 SPECIFIC AIMS

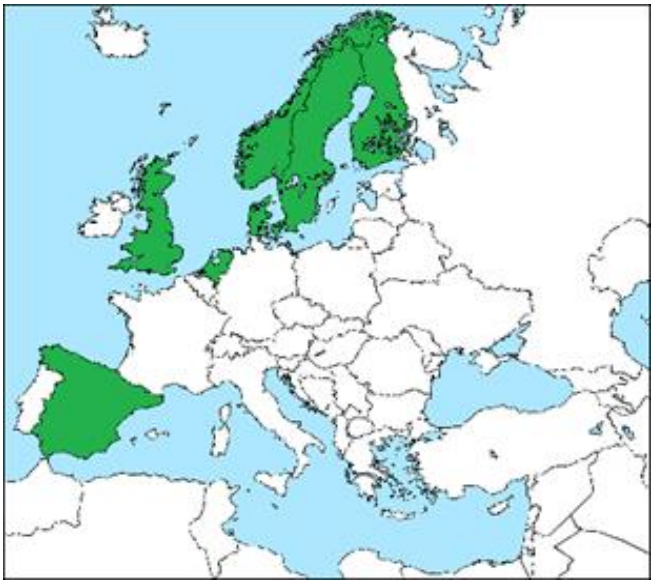
- To study the clinical spectrum and the natural course of POLG disease in a large cohort of patients to provide a detailed description of the disease's phenotypic-spectrum and a reliable clinical classification which can be used both in paediatric and adult populations.
- To identify robust diagnostic and prognostic biomarkers which may help to facilitate the diagnosis and to predict the outcome of the disease.

4. METHODS

4.1 STUDY DESIGN AND POPULATION

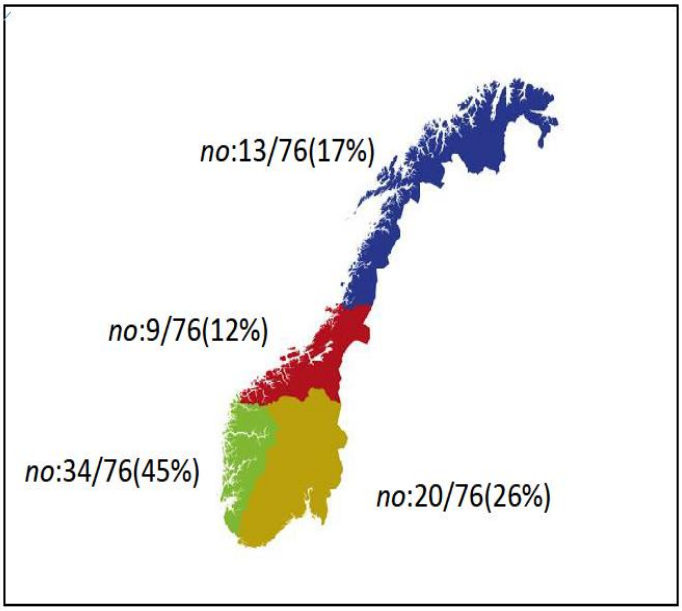
In these multinational, retrospective studies 159 patients were recruited from 13 centres in seven European countries: Norway (Haukeland University Hospital, Oslo University Hospital, St. Olav's Hospital and University Hospital of Northern Norway); United Kingdom (Great Ormond Street Hospital, London and Welcome Trust Centre for Mitochondrial Research, Institute of Neuroscience, Newcastle University); Sweden (Centre for Inherited Metabolic Diseases, Karolinska University Hospital, Stockholm and The Queen Silvia Children's Hospital, University of Gothenburg), Denmark (Department of Clinical Genetics, Copenhagen University Hospital); Finland (Children's Hospital, Helsinki University Hospital and Clinic for Children and Adolescents, Oulu University Hospital); Netherlands (Department of Genetics and Cell Biology, Maastricht University, Maastricht) and Spain (Sant Joan de Déu Children´s Hospital, Barcelona) (figure 7).

Figure 7: The European countries participating in this study highlighted in green.



The Norwegian patients were recruited from the National Norwegian POLG Registry, (www.polgregister.no). Collaboration was established with all Norwegian university hospitals and a local investigator responsible for data collection was allocated in each centre. By June 2018, 76 patients had been enrolled in the registry. Distribution of patients according to the health-regions (Central, Northern, Southern and Eastern and Western Norway) is provided in figure 8.

Figure 8: Distribution of patients with POLG disease in Norway according to the four health regions; Central: red, Northern: blue, Southern and Eastern: yellow, Western: green.



The majority of patients included in this study were Northern European (*n*=150), but there were three from Iraq, two from Cyprus and one each from Croatia, Pakistan, Spain and the United Arab Emirates.

In view of the rarity of MCHS, and to provide a better understanding of this particular phenotype, a systematic literature review (using search terms “POLG”, “mitochondria”, “Alpers”, “infantile hepatocerebral syndromes”, “mtDNA depletion”, “myocerebrohepatopathy syndrome” performed in PubMed, June 2016) was used to identify previously published cases. Cases fulfilling the criteria for MCHS (106) and confirmed biallelic pathogenic *POLG* variants were included. These additional MCHS cases were just included in paper I.

A large database of patients with POLG disease was assembled and analysed in step-wise fashion. Details regarding number of patients, recruiting-centres and period of data entry for each part of the study are provided in table 5. In paper I, only data from individuals with disease onset before 12 years of age was included, while in papers II, III and IV available data from all the individuals, regardless of the age of onset were included. Patients (n=4) recruited from the Centre for Mitochondrial Research, Newcastle, were only included in paper IV.

Table 5: Recruiting-centres, number of patients and period of data entry.

Study part	Recruiting centres	Number of patients	Data-entry period
Paper I			May 2015- July 2016
	Norway:	8	
	Haukeland University Hospital, Bergen		
	University Hospital of Northern Norway, Tromsø		
	United Kingdom:	19	
	Great Ormond Street Hospital, London		
	Total	27	
Paper II			May 2015- December 2017
	Norway:	76	
	Haukeland University Hospital, Bergen University Hospital of Northern Norway, Tromsø St. Olav's Hospital, Trondheim Oslo University Hospital, Oslo		
	United Kingdom:	19	
	Great Ormond Street Hospital, London		
	Sweden:	44	
	Karolinska University Hospital, Stockholm		
	The Queen Silvia Children's Hospital, Gothenburg		
	Denmark:	5	
	Copenhagen University Hospital, Copenhagen		
	Finland:	8	
	Helsinki University Hospital, Helsinki Oulu University Hospital, Oulu		
	Netherlands:	2	
	Maastricht University, Maastricht		
	Spain:	1	
	Sant Joan de Déu Children´s Hospital, Barcelona		
	Total	155	
Paper III			May 2015- June 2017
	Norway:	50	
	Haukeland University Hospital, Bergen University Hospital of Northern Norway, Tromsø St. Olav's Hospital, Trondheim Oslo University Hospital, Oslo		
	United Kingdom:	9	
	Great Ormond Street Hospital, London		
	Sweden:	19	
	Karolinska University Hospital Stockholm The Queen Silvia Children's Hospital, Gothenburg		
	Finland:	5	
	Helsinki University Hospital, Helsinki Oulu University Hospital, Oulu		
	Total	83	
Paper IV			May2015- December2016
	Norway:	45	
	Haukeland University Hospital, Bergen University Hospital of Northern Norway, Tromsø St. Olav's Hospital, Trondheim Oslo University Hospital, Oslo		
	United Kingdom:	16	
	Great Ormond Street Hospital, London Centre for Mitochondrial Research, Newcastle		
	Total	61	

4.2 INCLUSION CRITERIA

Individuals diagnosed and followed at the participating centres were considered eligible if they had recessive disease and confirmed biallelic pathogenic *POLG* variants or dominant disease and a heterozygous, confirmed pathogenic variant. Individuals with novel variants considered pathological and clinical features consistent with POLG disease were also included.

4.3 DATA COLLECTION

Data were recorded on an electronic-case report form (eCRF) completed by the responsible investigator(s) at each centre and reviewed by the study-monitor (O.H). The eCRF was developed and tested in a pilot study of 10 patients conducted at Haukeland University Hospital, Bergen, Norway. The electronic version of the eCRF (WebCRF) was developed by the Unit for Applied Clinical Research Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway. (https://webcrf3.medisin.ntnu.no/).

4.4 STUDY DATA

Detailed clinical, biochemical, muscle biopsy, neurophysiological, neuroimaging and genetics data were obtained (Appendix 1). The date of disease onset was defined by the date of symptom(s) necessitating the patient's first medical evaluation. The age of onset of each individual symptom was also identified. End of follow up was defined as the date of the patients' last visit to the treating centre or death. Preterm birth was defined as birth before a gestational age of 37 completed weeks. Microcephaly was defined as head circumference more than two standard deviations below the mean for age and sex.

Anaemia was defined as an abnormally low haemoglobin value according to age and sex adjusted values (164). Renal tubulopathy was defined by the presence of at least three of the following: tubular acidosis with generalised aminoaciduria, tubular proteinuria, glycosuria, increase in urinary N-acetyl-beta-D-glucosaminidase (NAG)/creatinine ratio, or Fanconi syndrome. Liver involvement was defined by the presence of two or more of the following in at least two different time points: elevated

aspartate aminotransferase (ASAT), gamma-glutamyltransferase (GGT), bilirubin or ammonia, low serum albumin, or pathological histological findings on liver biopsy.

CSF protein and/or albumin values at disease onset and during the disease course were obtained. Since the reference normal range for CSF protein may be age dependent, we looked at the values according to age (165-167). Since albumin is produced exclusively in the liver, all albumin detected in CSF originates, by definition, from blood. The level of CSF albumin provides, therefore, a parameter with which to evaluate the permeability of the blood-CSF barrier. The ratio of CSF to serum albumin (Q-alb) corrects for the individual's albumin level and provides a reflection of the diffusion gradient of albumin (165, 168-170). This ratio was calculated as CSF albumin (milligram (mg)/L)/serum albumin (g/L). The reference range for Q-alb is also age dependent. The cut-offs used in this study were reported in supplementary file 2, paper III (171).

Hearing impairment was defined as abnormal auditory evoked response test. Therapy resistant epilepsy was defined using the International League Against Epilepsy (ILAE) definition (172).

POLG variant(s) for each case were identified either by targeted mutation analysis for specific common mutations (c.1399G>A, p.Ala467Thr and c.2243G>C, p.Trp748Ser, and Gly848Ser) or by sequence analysis of all coding regions of *POLG* gene.

4.5 STATISTICAL ANALYSIS

Data were analysed using SPSS (Statistical Package of Social Sciences). A two sided P value less than 0.05 was considered to be statistically significant. Categories were compared using Fisher's Exact tests when appropriate.

In paper II, Correspondence Analysis (CA) was performed to examine the relationship between two variables graphically in a multidimensional space. These variables were: A) groups of patients who were classified according to the age of disease onset (early onset, juvenile and adult, and late onset disease), and B) the age of onset of each individual symptom. This enabled examination of the clustering of symptoms around

each age group. Further, mosaic plots were performed to study the differences between these groups.

For survival analysis, the end-point was time to death which was defined as the time in months from the date of onset to the date of death. Univariate survival analysis was performed using log-rank test (Kaplan Meier) to compare differences in survival time between categories. Adobe illustrator CS6 and BioRender (biorender.com) were used to create and edit the figures.

4.6 ETHICAL STATEMENT

The ethical approval for the study was obtained from the Regional Committee for Medical and Health Research Ethics, Western Norway (REK 2014/1783-4). Each participating country obtained approval from their local ethical committee. The study was registered as an audit at Great Ormond Street Hospital, London, UK (Registration Number 1675). Anonymised data regarding Newcastle patients was provided courtesy of the MRC Mitochondrial Disease Patient Cohort (Ethics ref:13/NE/0326). All procedures followed were in accordance with the ethical standards of the responsible committee both in Norway and in the other collaborating countries and with the Helsinki Declaration of 1975, as revised in 2000.

5. RESULTS

5.1 Paper I: *The clinical spectrum and natural history of early-onset diseases due to DNA polymerase gamma mutations*

The primary aim was to study the natural history of paediatric onset POLG disease in a sufficiently large cohort of patients to provide a better and more detailed description of the clinical spectrum in this age group. Clinical, biochemical, neuro-imaging and genetic data of 27 individuals with recessive disease and biallelic variants in *POLG* in whom initial symptomatology manifested prior to the age of 12 years were obtained.

The median age of disease onset was 11 months (range 2 weeks to 7 years). Extensive phenotypic characterisation was performed. Six patients fulfilled diagnostic criteria for MCHS, 19 for AHS, and one had a MNGIE-like phenotype. One patient was unclassified owing to insufficient data. The majority presented with global developmental delay (*n*=24/24, 100%), hypotonia (*n*=22/23, 96%), liver dysfunction (n=23/25, 92%) and failure to thrive (*n*=24/27, 89 %). Seizures were also common (*n*=19/26, 73%) and were present in 89% (*n*=17/19) at disease onset. Focal and focal evolving to bilateral convulsive seizures with epileptiform activities predominantly seen over the occipital regions were the most commonly reported seizure types. Notably, none of the patients with the MCHS phenotype had seizures.

Lactate was elevated in blood in 60% of those in whom this was measured (n=12/20) and in CSF in 40% of those in whom CSF studies were performed (n=2/5). Abnormal respiratory chain enzyme activities were observed in 9 of 14 cases in whom muscle biopsy was performed. Interestingly, most AHS patients had normal respiratory chain enzyme activities or only an isolated deficiency of a single enzyme complex, while those with MCHS had multiple respiratory chain complex deficiencies.

The most common MRI findings were cortical focal lesions manifesting as T2/FLAIR hyperintensities involving cortical and subcortical areas, predominantly affecting the occipital lobes.

All cases had two biallelic pathogenic *POLG* variants identified either by targeted mutation analysis for specific common variants as p.Ala467Thr and p.Trp748Ser or by sequence analysis of all coding regions of *POLG*. Two patients (no. 23 and no. 26, supplementary file 1, paper I) with classical AHS phenotype and novel *POLG* gene variants (p.Lys498Thr, p.Gly621Asp) were included. All *POLG* gene variants reported in this part of the study and associated phenotypes are illustrated in Figure 2 paper I.

Survival analysis showed that median age at death was 15.8 months (range 1.0 to 184.6 months), whereas median time from disease onset to death was 4.9 months (range 0.5 to 181.2 months). The main causes of death were liver failure, followed by sepsis and status epilepticus. Median survival time of patients with disease onset ≤12 months was 3.6 months (range 0.5-181) compared to 10 months (range 1.4-82) for those with disease onset >12 months. Survival analysis by phenotype showed that the median survival time for patients with MCHS was 5 months (range 0.6-22) and 4 months (range 0.5- 181) for AHS.

This study showed the multi-systemic nature and natural history of early onset POLG disease and highlighted the existence of a disease group without seizures - i.e. those with the MCHS phenotype. The common founder variants p.Ala467Thr and p.Trp748Ser were present in only one of our cases with MCHS and a review of the literature showed only one additional case with this variant, confirming the need to sequence the whole *POLG* gene rather than perform targeted variant analysis. Further, we extended the phenotypic spectrum of childhood POLG disease to include a phenotype with prominent gastro-intestinal symptoms mimicking mitochondrial neurogastro-intestinal encephalomyopathy (MNGIE), in addition to the AHS and MCHS phenotypes. Based on the results of this study, we observed that early onset POLG disease could be classified simply into those with and those without epilepsy.

5.2 Paper II: *Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset: studies using a cohort of 155 cases*

The aim of this study was to provide a simple clinical classification that would facilitate early clinical recognition of patients with POLG disease. To do this, we systematically reviewed detailed longitudinal data from 155 patients both at disease onset and later during the disease course. The cohort included patients with disease onset from birth to late adulthood, providing us with a unique opportunity to study the clinical spectrum of the disease during the whole life span.

The age of onset of each individual symptom was identified. Median age at disease onset for the whole study cohort was 10 years (range: birth – 71 years). Fifty-four percent (n=83/155) had onset prior to the age of 12 years, 34% (n=53/155) had onset between 12 and 40 years of age, and 12% (n=19/155) had onset after the age of 40 years. Disease debut was apparently spontaneous in 113/155 (73%), followed an infectious illness in 32/155 (21%) and not clearly reported in 10/155 (6%) of the patients.

Neurological (90%, n=139/155), ophthalmological (74%, n=112/151) and gastrointestinal (63%, n=92/146) symptoms were the predominant clinical features. Epilepsy was reported in 69% (n=107/155), with focal and focal evolving to bilateral tonic-clonic seizures being the most common seizure types (92%, n=94/102). Ataxia (63%, n=87/138), peripheral neuropathy (53%, n= 65/123), and hypotonia (50%, n=68/135) were frequently reported. Nystagmus (38%, n=55/146), PEO (38%, n=56/146) and ptosis (34%, n=51/149) were the most commonly reported ophthalmological features. Liver involvement was identified in 64% (n=96/151) of the patients. More than half of the study cohort had feeding difficulties (52%, n=75/145), regardless of the age of onset. A detailed description of the clinical features, laboratory, muscle-biopsy, neuro-physiology and neuro-imaging findings is provided in table 2 and supplementary tables 1 and 2, paper II.

Our data confirmed that POLG disease comprises a continuum of clinical features rather than a set of separate clinical identities (see figure 1 paper II). Nevertheless, by grouping the patients simply using age of onset, we could identify clear phenotypic and prognostic

differences (table 3 paper II). Liver involvement (87%), seizures (84%) and feeding difficulties (84%) were the major features in those with onset prior to the age of 12 years, while ataxia (90%), peripheral neuropathy (84%) and seizures (71%) occurred most in those with onset between the age 12 and 40 years, and ptosis (95%), progressive external ophthalmoplegia (89) and ataxia (58%) in those with onset after the age of 40 years. Early onset was associated with a worse prognosis. Cluster analysis showed there was obvious clustering of the symptoms around the three different age groups and further mosaic plots confirmed statistically significant differences in the phenotypes seen in the above mentioned groups (supplementary figure 2, paper II).

A total of 41 different *POLG* variants were identified in the 155 individuals described in this study. Ninety patients had compound heterozygous variants, 59 had homozygous variants and six patients had a heterozygous variant associated with autosomal dominant disease, mainly adPEO. The majority of patients with early onset disease (70%) had compound heterozygous pathogenic *POLG* variants (regardless of the variant types), while 62% of those with juvenile/adult onset had homozygous pathogenic variants. *POLG* gene variants reported in this part of the study are summarized in supplementary figure 3, paper II.

The main cause of death for the whole cohort was liver failure, followed by infection/sepsis, multi-organ failure and SE, and was unknown in 13% of the individuals. The presence of epilepsy was associated with significantly worse survival, and the median survival time from seizure onset to death was 37 months (range < 1 - 487). Survival after the onset of seizures in those with early onset disease was significantly worse than those who developed seizures as part of juvenile/adult onset disease. Analysis also showed that patients with pathogenic compound heterozygous *POLG* variants had significantly worse survival than those with pathogenic homozygous variants. Further, individuals with liver involvement showed a significantly worse survival than those without liver impairment (supplementary figure 4, paper II).

5.3 Paper III: *Elevated cerebrospinal fluid protein in POLG-related epilepsy: Diagnostic and prognostic implications*

Since epilepsy is common in individuals with POLG disease and the disruption of blood brain barrier (BBB) has been described in many neurological disorders that include epilepsy (Table 1, paper III), the aim of this study was to assess whether BBB dysfunction occurs in POLG disease and what clinical implications it has for patients.

Our study cohort contained 83 patients with details of CSF protein/albumin. We used the presence of a raised CSF/serum ratio of albumin (Q-alb) to evaluate the integrity of BBB.

This study demonstrated that elevated CSF protein was a common feature of POLG disease; raised CSF protein was observed in 70% of patients in whom data were available (*n*=58 /83) and was, moreover, associated with the most severe phenotypes. The finding of a significantly elevated Q-albumin ratio indicated a clear dysfunction of the BBB. We found that the majority of those with epilepsy (*n*=50/66, 76%) had raised CSF protein and interestingly, this preceded seizure debut in 75% (*n*=15/20). This study showed that elevated CSF protein can be used as a biomarker both to facilitate early diagnosis and to identify those at high risk of developing epilepsy.

5.4 Paper IV: *The presence of anaemia negatively influences survival in patients with POLG disease*

Mitochondria are known to play an important role in iron metabolism and thus haematopoietic cell homeostasis. Little attention has been given to the haematological manifestations in patients with mitochondrial disease particularly those with POLG disease. Recent studies performed in the POLG *mutator* mouse model demonstrated that variants in *POLG* can also drive haematopoietic abnormalities including anaemia (173-177). This study aimed to determine the frequency of and outcomes associated with anaemia in a large cohort of patients with POLG disease.

Approximately two third of the patients (n= 41/61, 67%) enrolled in this study developed anaemia at some stage and, importantly, almost one in four (n=14/61, 23 %) had anaemia already at presentation. This was particularly true for patients with early onset POLG disease (AHS and MCHS phenotypes) who had a high frequency of anaemia (n= 18/ 25, 72%) with 35% (n=8/23) having anaemia at presentation. Details regarding the number of individuals with anaemia stratified according to age group is provided in table 2, paper IV.

Survival analysis (Figure 1, paper IV) revealed that the presence of anaemia was associated with a significantly worse survival. This part of the study demonstrated for the first time that anaemia is indeed a feature of POLG disease in humans and is associated with significantly worse survival and can be used as a predictor for poor prognosis.

6. DISCUSSION

The aim of this study was to provide a thorough description of the natural history and the phenotypic spectrum of POLG disease, and to elaborate a robust, but simple clinical classification to guide clinicians and help predict the prognosis.

The cornerstone of clinical diagnosis is a relevant clinical, phenotypic classification that is useful in everyday clinical practice. This, however, is challenging in mitochondrial disorders due to poor phenotype:genotype correlation, namely that defects in a single gene, such as *POLG,* may give rise to multiple clinical phenotypes. This means that even affected individuals in the same family, sharing the same pathogenic variant may present with different phenotypes, and that unrelated individuals with different pathogenic variants may share the same phenotypes (83, 84, 100). Understanding the natural history of a disease is essential to address these challenges since this will provide information about the evolution of clinical features and the related morbidity and mortality.

Classification of POLG disease has developed haphazardly and resulted in a plethora of eponymous and syndromic categories (Table 3). This has made clinical recognition challenging and can, potentially, have resulted in delayed or even missed diagnosis. Since data describing the natural history of POLG disease in a sufficiently large cohort of patients were lacking, we established the largest known cohort of individuals with POLG disease by recruiting individuals from the National Norwegian POLG Registry and from our international collaborating centres. Clinical, laboratory, neuro-physiological and neuroimaging findings were reviewed both at the disease onset and later during the disease course. This enabled us to describe in detail the clinical spectrum and the natural history of the disease in a sufficiently large number of patients. Further, data from our thorough national history studies allowed us to classify POLG disease more simply than the earlier attempts. We believe that such a classification is easier to implement in clinical practice and will facilitate early clinical recognition. We were also able to provide clinical, biochemical and genetic prognostic indicators of the disease.

6.1 NATURAL HISTORY AND THE PHENOTYPIC SPECTRUM OF POLG DISEASE

6.1.1 Paper I: *The clinical spectrum and natural history of early-onset diseases due to DNA polymerase gamma mutations*

In this study, we focused on early onset disease. We looked at paediatric patients with disease onset prior to the age of 12 years and generated a comprehensive clinical and laboratory description of the features present at onset and later during the disease course. In addition, we reviewed the neurophysiological, histopathological and neuro-imaging findings and performed a phenotype-genotype correlation study.

We found that the majority of patients with early onset POLG disease had relatively diffuse clinical manifestations such as hypotonia, failure to thrive, developmental delay and seizures. These findings highlight the challenges associated with making the diagnosis of POLG disease in the paediatric population. These non-specific clinical manifestations overlap with the manifestations of many other disorders including mitochondrial and non-mitochondrial disorders. For example, pathogenic variants in nuclear genes such as *PARS2, NARS2, FARS2, SURF1, PDHA1 and ADCK3* (83, 178-180), or those affecting mtDNA stability (summarized in table 2), may all mimic POLG phenotypes clinically. Defects in mtDNA such as the m.3243A>G mutation causing MELAS (181), may also mimic the early and juvenile forms of POLG disease. The differential diagnosis of non-mitochondrial disorders resembling POLG disease is also wide and includes febrile infection related epilepsy syndrome (FIRES) (182), autoimmune encephalitis/Rasmussen encephalitis with EPC (183), and other inborn errors of metabolism with recurrent liver failure such as that caused by pathogenic mutations in *NBAS* gene (184) or neonatal hypoglycaemia and non-ketotic hyperglycinaemia (185).

Survival analysis showed that early onset disease is associated with high mortality (n=22/26, 85%) and the median survival time from disease onset to death was 5 months, which highlights the rapid disease progression.

The majority of individuals in this paediatric cohort were classified as AHS or MCHS. We also found one patient who fulfilled the criteria for MNGIE and were, therefore, able to expand the POLG phenotypic spectrum in this age category. One patient remained unclassified owing to insufficient data. Our findings were also in broad agreement with the earlier understanding that MCHS and AHS can be considered as two separate entities; we found clear differences in the age of onset and the survival between these two patient groups. Nevertheless, since few of the patients had liver biopsy performed (liver biopsy was only performed in 3 cases), it was not possible to differentiate these two phenotypes as previously suggested (106). This probably reflects everyday clinical practice where liver biopsy is rarely performed in early onset POLG disease due to the associated risks and is contraindicated in the case of severe liver dysfunction. We did find one important feature that differentiated these two phenotypes: none of the individuals with MCHS phenotype had epilepsy (Table 3, paper I). This was true not only in our cohort, but also when we collected all previously published cases of MCHS (Table 2, paper I). Based on these findings, we concluded that the previously described clinical and histopathological criteria used to differentiate between AHS and MCHS phenotypes were not applicable and we suggested a simplified classification of early onset disease into those with or without epilepsy.

Patients with MCHS who underwent biochemical measurements also showed the most severe respiratory chain defects (supplementary file 2, paper I). We hypothesised that these patients were the more severely affected and, possibly because of this, died before the onset of seizures. Discordance in the outcome observed in one sibling pair (cases 15 and 16) provides further evidence for this hypothesis. Case 15 with an AHS phenotype died at 9 months with intractable seizures whereas his sister (case 16) with a MCHS phenotype died at 5 months and never developed seizures.

Our data also led us to believe that the MCHS phenotype (i.e. early onset disease without epilepsy) may be under diagnosed due to the presence of non-specific clinical features and absence of epilepsy, the most common factor that triggers the clinical suspicion of POLG disease in the paediatric population. Thus, we feel that highlighting the

possibility of POLG disease arising in the absence of epilepsy, will improve the clinical ascertainment of patients in this age group.

When we looked at the genotypes in the group of MCHS patients, we found that the common variants (c.2243G>C, p.Trp748Ser and c.1399G>A, p.Ala467Thr) occurred only once (patient 16, paper I) in our MCHS group. Moreover, we found only one other previously published MCHS case with these variants (186). These findings suggest that screening only for common *POLG* mutations in children younger than 12 years with unexplained encephalopathy, or multi-system neurological disorders, as previously suggested (91, 144), will be unsuccessful.

6.1.2 Paper II: *Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset: studies using a cohort of 155 cases*

In the second study, we wished to extend our work to include all age groups and at the same time include sufficient numbers of patients to enable us to make robust statements concerning the natural history and the clinical spectrum of POLG disease. To do this we used all the available data from the National Norwegian POLG Registry, data from our ongoing collaboration in the Mitochondrial Clinical Research Network (MCRN) (www.mcrnet.org) and data from patients in our first study that were recruited from Great Ormond Street Hospital, London, UK. We were able to recruit 155 patients and thus establish the largest known cohort of patients with POLG disease. This large multicentre/international cohort gave us a unique opportunity to study the natural history of POLG disease through all ages, to increase the power of statistical analysis, and eliminate any potential geographical bias.

Since our cohort contained longitudinal data, including clinical, laboratory and neuro-imaging data at disease onset and during the disease course, we could show that POLG disease comprised a continuum of clinical features (Figure 1, paper II). These data showed clearly that features such as ataxia, seizures, liver dysfunction and peripheral neuropathy could arise at any point from infancy to older age. Indeed, it was clear that many of the features that were associated with age-defined phenotypes/syndromes could

arise at different times and overlap with one another. This suggested to us that POLG disease was a continuum rather than a set of separate clinical identities (Table 1).

While we felt that the finding of a continuum of features mitigated against multiple different clinical entities, we observed that when we looked at the median age of onset of the major features, there was a tendency for these to cluster according to age (Figure 1 paper II). We, therefore, re-analysed the data using three age groups (Table 3, paper II). Based on these findings, we showed that the clinical spectrum of POLG disease could be described by stratifying the patients simply according to the age of disease onset. 1.) An early onset disease (onset prior to the age of 12 years) - liver involvement, feeding difficulties, seizures, hypotonia, and muscle weakness were the most common/ easily recognizable clinical features and this group had the worst prognosis. 2.) A juvenile/adult onset (onset from 12 - 40 years of age) - characterized by peripheral neuropathy, ataxia, seizures and SLE. Patients in this group carried a better prognosis than the early onset group. 3.) A late onset disease (onset after the age of 40 years) - characterized by ptosis and PEO. Other features such as peripheral neuropathy ataxia and muscle weakness may also occur. This group had the best prognosis.

To confirm our findings, we performed cluster analysis which showed a clear clustering of the symptoms around the age groups mentioned above (supplementary figure 1, paper II). Further, mosaic plots confirmed statistically significant differences in the phenotypes observed in these groups (supplementary figure 2, paper II).

Based on our age groups, clear clinical patterns could be identified and these dictated which investigations were appropriate and useful to reach the diagnosis. We used these data to generate a diagnostic algorithm (Figure 3, paper II) to show how recognition of the key clinical features could be used to direct the diagnostic investigations in the different age groups.

We believe that our simple clinical classification of POLG disease into these three groups - early onset, juvenile to adult, and late onset disease - will facilitate early clinical recognition and provide data that will help predict the outcome of patients with POLG disease.

Age of disease onset was clearly correlated to the survival time (Table 3, paper II), the earlier the onset, the worse the prognosis. Moreover, survival analysis showed that the presence of epilepsy was significantly associated with worse prognosis (Figure 2, paper II). Further, individuals who developed liver involvement showed significantly worse survival than those without liver involvement (supplementary figure 4, paper II). Regarding the impact of the genotype on survival, we found that individuals with compound heterozygous *POLG* variants had a worse prognosis compared to those with homozygous variants, regardless of variant type (Figure 2, paper II). We believe that data concerning the features mentioned above, age of onset, seizure, liver involvement and/or the identification of specific genotype (i.e. compound heterozygous), can inform everyday clinical practice and help us guide patients/families regarding the prognosis of POLG disease.

6.2 NOVEL DIAGNOSTIC AND PROGNOSTIC BIOMARKERS

Biomarkers (biological markers) are defined in general as: '*A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*' (187). In mitochondrial disorders, biomarkers have been used to facilitate early diagnosis, selecting patients who should undergo more invasive diagnostic procedures and providing information about disease outcome (188).

Our studies in POLG disease showed that laboratory biomarkers commonly used in the initial diagnostic work up such as blood and CSF lactate, the presence of ragged-red/COX-negative fibres and abnormal respiratory chain activities in the skeletal muscle had low diagnostic sensitivity, being present in less than 50% of the individuals. This is particularly true in the paediatric population.

In the third and fourth studies of this thesis, we examined two novel biomarkers and evaluated their impact on the management of individuals with POLG disease.

6.2.1 Paper III: *Elevated cerebrospinal fluid protein in POLG-related epilepsy: Diagnostic and prognostic implications*

This study investigated whether POLG disease is associated with disruption of BBB and, if so, what the diagnostic and/or prognostic implications were for the affected individuals.

Clinical, laboratory and genetic data of 83 individuals were available for this study. The laboratory data included CSF protein and/or albumin values. The study revealed that more than two thirds of those studied (70%) had raised CSF protein and/or albumin. When we used the ratio of CSF/serum albumin (Q-alb) to evaluate the integrity of the BBB, we found markedly raised levels (median value of 21.5 x 10^{-3}) in the 18 individuals in whom these were measured. Indeed, the Q-alb ratios were higher in individuals with POLG disease than in other disorders in which BBB dysfunction also occurs (Table 1, paper III) suggesting a profound disruption of BBB integrity. Further, the availability of longitudinal clinical and laboratory data allowed us to follow disease progression and enabled us to show that there was a progressive deterioration of BBB integrity as both the proportion of those with abnormal raised CSF protein and the actual CSF protein value increased with time.

Raised CSF protein was also associated with worse prognosis (regardless of the age of disease onset). Median survival time for those with raised CSF protein was markedly decreased (13 months) compared to those with normal CSF protein (32 months). Further, our analysis showed that raised CSF protein mainly occurred in patients with epilepsy (76%) and interestingly, its presence preceded the onset of the seizures (n= 15/20). This finding has important clinical implications and suggests that raised CSF protein can be used as a biomarker to identify those at risk of developing epilepsy.

We also found that the proportion of individuals with elevated protein/albumin was higher than the proportion of patients having abnormalities of other commonly used diagnostic biomarkers (Table 3, paper III). This suggests that elevated CSF protein has a higher diagnostic sensitivity than tests such as blood lactate and may be useful as a diagnostic biomarker.

The role of mitochondria in the integrity of the BBB has been investigated; *in vitro* inhibition of complex I with rotenone, impairing ATP production using carbonyl cyanide 4- (trifluoromethoxy) phenylhydrazone (FCCP) or inhibiting complex V with oligomycin can result in disruption of BBB integrity and increase its permeability (189). Further, inhibition of mitochondrial respiratory chain enzyme activity in mice by epidural administration of rotenone impaired BBB integrity and was associated with marked increase in BBB permeability (189). Disruption of the BBB permits circulating large molecular proteins such as albumin to enter into the brain microenvironment. Uptake of these molecules by neurons or astrocytes (190) is followed by down-regulation of extracellular potassium buffering capacity (191), which in turn facilitates N-methyl-D-aspartate receptor mediated neuronal hyperexcitability (192, 193), and the release of proinflammatory cytokines (190, 194, 195). All of these mechanisms may lead either to seizures or lower the threshold for seizure initiation (192, 196, 197). Based on our findings, and work by others (189, 190, 192, 197), we hypothesized that mitochondrial dysfunction induced by pathogenic variants in *POLG* gene leads to disruption of the BBB tight junctions and leakage of proteins such as albumin. Uptake of albumin by astrocytes and neurons initiates a cascade of events that contribute to seizure development (Figure 1, paper III) and subsequent worsening of the BBB dysfunction.

6.2.2 Paper IV: *The presence of anaemia negatively influences survival in patients with POLG disease*

This study was inspired by previous work showing that polγ-deficient mice manifested haematopoietic abnormalities including anaemia (173-177). The aim of the study was to investigate whether anaemia was a feature of POLG disease in humans.

Haematological abnormalities associated with POLG disease have not previously been reported. We assumed, therefore, it was likely that the serious nature of the neurological and hepatic manifestations may have obscured the significance of the haematological abnormalities. In the absence of human data, we analysed the frequency of anaemia in the 61 individuals in whom data were available when this study was performed.

We showed that anaemia is indeed a feature of POLG disease in humans with approximately two third of the individuals studied developing anaemia during the course of their disease, and almost one in four having anaemia at disease onset. The frequency of anaemia was even higher (72%) in individuals with early onset disease, and 35% of these had anaemia at the disease onset. This may reflect the severity of the disease in this age group. Further, survival analysis showed that the presence of anaemia was significantly associated with worse survival (Figure 1, paper IV). This finding has an important clinical implication as the presence of anaemia can be used as a negative prognostic biomarker.

When thinking about how the anaemia arose in POLG disease, we hypothesised that it was due to deceased RBC production based on the following observations: A) None of the individuals enrolled in this study had any clinical or laboratory evidence of haemolysis or blood loss at the time that anaemia was recorded. B) Those who developed terminal multi-organ failure already had anaemia registered at an earlier time point. Individuals who developed anaemia due to bleeding as a part of multi-organ failure were not included. C) The presence of anaemia at disease onset argues strongly against an "anaemia of chronic disease" or it being due to the influence of therapy e.g. AEDs. D) Only 35 % (5/14) of those with anaemia at disease onset had evidence of liver impairment (three with only mild elevation of ASAT or ALAT, and two with early onset disease without epilepsy with clear evidence of liver failure), suggesting liver dysfunction was most probably not the cause of the anaemia. Lastly, our hypothesis is supported by the animal studies showing that *POLG* mutation is associated with anaemia, haematopoietic progenitor dysfunction and erythroid dysplasia (173-177).

We were unable to classify the type of anaemia in the majority of individuals enrolled in this study owing to insufficient data, however available data suggested iron deficiency was the major cause.

The question of whether we are missing a potential therapeutic opportunity by not treating anaemia remains unresolved. A study using matched quantitative genomic and proteomic analysis in mouse muscle cells showed iron deprivation resulted in a rapid,

dose-dependent decrease of mitochondrial proteins and oxidative capacity which was fully reversed when iron was reintroduced (198). A subsequent study performed by the same group showed that the transcriptional changes were accompanied by alterations to histone acetylation and methylation levels that were largely reversible by reintroduction of iron (199). Prospective studies are needed to investigate whether treating anaemia and optimizing iron level will improve the survival and decrease the morbidity in patients with POLG disease.

6.3 LIMITATIONS OF THE PRESENT STUDIES:

As shown by our studies, one strength of retrospective studies is the ability to accumulate data from a large number of patients. Another strength is that patients are unselected. A major weakness of such studies is that there is often a great deal of missed data, which may have the effect of reducing the size and power of the study. This disadvantage would be compounded if the missing data were not distributed randomly.

Our study population was diverse enough to include all the known POLG clinical phenotypes. This provided us with a unique opportunity to produce robust and statistically significant conclusions. Nevertheless, missing data did influence some of our sub-analyses such as in paper III where CSF/serum ratio of albumin could only be measured in 18 of the 83 individuals enrolled in the study. Another example of this was seen in paper IV where we were unable to identify the cause of anaemia in the majority of the individuals, again due to missing data.

The clinical forms used to acquire data were completed by many investigators based in different centres. This too might have generated discrepancies in the data collected. We were, however, able to minimize this potential disadvantage by having regular study-meetings in which we first went through the study form in detail, and then discussed the quality of the collected data and the results of the studies as the project progressed. Further, all the forms were quality checked by O.H.

There is also the question of ethnicity. Individuals included in this study were mainly of Northern European descent. While this may limit the impact of this study to other ethnic groups, we feel that the numbers included in our studies provide a counterbalance. Our

cohort is the largest known group of patients with POLG disease and included all the known phenotypes previously associated with POLG disease and more than 40 different *POLG* variants. Moreover, there is no evidence in the literature of marked ethnic differences in phenotypic expression of POLG disease. We can therefore suppose that our findings are relevant to patients with POLG disease, regardless of the ethnic background.

7. CONCLUSION

The studies performed as part of the PhD thesis allow us to make some important observations. We found that the clinical data were best explained by POLG disease being a continuum rather than a collection of separate phenotypes. Nevertheless, we did see that age played an important role: we found that by classifying patients simply according to age of onset it was possible to elucidate three groups; early onset, juvenile /adult onset and late onset disease with clear phenotypic and prognostic differences. We hope that our classification and thorough description of the natural history will help facilitate early clinical recognition of the disease, help physicians to direct the laboratory investigations and be useful in predicting the outcome of patients with POLG disease (Figure 3, paper II).

Highlighting the existence of early onset POLG disease without seizures will hopefully improve diagnosis of this subgroup of patients with rapidly fatal disease. Since affected individuals rarely have the so-called 'common' *POLG* variants, we state the need for full screening of the *POLG* gene. This applies equally to patients in the neonatal period and during childhood with unexplained encephalopathy and multi-system disorders with or without epilepsy.

Our studies also enabled us to identify commonly available prognostic biomarkers such as CSF protein and haemoglobin concentration. Further, we showed that age of disease onset had important negative impact on the severity of the disease and that patients with

epilepsy and/or liver impairment, and those who were compound heterozygous for POLG variants had a significantly worse prognosis.

In light of the difficulties associated with performing randomized controlled clinical trials in rare disorders such as POLG disease, we believe our natural history data, based on a large number of individuals, will help physicians to establish a clinical baseline for comparison in single-arm clinical trials for potential novel therapies in the future.

8. FUTURE PROSPECTS

The available data from this large cohort of individuals with POLG disease is still not completely analysed. The impact of gender is an important question we need to address: based on clinical observations, females with POLG disease appear more severely affected during puberty and pregnancy. Further, we need to analyse our data regarding the efficacy of AED(s) to reduce seizure frequency and severity so that we may provide an evidence based guideline for treatment of epilepsy in patients with POLG disease.

Previous studies have shown conflicting results regarding the effect of nutritional supplements such as co-enzyme Q_{10}, carnitine and vitamin cocktails, which are widely used in the treatment of mitochondrial disorders. Using our cohort, we hope to address the question of whether these supplements have any positive effect to stop the disease progression, reduce seizure frequency or improve the survival in patients with POLG disease.

Another important area that has not been sufficiently studied is the quality of life in patients with POLG disease. We are currently conducting a study using standardized questionnaires to evaluate the quality of life and mental health status of individuals with POLG disease.

Our ongoing collaboration with the members of the MCRN (Mitochondrial Clinical Research Network) and with the Mitochondrial Research Group, Genetics and Genomic Medicine Program at UCL Great Ormond Street Institute of Child Health, will enable us to continue our clinical studies within the field of mitochondrial medicine.

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10. APPENDIX

POLG study – Clinical record form (CRF)

POLG related disorders

CASE REPORT FORM (CRF)

Site ID: _ _	Patient ID: _ _ _ _	Date of registration: _ _ /_ _ / _ _ _ _
		Date of re-entry: _ _ /_ _ /_ _ _ _
Mito-Server ID: _ _ _		Bio-bank ID: _ _ _
Registration performed by: _ _ _ _		Registration controlled by: _ _ _ _

Inclusion Criteria

Genetically verified pathologic *POLG* mutations ☐ Yes ☐ No

Demographics

- Date of birth:_ _ / _ _ /_ _ _ _ (dd/ mm/yyyy)
- Gender: ☐ male ☐ female ☐ unknown
- Ethnic group:

  ☐ Caucasian ☐ Black ☐Asian ☐ other, specify _____ ☐unknown
- Resident country: _____, ☐ unknown
- Resident county (for Norwegian patients only) : _____, ☐ unknown
- Hospital name (for Norwegian patients only) : _____, ☐ unknown

Family History

- Parental consanguinity: ☐ yes ☐ no ☐ Unknown

  If yes specify: _____
- Presence of a known mitochondrial disease phenotype in relative:

  ☐ yes ☐ no ☐ unknown

  *If yes specify:*_____
  - Phenotype: specify,_____, ☐ unknown
  - Gender: ☐ male ☐ female ☐ unknown
  - Origin of relatedness: ☐ paternal ☐ maternal ☐ both ☐ unknown
  - Degree of relatedness: ☐ 1st ☐ 2nd ☐ other _____ ☐ unknown

Disease onset and last follow up Dates

- Onset date : _ _ /_ _ / _ _ _ _ (dd/mm/yyyy)
- Last follow up: _ _ / _ _ / _ _ _ _ (dd/ mm/yyyy)

Growth

- At disease onset :
  - Weight: ____ (kg) ☐ unknown
  - Height: ____ (cm) ☐ unknown
  - Head cf. : ____ (cm) ☐ unknown
- At last follow up:
  - Weight: ____ (kg) ☐ unknown
  - Height: ____ (cm) ☐ unknown
  - Head cf. : ____ (cm) ☐ unknown

Diagnostic Testing For Suspected Mitochondrial Disease

- Muscle biopsy: ☐ performed, date _ _/_ _ /_ _ _ _ (dd/ mm/yyyy) ☐ not done
- Liver biopsy: ☐ performed, date _ _/_ _ /_ _ _ _ (dd/mm/yyyy) ☐ not done
- Fibroblast: ☐ performed, date_ _/ _ _ /_ _ _ _ (dd/mm/yyyy) ☐ not done
- Genetic investigation:☐ performed, date _ _/_ _ /_ _ _ _ (dd/mm/yyyy) ☐ not done
- Other test: ☐ performed, date _ _ /_ _ /_ _ _ _ (dd/mm/yyyy) ☐ not done

Genetic Findings

- *POLG* mutations :
  Specify: _____

Perinatal History

- Mother age at birth: _ _ (years) ☐ unknown
- Gestational age (weeks) : ☐ < 35 ☐ 35-37 ☐ 38-41 ☐ > 41 ☐ unknown
- Intra-uterine growth retardation: ☐ yes ☐ no ☐ unknown
- Placenta pathology: ☐ yes ☐ no ☐ unknown
- Birth weight: ____ kg ☐ AGA ☐ SGA ☐ LGA ☐ unknown
- Head cf. at birth: ___cm ☐ normal ☐ microcephaly ☐ macrocephaly ☐ unknown
- APGAR 1-5-10 : ____-____-____ ☐ unknown
- Pathological signs at birth: ☐ yes ☐ no ☐ unknown
  If yes:
  - Need for resuscitation: ☐ yes ☐ no ☐ unknown
  - Hypotonia: ☐ yes ☐ no ☐ unknown
  - Hypertonia: ☐ yes ☐ no ☐ unknown
  - Seizures: ☐ yes ☐ no ☐ unknown
  - Respiratory complications: ☐ yes ☐ no ☐ unknown
  - Cardiac complications: ☐ yes ☐ no ☐ unknown
  - Dysmorphic features: ☐ yes ☐ no ☐ unknown
  - Hyperlactatemia: ☐ yes ☐ no ☐ unknown
  - pH value at birth : _____ ☐ unknown
  - Base access value at birth : ____ ☐ unknown
  - Hyperglycaemia: ☐ yes ☐ no ☐ unknown
  - Hypoglycaemia: ☐ yes ☐ no ☐ unknown
  - Feeding difficulties : ☐ yes ☐ no ☐ unknown
  - Vomiting : ☐ yes ☐ no ☐ unknown
  - Anaemia: ☐ yes ☐ no ☐ unknown
  - Other : ☐ yes ☐ no ☐ unknown
    If yes, specify: _____

Disease Debut

- Spontaneous: ☐ yes ☐ no ☐ unknown
- Post infectious: ☐ yes ☐ no ☐ unknown
- Post epileptic: ☐ yes ☐ no ☐ unknown
- Post puberty: ☐ yes ☐ no ☐ not applicable ☐ unknown
- Post Menarche: ☐ yes ☐ no ☐ not applicable ☐ unknown
- Other: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____

Registered Mitochondrial disease diagnosis

- ICD code : __ _ _ _ ☐ unknown
- Date of definitive diagnosis: _ _ /_ _/_ _ ☐ unknown
- Alpers-Hunttenlocher syndrome: ☐ yes ☐ no ☐ unknown
- Myocerebrohepatopathy spectrum: ☐ yes ☐ no ☐ unknown
- Myoclonic epilepsy myopathy sensory ataxia: ☐ yes ☐ no ☐ unknown
- Ataxia Neuropathy spectrum: ☐ yes ☐ no ☐ unknown
- Progressive external ophthalmoplegia(PEO): ☐ yes ☐ no ☐ unknown
  - Autosomal recessive PEO: ☐ yes ☐ no ☐ unknown
  - Autosomal dominant PEO: ☐ yes ☐ no ☐ unknown
  - Unclassified: ☐ yes ☐ no ☐ unknown
- Other: ☐ yes ☐ no ☐ unknown
  If yes specify: _____
- Unclassified : ☐ yes ☐ no ☐ unknown

Clinical Features

- Neurological disorders:
  If yes specify:
  - Seizures: ☐ yes ☐ no ☐ unknown
    If yes, specify:
    - Generalized: ☐ yes ☐ no ☐ unknown
      If yes, Debut: ☐ at onset ☐ later, Date_ _ / _ _/_ _ ☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown

      Infantile spasms: ☐ yes ☐ no ☐ unknown
      If yes, Debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown
      Myoclonus epilepsy: ☐ yes ☐ no ☐ unknown
      If yes, Debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _ ☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown
      Status epilepticus: ☐ yes ☐ no ☐ unknown
      If yes, Debut: ☐ at onset ☐ later, Date _ _/ _ _/_ _☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown
    - Focal: ☐ yes ☐ no ☐ unknown
      If yes, Specify: _____
      Debut: ☐ at onset ☐ later, Date _ _/ _ _/_ _☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown

      Epilepsia partialis continue: ☐ yes ☐ no ☐ unknown
      If yes, Debut: ☐ at onset ☐ later, Date _ _ /_ _/_ _☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown
    - Other: ☐ yes ☐ no ☐ unknown
      If yes, Specify: _____
      Debut: ☐ at onset ☐ later, Date _ _ /_ _/_ _ ☐ unknown
      Present at last follow up: ☐ yes ☐ no ☐ unknown

- Therapy resistant: ☐ yes ☐ no ☐ unknown
  If yes, specify; _____

  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Cerebrovascular: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Stroke: ☐ yes ☐ no ☐ unknown
    If yes, Specify: _____

    Debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Stroke like episodes: ☐ yes ☐ no ☐ unknown
    If yes, Specify: _____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Migraine: ☐ yes ☐ no ☐ unknown
    If yes, Specify: _____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown
- Motor: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Limb weakness: ☐ yes ☐ no ☐ unknown
    If yes, specify character: _____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Muscle atrophy: ☐ yes ☐ no ☐ unknown
    If yes, specify character: _____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Hypotonia: ☐ yes ☐ no ☐ unknown
    If yes, specify character: _____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

- Hypertonia: ☐ yes ☐ no ☐ unknown
  If yes, specify character: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Dystonia: ☐ yes ☐ no ☐ unknown
  If yes, Specify: _____
  Debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Ataxia: ☐ yes ☐ no ☐ unknown
  If yes, Specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Spasticity : ☐ yes ☐ no ☐ unknown
  If yes, Specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Weakness ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Bradykinesia: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Chorea/athetosis: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Other motor disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Other: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Hearing impairment: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
    Sensorineural hearing loss: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Peripheral neuropathy: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Fatigue: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Exertional myalgia: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Other not otherwise specified: ☐ yes ☐ ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown

- Psychomotor developmental disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Global developmental delay: ☐ yes ☐ no ☐ unknown
    If no, specify:
    ☐Gross motor delay ☐ fine motor delay ☐ speech ☐ social ☐ Unknown

- Neuropsychiatric and cognitive disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Depression: ☐ yes ☐ no ☐ unknown
    If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Psychosis: ☐ yes ☐ no ☐ unknown
    If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown

  - Dementia: ☐ yes ☐ no ☐ unknown
    If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up : ☐ yes ☐ no ☐ unknown

  - Visual hallucination: ☐ yes ☐ no ☐ unknown
    If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up : ☐ yes ☐ no ☐ unknown

  - Mental retardation: ☐ yes ☐ no ☐ unknown
    If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    WPPSI III test☐ performed ☐ not performed ☐ unknown
    WISC IV test ☐ performed ☐ not performed ☐ unknown
    WAIS test ☐ performed ☐ not performed ☐ unknown
    WMS test ☐ performed ☐ not performed ☐ unknown

- Ophthalmological disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Ptosis: ☐ yes ☐ no ☐ unknown
    If yes, symmetry: ☐ bilateral ☐unilateral ☐ unknown
    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown

- Progressive external ophthalmoplegia: ☐ yes ☐ no ☐ unknown

  If yes, symmetry: ☐bilateral ☐unilateral ☐unknown

  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Cataract: ☐ yes ☐ no ☐ unknown

  If yes, symmetry: ☐bilateral ☐unilateral ☐unknown

  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Strabismus: ☐ yes ☐ no ☐ unknown

  If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  Present at last follow up : ☐ yes ☐ no ☐ unknown

- Nystagmus: ☐ yes ☐ no ☐ unknown

  If yes, debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Pigmentary retinopathy: ☐ yes ☐ no ☐ unknown

  If yes, specify:_____

  Debut: ☐ at onset ☐ later, Date_ _/ _ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Visual impairment: ☐ yes ☐ no ☐ unknown

  If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Cortical blindness : ☐ yes ☐ no ☐ unknown

  If yes, debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Other ophthalmological disorder: ☐ yes ☐ no ☐ unknown

  If yes, specify:_____

  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

  State at last follow up: ☐ yes ☐ no ☐ unknown

- Cardiac disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Cardiomyopathy: ☐ yes ☐ no ☐ unknown
    If yes, specify;

    ☐ Dilated cardiomyopathy

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

    ☐ Hypertrophic cardiomyopathy

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

    ☐ Hypertension

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

    ☐ Other____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

    ☐Unknown

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

  - Other cardiac disorder: specify _____
    If yes, Specify: _____

    Debut: ☐ at onset ☐ later, Date _ _/ _ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown
- Endocrine disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Diabetes mellitus type 1: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____

    Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown

    Present at last follow up: ☐ yes ☐ no ☐ unknown

- Diabetes mellitus type 2: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Exocrine pancreatic insufficiency: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Hypogonadism: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ / _ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Delayed puberty: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Irregular menses: ☐ yes ☐ no ☐ not applicable ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Repeated miscarriage: ☐ yes ☐ no ☐ not applicable ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Adrenal insufficiency: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Growth hormone deficiency: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Other endocrine disorder : ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown

- Gastrointestinal and nutritional disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Failure to thrive: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown

  - Feeding difficulties: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ / _ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Gastrointestinal dysmotility: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Chronic diarrhoea : ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Vomiting: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Other gastrointestinal disorder : ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown

- Hematologic disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Anaemia: ☐ yes ☐ no ☐ unknown
    If yes, specify: ☐Microcytic ☐ Macrocytic ☐ normocytic ☐ unknown
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Leukopenia: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Thrombocytopenia: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Other haematological disorders: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
- Other disorders: ☐ yes ☐ no ☐ unknown
  If yes, specify:
  - Renal disorder: ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Hepatic disorder : ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown
  - Respiratory disorder : ☐ yes ☐ no ☐ unknown
    If yes, specify: _____
    Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
    Present at last follow up: ☐ yes ☐ no ☐ unknown

- Other disorder not otherwise specified: ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
  Debut: ☐ at onset ☐ later, Date _ _/_ _ /_ _ ☐ unknown
  Present at last follow up: ☐ yes ☐ no ☐ unknown
- Smoking : ☐ yes ☐ no ☐ unknown
  If yes, specify: ☐ at onset ☐ later, Date _ _/_ _/_ _ ☐ unknown
  Smoking at last follow up: ☐ yes ☐ no ☐ unknown

Disease Course

- Has the patient experienced acute exacerbation(s)/relapse(s):
  ☐ yes ☐ no ☐ unknown
  - Main cause : ☐ infection ☐ side effect of medication
    ☐ other, specify_____ ☐unknown
  - Treatment required :
    ☐ outpatient care ☐hospitalization ☐ICU ☐ unknown
  - Type(s) of exacerbation symptoms: ☐Motor ☐seizures
    ☐ cognitive ☐other, specify_____. ☐unknown

Survival Status

- Last known status : ☐alive ☐deceased ☐ unknown/ lost to follow up
  If deceased, specify
  - Date of death _ _ / _ _ / _ _ _ _ (dd/ mm/yyyy)
  - Cause of death : ☐Status epilepticus ☐Liver failure ☐Multi-organ failure
    ☐Sepsis ☐other_____ ☐ unknown
  - Post-mortem investigation perform ☐ yes ☐ no ☐ unknown

Laboratory Findings

- Respiratory chain enzyme activity : ☐ assayed ☐ not assayed/unknown

  If assayed, Date: _ _/ _ _/ _ _ ☐normal ☐abnormal
  If abnormal, specify: _____

	In muscle	Ref. Range	In liver	Ref. Range	In Fibroblast	Ref. Range
Complex I						
Complex II+III						
Complex IV						
Complex V						

- Blue native gel perform : ☐ yes ☐ no ☐ unknown
  If yes, specify: _____
- At onset :
  - Absolute values in blood/ serum:
    - pH: ____ , ref. __-__ ☐normal ☐not taken/ unknown
    - Base excess: : ____ , ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - Lactate: ____ mmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - Pyruvate: ____ mmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - Glucose : ____ mmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - Creatinine kinase: ____ IU/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - Albumin: ____ g/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - ASAT: ____ IU/L , ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
    - ALAT: ____IU/L , ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown

- GGT : ____ IU/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Hb: ____ g/dL, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- MCV ____ fl, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Vit B12 ____ pmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Folate ____ nmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
-
- Ferritin: ____ microgram/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Iron: ____ mircromol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown

➢ Absolute values in CSF:

- Lactate: ____ mmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Pyruvate: ____ mmol/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Albumin: ____ mg/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown
- Protein: ____ g/L, ref. __-__ ☐normal ☐abnormal ☐not taken/ unknown

- Most abnormal values noted over disease course:

➢ Absolute values in blood/ serum :

- pH: ____ , ref. __-__ , date: _ _/ _ _/ _ _ ☐normal ☐abnormal ☐not taken/ unknown
- Base excess: : ____ , ref. __-__ , date: _ _/ _ _/ _ _ ☐normal ☐abnormal ☐not taken/ unknown

- Lactate: ____ mmol/L, ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown
- Pyruvate: ____ mmol/L , ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown
- Glucose : ____ mmol/L, ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown

- Creatinine kinase: ____ IU/L, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal

  ☐abnormal ☐not taken/ unknown
- Albumin: ____ g/L, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal

  ☐abnormal ☐not taken/ unknown
- ASAT: ____ IU/L, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal

  ☐abnormal ☐not taken/ unknown
- ALAT: : ____ IU/L, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal

  ☐abnormal ☐not taken/ unknown
- Hb: ____ g/dL, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal

  ☐abnormal ☐not taken/ unknown
- MCV ____ fL, ref. __-__ ☐normal ☐abnormal

  ☐not taken/ unknown
- Vit 12 ____ pmol/L, ref. __-__ ☐normal ☐abnormal

  ☐not taken/ unknown
- Folate ____ nmol/L, ref. __-__ ☐normal ☐abnormal

  ☐not taken/ unknown
-
- Ferritin: ____ mircromol/L, ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown
- Iron: ____ mircromol/L, ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown

➢ Absolute values in CSF:

- Lactate: ____ mmol/L, ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown
- Pyruvate: ____ mmol/L, ref. __-__ , date: _ _/ _ _/ _ _

  ☐normal ☐abnormal ☐not taken/ unknown

- Albumin: ____ mg/L, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal ☐abnormal ☐not taken/ unknown
- Protein: ____ g/L, ref. __-__ , date: _ _/ _ _/ _ _ ☐normal ☐abnormal ☐not taken/ unknown

- Pathological total or free carnitine: ☐ yes ☐ no ☐ unknown
  If yes, date of the test : _ _/ _ _/_ _, absolute value:_____ ref: __-___
- Pathological acyl carnitine: ☐ yes ☐ no ☐ unknown
  If yes, date of the test : _ _/_ _ /_ _ (, absolute value:_____ ref: __-___

- Muscle pathology abnormality: ☐ yes ☐ no ☐ unknown / not perform
  If yes
  - Date: _ _/ _ _/ _ _ (dd/ mm/yy)

Regged red fibers	SDH positive fibers	COX negative fibers	Excess lipid
□ yes □ no □ unknown	□ yes □ no □ unknown	□ yes □ no □ unknown	□ yes □ no □ unknown

  - Other Specify:_____
  - Electron microscopy performed : ☐ yes ☐ no ☐ unknown / not perform
  - If yes , specify _____
- Liver pathology abnormality: ☐ yes ☐ no ☐ unknown / not perform
  If yes
  - Date: _ _/ _ _/ _ _ (dd/ mm/yy)
  - Specify:_____

- Pathological EEG: ☐ yes ☐ no ☐ unknown / not perform
  If yes:
  - Date_ _/ _ _/_ _ (dd/mm/yy)
  - Finding _____
  - Epi.activity: ☐ yes ☐ no ☐ unknown

  If yes : ☐frontal☐ parietal ☐temporal ☐occipital ☐ multifocal ☐unknown
  - Repeated examination: ☐ no ☐progression☐ no clear change ☐unknown
- Pathological EMG: ☐ yes ☐ no ☐ unknown / not perform
  If yes:
  - Date _ _ /_ _/_ _ (dd/mm/yy)

- Finding: ☐ Myopathic ☐ neuropathic ☐ other _____ ☐ unknown

- Pathological nerve conduction : ☐ yes ☐ no ☐ unknown / not perform
  If yes:
  - Date _ _/_ _/_ _ (dd/ mm/yy)
  - Finding: ☐ axonal ☐demyelinating ☐combination ☐other____ ☐ unknown

- Pathological VER : ☐ yes ☐ no ☐ unknown / not perform
  If yes: Date _ _/_ _/_ _ (dd/ mm/yy)

- Pathological ERG : ☐ yes ☐ no ☐ unknown / not perform
  If yes: Date _ _/_ _/_ _ (dd/ mm/yy)

MtDNA Findings

- Genetic analysis method :

  ☐ Long PCR ☐ Real Time PCR ☐Other, specify _____

Tissue	mtDNA deletion (YES / NO / % if available)	mtDNA depletion (YES / NO / % if available)
Skeletal muscle	☐yes☐ no, %:	☐yes ☐no; %:
Liver	☐yes☐no, %:	☐yes☐no, %:
Brain	☐yes☐no, %:	☐yes☐no, %:
Other	☐yes☐no, %:	☐yes☐no, %:
Other	☐yes☐no, %:	☐yes☐no, %:

Imaging

- First cerebral MRI : date :_ _/ _ _/ _ _ (dd/ mm/yy)

  Finding: ☐ yes ☐ no ☐ unknown / not perform

  If abnormal please complete the below **MRI registration form** :

Region	Signal abnormality				Atrophy
☐Putamen	☐ No signal abnormality				☐ no atrophy
	T1:☐increased ☐decreased	☐ Bilateral ☐Unilateral	T2:☐increased ☐decreased	☐Bilateral ☐Unilateral	☐ bilateral ☐ unilateral
☐Caudate nucleus	☐ No signal abnormality				☐ no atrophy
	T1:☐increased ☐decreased	☐ Bilateral ☐Unilateral	T2☐increased ☐decreased	☐ Bilateral ☐Unilateral	☐ bilateral ☐ unilateral
☐Global pallidus	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐ increased ☐ decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐ Thalamus	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐N subthalamus	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐ Midbrain	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐Pons	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐medulla	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐Grey matter	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2:☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐White matter	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐vermis	☐ No signal abnormality				☐ no atrophy

	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐ peduncles	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐Dentae nucleus	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐cerebral cortex	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐supraten torial white matter	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐corpus callosum	☐ No signal abnormality				☐ no atrophy
	T1:☐ increased ☐ decreased	☐ bilateral ☐ unilateral	T2☐increased ☐decreased	☐ bilateral ☐ unilateral	☐ bilateral ☐ unilateral
☐ other					

- 2nd cerebral MRI: ☐ performed ☐ unknown / not perform
  If performed, date :_ _/ _ _/ _ _ (dd/ mm/yy)

  ☐ progression,_____ ☐ regression,_____ ☐ no changed
  Other notes :

  If this is the first abnormal MRI please complete the MRI registration form

- Last cerebral MR: ☐ performed ☐ unknown / not perform
  If performed, date :_ _/ _ _/ _ _ (dd/mm/yy)

  ☐ progression,_____ ☐ regression,_____ ☐ no changed
  If this is the first abnormal MRI please complete the MRI registration form
  Other notes:

- MRI of the thigh: ☐ performed ☐ unknown / not perform
  If performed, date : _ _/ _ _/ _ _ (dd/mm/yy)
  Specify:_____
- MRS: ☐ yes ☐ no ☐ unknown / not perform
  If normal , date :_ _/ _ _/ _ _ (dd/ mm/yy)
  If abnormal , date :_ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ elevated lactate region _____

  ☐ elevated choline region _____

  ☐ elevated succinate region _____

  ☐ decreased NAA region _____

  ☐ other, specify _____ region _____
- CT finding 1st CT : ☐ normal ☐ abnormal ☐ unknown / not perform
  If normal, date : _ _/_ _/ _ _ (dd/ mm/yy)
  If abnormal, date : _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ calcifications region _____

  ☐ high attenuation region _____

  ☐ low attenuation region _____

  ☐ other, specify _____ region _____

Treatment

- Antiepileptic treatment: ☐ yes ☐ no ☐ unknown

  ☐ single antiepileptic drug, specify _____ date started : _ _/ _ _/_ _

  ☐ combination of antiepileptic drugs , specify _____

  ☐ antiepileptic drugs used with no effect : _____, _____,_____,_____,_____

  ☐ valproate , date started: _ _/ _ _/_ _.

  ☐ on-going antiepileptic drugs: effect : □ no apparent □positive □ unknown

  ☐ discontinued, reason(s):_____

- Ketogenic diet : ☐ yes ☐ no ☐ unknown
  If yes : date started : _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect : ☐ no apparent ☐positive ☐ unknown

  ☐ discontinued, reason(s):_____

- Coenzyme Q10: ☐ yes ☐ no ☐ unknown
  If yes : date started : _ _/ _ _ / _ _ (dd/mm/yy)

  ☐ on-going: effect : ☐ no apparent ☐positive ☐ unknown

  ☐ discontinued, reason(s):_____

- Thiamine: ☐ yes ☐ no ☐ unknown
  If yes : date started : _ _/ _ _ / _ _ (dd/mm/yy)

  ☐ on-going: effect : ☐ no apparent ☐positive ☐ unknown

  ☐ discontinued, reason(s):_____

- Nicotinamide: ☐ yes ☐ no ☐ unknown
  If yes : date started : _ _ / _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect : ☐ no apparent ☐positive ☐ unknown

  ☐ discontinued, reason(s):_____

- Riboflavin: ☐ yes ☐ no ☐ unknown
  If yes: date started : _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect: ☐ no apparent ☐positive ☐ unknown

  ☐ discontinued, reason(s):_____

- Carnitine: ☐ yes ☐ no ☐ unknown
  If yes: date started : _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect: ☐ no apparent ☐positive ☐ unknown

☐ discontinued, reason(s):_____

- Folic acid: ☐ yes ☐ no ☐ unknown
  If yes: date started : _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect: ☐ no apparent ☐positive ☐ unknown

  ☐ discontinued, reason(s):_____
- Antidiabetic treatment: ☐ yes ☐ no ☐ unknown
  If yes: date started: _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐on-going: effect: ☐ no apparent ☐positive ☐ unknown

  ☐discontinued, reason(s):_____
- Oral contraceptive: ☐ yes ☐ no ☐ unknown
  If yes: date started: _ _/ _ _ / _ _ (dd/ mm/yy)

- Other: ☐ yes ☐ no ☐ unknown
  If yes: date started: _ _ / _ _ (mm/yy)

  ☐on-going: effect: ☐ no apparent ☐positive ☐ unknown

  ☐discontinued, reason(s):_____
- Other: ☐ yes ☐ no ☐ unknown
  If yes: date started: _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect: ☐ no apparent ☐ positive ☐ unknown

  ☐ discontinued, reason(s):_____
- Other: ☐ yes ☐ no ☐ unknown
  If yes: date started: _ _/ _ _ / _ _ (dd/ mm/yy)

  ☐ on-going: effect: ☐ no apparent ☐ positive ☐ unknown

  ☐ discontinued, reason(s):_____

Other details :

Investigator signature

I have carefully checked all the data recorded in the present case report form and I confirm that they are true, complete and accurate to the best of my knowledge.

Investigator name: _____

Registration completed: yes ☐ no ☐

Signature date: _____

11. Original publications (PAPERS I, II, III and IV)

PAPER II

Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset; studies using a cohort of 155 cases.

Hikmat O, Naess K, Engvall M, Klingenberg C, Rasmussen M, Tallaksen CME, Brodtkorb E, Ostergaard E, de Coo I.F.M, Pias-Peleteiro L, Isohanni P, Uusimaa J, Darin N, Rahman S, Bindoff LA.

J Inherit Metab Dis. DOI: 10.1002/jimd.12211.

Erratum: Page 7; 3.6 Genetic findings: the correct cDNA position is c.1399G>A (p.Ala467Thr).

Received: 14 August 2019 | Revised: 2 January 2020 | Accepted: 3 January 2020

DOI: 10.1002/jimd.12211

ORIGINAL ARTICLE

JIMD SSIEM WILEY

Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset; studies using a cohort of 155 cases

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Funding information
Helse Vest Regionalt Helseføretak, Grant/Award Number: 91144; NeMO foundation, Grant/Award Number: 17_P19; Lily Foundation; NIHR Great Ormond Street Hospital Biomedical Research Centre; Great Ormond Street Hospital Children's Charity

Communicating Editor: Saskia Brigitte Wortmann

Summary

Background: Variants in *POLG* are one of the most common causes of inherited mitochondrial disease. Phenotypic classification of POLG disease has evolved haphazardly making it complicated and difficult to implement in everyday clinical practise. The aim of our study was to simplify the classification and facilitate better clinical recognition.

Methods: A multinational, retrospective study using data from 155 patients with *POLG* variants recruited from seven European countries.

Results: We describe the spectrum of clinical features associated with *POLG* variants in the largest known cohort of patients. While clinical features clearly form a continuum, stratifying patients simply according to age of onset—onset prior to age 12 years; onset between 12 and 40 years and onset after the age of 40 years, permitted us to identify clear phenotypic and prognostic differences. Prior to 12 years of age, liver involvement (87%), seizures (84%), and feeding difficulties (84%) were the major features. For those with onset between 12 and 40 years, ataxia (90%), peripheral neuropathy (84%), and seizures (71%) predominated, while for those with onset over 40 years, ptosis (95%), progressive external ophthalmoplegia (89%), and ataxia (58%) were the major clinical features. The earlier the onset the worse the prognosis. Patients with epilepsy and those with compound heterozygous variants carried significantly worse prognosis.

Conclusion: Based on our data, we propose a simplified POLG disease classification, which can be used to guide diagnostic investigations and predict disease course.

KEYWORDS
Alpers syndrome, epilepsy, mitochondrial disease, POLG, stroke-like episodes

1 | INTRODUCTION

Mitochondria are intracellular organelles found in almost all human cells. Their key function is the production of adenosine triphosphate through the process of oxidative phosphorylation performed by the mitochondrial respiratory chain (MRC). The MRC comprises more than 90 proteins organised into five complexes (I-V). Mitochondrial DNA (mtDNA) codes for 13 proteins while the remaining proteins are encoded by nuclear DNA.[1] The enzyme that replicates and repairs mtDNA, polymerase γ,[2] is a heterotrimer comprising a catalytic subunit (POLG) and two accessory subunits (POLG2). Mutations in *POLG* (OMIM * 174763), the nuclear gene encoding the catalytic subunit, interfere with mtDNA maintenance.[2,3]

Variants in *POLG* are the single most common cause of inherited mitochondrial disease.[4] The first *POLG* variant associated with disease was described in a family with autosomal dominant progressive external ophthalmoplegia (PEO[5]), but since then, more than 190 disease-causing variants have been identified (http://tools.niehs.nih.giv/polg). *POLG* variants are associated with a wide spectrum of overlapping phenotypes ranging from devastating fatal neonatal disease to a mild late onset disease with myopathy and PEO. A summary of the major *POLG*-related phenotypes reported in the literature[4,6-22] is provided in Table 1.

The clinical features of POLG disease are extremely heterogeneous making early clinical recognition challenging. The increasing numbers of terms that have been used to describe the clinical phenotypes (Table 1) have added to this confusion.

The clinical reports published so far were based on small numbers of patients and did not describe the clinical spectrum through the whole life span. Longitudinal studies describing the natural history of the disease in a large cohort of patients are still lacking.

TABLE 1 Summary of the major syndromes associated with *POLG* mutations reported in the literature

	Phenotype nomenclatures (reference)	Major clinical features	Age of onset
1	Myocerebrohepatopathy (MCHS)[9,18,22]	Myopathy, hypotonia, developmental delay, encephalopathy, liver failure.	Neonate, early infancy
2	Alpers-Huttenlocher Syndrome (AHS)[9,11,17]	Encephalopathy, psychomotor regression, refractory epilepsy liver dysfunction	Infancy, childhood, adolescence
3	Alpers syndrome[4,13,14]	Synonym of AHS	As in AHS
4	Alpers-Huttenlocher like[21]	Synonym of AHS	As in AHS
5	Infantile hepatocerebral syndrome[9]	Includes AHS and MCHS	Neonate, infancy, childhood
6	Infantile mitochondrial DNA depletion syndrome[19]	Includes both AHS and MCHS	Infancy, childhood
7	Leigh like[19]	Psychomotor retardation, hypotonia, extrapyramidal dysfunction, symmetrical hyperintensities on T2 weighted images in basal ganglia, brain stem, thalamus	Infancy
8	Mitochondrial Neuro-Gastro-Intestinal Encephalopathy (MNGIE) like[13,14,20]	Severe gastrointestinal dysmotility, encephalopathy, ptosis ophthalmoplegia, peripheral neuropathy	Childhood, adolescence adulthood
9	Myoclonus, epilepsy, myopathy, and sensory ataxia (MEMSA)[4]	Epilepsy, myopathy, ataxia, liver dysfunction, headache and stroke-like episodes	Adolescence, adulthood
10	Spinocerebellar ataxia with epilepsy (SCAE)[4,7]	Now incorporated under MEMSA umbrella	As in MEMSA
11	Mitochondrial spinocerebellar ataxia with epilepsy (MSCAE)[6]	Now incorporated under MEMSA umbrella	As in MEMSA
12	Ataxia neuropathy spectrum (ANS)[4,7]	Ataxia, neuropathy, psychiatric symptoms, cognitive impairment, epilepsy, and ophthalmoplegia	Adolescent and adult
13	Mitochondrial recessive ataxia syndrome (MIRAS)[10]	Now incorporated under ANS umbrella	As in ANS
14	Sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO)[4]	Now incorporated under ANS umbrella	As in ANS
15	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) like phenotype[23]	Headache, seizures, stroke-like episodes as in MEMSA	Adult
16	Recessive Charcot-Marie tooth like[12]	Axonal polyneuropathy, muscle weakness, wasting, tremor nystagmus, dysarthria, dysmetria, and dysdiadochokinesis.	Adult
17	Parkinsonism[8,16]	Tremor, rigidity, hypo/bradykinesia, balance disturbance	Adult
18	Autosomal recessive progressive external ophthalmoplegia (arPEO)[5,7]	Ptosis, ophthalmoparesis, may be associated with ataxia and myopathy	Adult, elderly
19	Autosomal dominant progressive external ophthalmoplegia (adPEO)[5,7]	Ptosis, ophthalmoparesis, myopathy, neuropathy, ataxia	Adult-elderly
20	Chronic progressive external ophthalmoplegia plus(CPEO+)[7]	Synonym of adPEO	Adult-elderly

In this study, we aimed to describe the natural history of POLG disease in the largest cohort of patients with confirmed *POLG* variants, focusing on the clinical features and the biomarkers which may predict the long-term prognosis. We aimed to provide a simpler clinical classification to facilitate early clinical recognition of patients with POLG disease.

2 | METHODS

2.1 | Study design, population, and data collection

We performed a multinational, retrospective study of patients from 12 centres in seven European countries:

Norway (Haukeland University Hospital, Oslo University Hospital, St. Olav's Hospital and University Hospital of Northern Norway); United Kingdom (Great Ormond Street Hospital, London); Sweden (Centre for Inherited Metabolic Diseases, Karolinska University Hospital, Stockholm and The Queen Silvia Children's Hospital, University of Gothenburg); Denmark (Department of Clinical Genetics, Copenhagen University Hospital); Finland (Children's Hospital, Helsinki University Hospital and Clinic for Children and Adolescents, Oulu University Hospital); Netherlands (Department of Genetics and Cell Biology, Maastricht University, Maastricht); and Spain (Sant Joan de Déu Children's Hospital, Barcelona). Patients diagnosed and followed at the participating centres were considered eligible if they had recessive disease and confirmed biallelic pathogenic/likely pathogenic *POLG* variants or dominant disease and heterozygous confirmed pathogenic variants. Data entry was completed in December 2017.

Detailed clinical, biochemical, muscle biopsy, neurophysiological, neuroimaging, and genetic data were obtained by using an electronic-case report form completed by the responsible investigator(s) at each centre and reviewed by the study-principal investigator (O.H.).

The date of disease onset was defined by the date of the first symptom(s) requiring medical evaluation. End of follow up was defined as the date of the patient's last visit to the follow-up centre or death. Available longitudinal data, both at disease onset and later during the disease course, were collected. Liver involvement was defined by the presence of two or more of the following parameters in at least two different time points; elevated aspartate aminotransferase, gamma-glutamyltransferase, bilirubin or ammonia, low serum albumin, or pathological histological findings of liver biopsy. The presence of anaemia and abnormal cerebrospinal fluid (CSF) protein and/or albumin was identified as described in previous publications.[13,14,24] We use the recent International League Against Epilepsy (ILAE) classification[25] for seizure classification.

2.2 | Data and statistical analysis

Detailed descriptive data analysis was performed on the entire study cohort using SPSS (Statistical Package of Social Sciences), Version 23.0. A two sided *P* value less than .05 was considered to be statistically significant. Mosaic plots was performed by using R (The R foundation for statistical computing), version 3.6.1.

In order to simplify the clinical classification patients were grouped according to the age of disease onset into three groups: (a) those with disease onset prior to the age of 12 years (before puberty), (b) those with disease onset between 12 and 40 years, and (c) those with disease onset after the age of 40 years. The age of onset of each individual symptom was recorded and classified according to these three defined age-groups. Correspondence analysis was performed to examine the relationship between two variables (groups of patients who were classified according to the age of onset as described and the age of onset of each individual symptom) graphically in a multidimensional space; this allowed examination of the clustering of symptoms around each age group. Further, mosaic plots was performed to study the differences between the above mentioned groups. The study cohort was also classified according to the presence or absence of epilepsy, regardless the age of onset.

For survival analysis, the end-point was time to death which was defined as the time in months from the date of disease onset to the date of death. Univariate survival analysis was performed using log-rank test (Kaplan-Meier) to compare differences in survival time between categories.

3 | RESULTS

3.1 | Demography

One hundred and fifty-five patients, (males n = 76 [49%], females n = 79 [51%]) with confirmed pathogenic *POLG* variants were identified. Seventy-six were diagnosed in Norway, 44 in Sweden, 19 in the United Kingdom, 8 in Finland, 5 in Denmark, 2 in The Netherlands, and 1 in Spain. The majority of patients were Northern European (n = 146), while three patients were from Iraq, two from Cyprus and one from Croatia, Pakistan, Spain, and the United Arab Emirates.

3.2 | Major clinical features

Median age at disease onset for the whole study cohort was 10 years (range: birth—71 years). Disease onset prior to the age of 12 years occurred in 54% (n = 83/155), between 12 and 40 years of age in 34% (n = 53/155), and after the age of 40 years in 12% (n = 19/155) had. Disease debut was apparently spontaneous in 113/155 (73%), followed an infectious illness in 32/155 (21%) and not clearly reported in 10/155 (6%) of the patients.

Neurological (90%, n = 139/155), ophthalmological (74%, n = 112/151), and gastrointestinal symptoms (63%, n = 92/146) were the most predominant clinical features. Epilepsy was reported in 69% (n = 107/155), with focal and focal evolving to bilateral tonic-clonic seizures being

TABLE 2 Major clinical features of patients reported in this study

Major clinical features	Number of patients	Number of patients at onset	Number of patients later
1. Neurological			
Seizure	107/154 (69%)	73/106 (69%)	33/106 (31%)
Focal	94/102 (92%)	60/91 (66%)	31/91 (34%)
Focal evolving to bilateral tonic-clonic	85/100 (85%)	43/85 (51%)	42/85 (49%)
Myoclonic	73/98 (74%)	26/71 (37%)	45/71 (63%)
Epilepsia partialis continua	52/91 (57%)	15/52 (29%)	37/52 (71%)
Convulsive status epilepticus	79/101 (78%)	30/78 (38%)	48/78 (62%)
Others[a]	11/80 (14%)	5/10 (50%)	5/10 (50%)
Ataxia	87/138 (63%)	53/85 (62%)	32/85 (38%)
Hypotonia	68/135 (50%)	50/66 (76%)	16/66 (24%)
Limb weakness	89/125 (71%)	33/83 (40%)	50/83 (60%)
Migraine-like headache	52/143 (36%)	38/52 (73%)	14/52 (27%)
Peripheral neuropathy	65/123 (53%)	23/63 (36%)	40/63 (64%)
Sensorineural hearing loss	16/146 (11%)	9/16 (56%)	7/16 (44%)
2. Ophthalmological			
Ptosis	51/149 (34%)	28/49 (57%)	21/49 (43%)
Progressive external ophthalmoplegia	56/146 (38%)	24/56 (43%)	32/56 (57%)
Nystagmus	55/146 (38%)	29/53 (55%)	24/53 (45%)
Cataract	11/148 (7%)	2/9 (22%)	7/9 (78%)
Cortical blindness	32/111 (29%)	17/29 (59%)	12/29 (41%)
Pigmentary retinopathy	3/140 (2%)	1/2 (50%)	1/2 (50%)
3. Gastrointestinal			
Feeding difficulties	75/145 (52%)	36/73 (49%)	37/73 (51%)
Vomiting	52/137 (38%)	31/49 (63%)	18/49 (37%)
Chronic diarrhoea	8/136 (6%)	2/8 (25%)	6/8 (75%)
Liver involvement	95/151 (64%)	35/93 (38%)	58/93 (62%)
Others[b]	7/128 (5%)	2/7 (29%)	5/7 (71%)
4. Endocrinological			
Diabetes mellitus type 1	1/151 (1%)	0/1 (0%)	1/1 (100%)
Diabetes mellitus type 2	2/151 (1%)	2/2 (100%)	0/2 (0%)
Adrenal insufficiency	2/151 (1%)	1/2 (50%)	1/2 (50%)
Growth hormone deficiency	2/147 (1%)	1/2 (50%)	1/2 (50%)
Others[c]	3/140 (2%)	0/3 (0%)	3/3 (100%)
5. Others			
Anaemia	77/136 (57%)	20/77 (26%)	57/77 (74%)
Renal disorders[d]	13/149 (8%)	3/12 (25%)	9/12 (75%)
Respiratory disorders[e]	18/149 (12%)	5/18 (28%)	13/18 (72%)

[a]One infantile spasms, eight absence, one atonic seizure, and one non convulsive status epilepticus.
[b]One Coeliac disease, one constipation, one paralytic ileus, one acute colon necrosis, one gastrointestinal bleeding, one colitis, and one milk protein intolerance.
[c]One hypothyroidism, one hypoparathyroidism, and one pseudo hypoparathyroidism.
[d]Seven renal tubular acidosis, five renal failure, and one renal stone.
[e]Two asthma, one chest deformity, two recurrent chest infections, two sleep apnoea, elven hypoventilation/respiratory insufficiency.

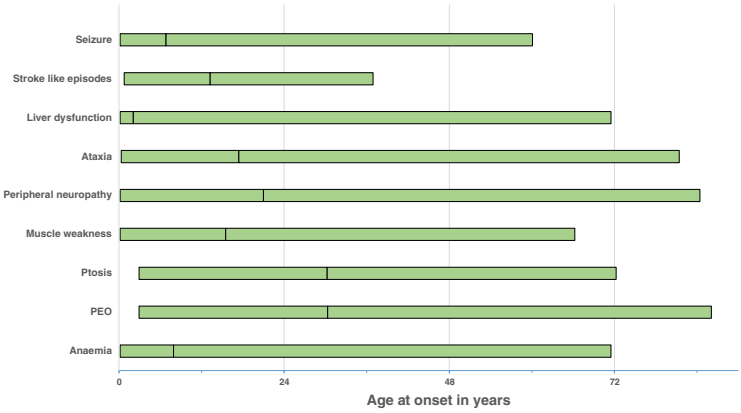

FIGURE 1 Age of onset of each individual symptom in patients with POLG disease. Range I, Median. PEO, progressive external ophthalmoplegia

TABLE 3 The onset of symptoms according to three age groups

Symptoms	<12 years 83/155 (54%)	12–40 years 53/155 (34%)	>40 years 19/155 (12%)
Seizures	69/82 (84%)	37/52 (71%)	1/19 (5%)
Ataxia	30/67 (45%)	46/51 (90%)	11/19 (58%)
Hypotonia	57/72 (79%)	9/44 (20%)	2/18 (11%)
Stroke-like episodes	26/73 (36%)	26/48 (54%)	None (0%)
Peripheral neuropathy	17/57 (30%)	38/45 (84%)	10/18 (65%)
Migraine-like headache	14/52 (27%)	36/52 (69%)	2/52 (4%)
Feeding difficulties	58/69 (84%)	13/47 (28%)	4/18 (22%)
Liver involvement	71/82 (87%)	23/49 (47%)	2/19 (11%)
Anaemia	49/71 (69%)	25/45 (56%)	3/16 (19%)
Ptosis	12/78 (15%)	21/50 (42%)	18/19 (95%)
PEO	7/83 (8%)	32/48 (67%)	17/19 (89%)
Survival time in months	19 (0.5–600)	151 (4–487)	191 (17–336)
Median (Range)			

Abbreviation: PEO, progressive external ophthalmoplegia.

the most common seizure types (92%, n = 94/102). Ataxia (63%, n = 87/138), peripheral neuropathy (53%, n = 65/123), and hypotonia (50%, n = 68/135) were frequently reported. Nystagmus (38%, n = 55/146), PEO (38%, n = 56/146), and ptosis (34%, n = 51/149) were the most commonly reported ophthalmological features. Liver involvement was identified in 64% (n = 96/151) of the patients. More than half of the study cohort had feeding difficulties (52%, n = 75/145), regardless of the age of onset. A detailed description of the clinical features is provided in Table 2.

3.3 | Age-related clinical features

We found clear evidence that the clinical features of POLG disease form a continuum (Figure 1) rather than distinct phenotypes (Table 1). Nevertheless, by grouping the patients into three groups according to the age of onset (early, juvenile/adult, and late onset groups), we could identify clear phenotypic and prognostic differences (Table 3). To confirm this finding, correspondence analysis was performed and demonstrated clear clustering of the symptoms around the three different age groups as illustrated in Figure S1. Further, mosaic plots showed there was a statistically significance differences in the phenotypes between the above mentioned groups (Figure S2).

3.4 | Laboratory, muscle biopsy, and neurophysiological findings

The percentage of those with raised lactate in serum and CSF at disease onset was 35% (n = 29/84) and 40% (n = 19/47), respectively. Abnormal elevated CSF protein at disease onset was reported in 68% (n = 44/68) of patients. In muscle biopsies, the presence of ragged-red fibres, COX-negative fibres, and abnormal respiratory chain activities was reported in fewer than the half of those who had been investigated (Table S1).

Electroencephalogram recordings showed that approximately half (54%, n = 58/107) of patients with epilepsy had epileptiform activities over the occipital lobes. Abnormal nerve conduction was observed in 70%

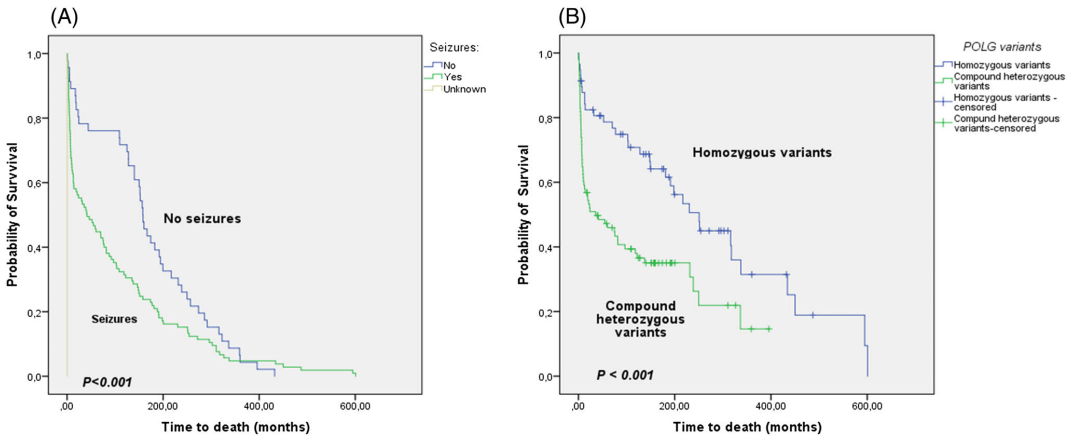

FIGURE 2 Survival analysis. A, Kaplan-Meier curve comparing survival of those with seizures and those without seizures and showed those with seizures carried significantly worse survival. B, Kaplan-Meier curve comparing survival of those with homozygous variants and those with compound heterozygous *POLG* variants and showed those with compound heterozygous variants carried significantly worse survival

(n = 43/61) of the individuals, the majority of those (81%, n = 35/43) had axonal neuropathy. None had a pure demyelinating neuropathy (Table S1).

3.5 | Neuroimaging findings

General cerebral atrophy (59%, n = 35/59) and cortical focal lesions (54%, n = 59/108) manifesting as T2/FLAIR hyperintensities affecting cortical and subcortical areas were the most frequently reported magnetic resonance imaging (MRI) abnormalities in the study group as a whole. These imaging findings were more prevalent in patients with epilepsy compared to those without epilepsy. A detailed description of MRI findings is provided in Table S2.

3.6 | Genetic findings

POLG variant(s) for each case were identified either by targeted variant analysis for specific common variants (c.1399G>C, p.Ala467Thr and c.2243G>C, p.Trp748Ser) or by sequence analysis of all coding regions of the *POLG* gene. All *POLG* variants identified in this study are illustrated in igure S3 and the individual mutation results are available on request.

A total of 41 different *POLG* variants were identified in the 155 individuals described in this study. Ninety patients had compound heterozygous variants, 59 had homozygous variants, and 6 patients had a heterozygous variant associated with autosomal dominant disease, mainly autosomal dominant progressive external ophthalmoplegia. The majority (n = 58/83) of patients with early onset disease (before the age of 12 years) had compound heterozygous pathogenic *POLG* variants (regardless of the variant types). The opposite was found in those with juvenile/adult onset disease in whom the majority (n = 32/52) had homozygous pathogenic variants. Frequency data for the homozygous variant c.1399G>C, p.(Ala467Thr, compound heterozygous variants c.1399G>C, p.(Ala467Thr)/c.2243G>C, p.(Trp748Ser), and the homozygous variant c.2243G>C, p.(Trp748Ser) for each of the three age groups are provided in Table S3.

3.7 | Survival analysis

Of the155 patients, 61 were alive at the time of data analysis and one had been lost to follow-up. Median age at death was 7.4 years (range 1 month to 91 years). The main cause of death was liver failure (32%, n = 30/93), followed by infection/sepsis (20%, n = 19/93), multiorgan failure (19%, n = 18/93), status epilepticus (14%, n = 13/93), one suicidal death. The cause of death was unknown in 13% (n = 12/93) of the individuals.

Further analysis showed that median survival time from disease onset to death was 19 months (range 0.5-600 months, interquartile range [IQR] 111) for those with disease onset prior to the age 12 years, 151 months (range 4-487, IQR 255) for those with disease onset between 12 and 40 years, and 191 months (range 17-336,

IQR 101) for those with disease onset after the age of 40 years.

The presence of epilepsy was associated with significantly worse survival ($P < .001$), and the median survival time from seizure onset to death was 37 months (range <1-487). Survival analysis also showed that patients with pathogenic compound heterozygous *POLG* variants had significantly ($P < .001$) worse survival compared to those with pathogenic homozygous variants, regardless of specific variant types (Figure 2). Further analysis showed that survival after the onset of seizures in those with early onset disease was significantly worse than those who developed seizures as part of juvenile/adult onset disease. Further, patients who developed liver involvement showed a significantly worse survival than those without liver impairment (Figure S4).

4 | DISCUSSION

We present the detailed description of 155 patients with confirmed pathogenic *POLG* variants focusing on the clinical features, but including laboratory, genetic, and neuroimaging findings. As far as we can ascertain, this is the largest cohort of patients with POLG disease so far described. In addition to the descriptive element, we have also analysed factors, which may predict the prognosis.

We defined the age of onset of each individual symptom and our data confirms that POLG disease comprises a continuum of clinical features rather than a set of separate clinical identities (Figure 1). Apart from PEO/ptosis, all other symptoms could start from infancy to adulthood. While hypotonia and feeding difficulties in infants are likely due to different pathological processes than these features appearing in adults, seizures, peripheral neuropathy, ataxia, muscle weakness, and hepatic disturbance have a similar basis and all could present at any age. PEO/ptosis starts later and appears mainly in patients with dominantly inherited disease or in those with juvenile/early adult onset disease who do not develop epilepsy or, less often, survive despite it. Stroke-like episodes appear to start slightly later than most other features. This may reflect the nature of the process[26,27] namely that these represent prolonged seizure activity or status epilepticus.

If we look at the median ages of onset, instead of looking at the age range, we do see a tendency for the features to cluster according to age. We, therefore, reanalysed the data using different age groups. Based on these findings, we found that the clinical spectrum of POLG disease was best described by grouping patients into three categories of early, juvenile/adult, and late onset. Early onset disease was classified as beginning prior to the age of 12 years. In these patients, liver involvement, feeding difficulties, seizures, hypotonia, and muscle weakness were the most dominant/important clinical features and this group had the worst prognosis. The juvenile/adult onset form (12-40 years of age) was characterised by peripheral neuropathy, ataxia, seizures, stroke-like episodes and, in patients with longer survival, PEO. This group carried a better prognosis than the early onset group. Late onset disease (after the age of 40 years) was characterised by ptosis and PEO, with additional features such as peripheral neuropathy ataxia and muscle weakness occurring frequently. This group had the best prognosis. Thus, while the clinical features associated with *POLG* variants can present at any age, age of disease onset provides both clues to the diagnosis and information about the outcome (Table 3).

The most frequently reported neurological features, included seizures, ataxia, and peripheral neuropathy. Focal evolving to bilateral tonic-clonic seizures were the most common seizure types, with epileptiform activities predominantly seen in occipital regions. These findings are consistent with previous reports ([8,10,13-15,28,29]), however, our results also showed that seizures were the most predominant clinical feature in patients with early onset disease (<12 years), common in those with juvenile/adult onset (12-40 years), but infrequent in those with late onset disease (>40 years). Ataxia, peripheral neuropathy, and migraine-like headache were most predominant in individuals with juvenile/adult onset disease, although reported in both early and late onset disease. Ptosis and PEO were common in late onset disease as reported previously,[22] however our data showed that the onset of ptosis and PEO occurred in all age groups. The onset of gastrointestinal features such as feeding difficulties and liver involvement occurred at any age, but was predominantly seen in patients with early onset disease.

Demographic data showed that more than half of the individuals included in this study had onset during childhood (prior to the age of 12), and the incidence of the disease decreased with age. Contrary to previous publications,[9,15] which demonstrate some male predominance, we observed no gender difference.

Survival analysis demonstrated a clear correlation between the age of disease onset and the survival time; earlier onset was associated with worse prognosis (Table 3). Further analysis showed that the presence of epilepsy was significantly associated with worse prognosis regardless of the age of disease onset, and individuals harbouring compound heterozygous *POLG* variants had worse prognosis compared to those with homozygous variants.

Our study showed that laboratory investigations which are commonly used in the initial diagnostic work-up of

mitochondrial disorders, for example, raised blood and CSF lactate, the presence of ragged-red/COX-negative fibres and abnormal respiratory chain activities in the skeletal muscle have low diagnostic sensitivity, being present in fewer than 50% of the individuals. As we showed in a previous publication,[24] elevated CSF protein was the most sensitive (68%, n = 44/65) laboratory diagnostic biomarker at disease onset.

The majority of the patients included in this study were of Northern European descent; thus, a possible limitation of this study is that it might not be possible to extrapolate our findings to other ethnic groups. However, we provide detailed description of all the known phenotypes associated with POLG disease related to more than 40 different *POLG* variants. Moreover, there is little evidence in the literature of marked ethnic differences in phenotypic expression of POLG disease. Based on the diverse genotypic background of our population, we consider that the findings of our study are relevant to patients with POLG disease, regardless of the ethnic background.

A simple and robust clinical classification is the cornerstone of early diagnosis. Such a classification, together with diagnostic investigations, should facilitate easy recognition of the disease and be useful for both experts and physicians with limited experience of the field. Current nomenclature describing the phenotypic spectrum of POLG disease (Table 1) is complicated and includes overlapping clinical syndromes. This makes implementation in everyday clinical practice difficult. A clear and accurate classification that describes the full spectrum of disease taking account of age-related features is essential not only for optimal management, but also for research

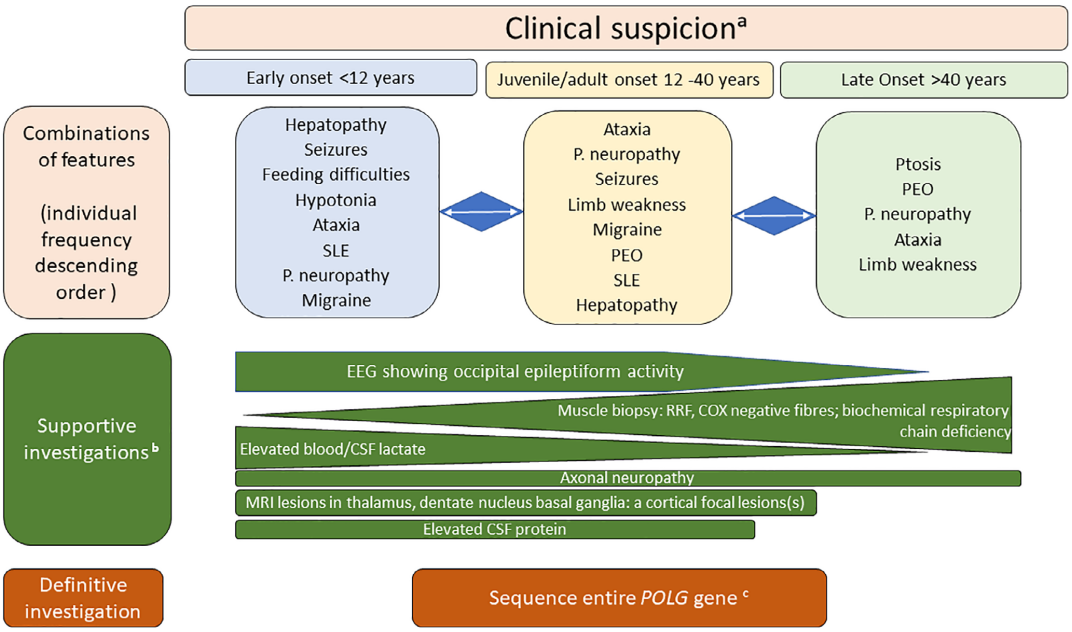

FIGURE 3 Diagnosing POLG disease; clinical suspicion and relevant investigations according to the age of onset. While we have shown that POLG clinical features form a continuum, but it is also clear that age plays a role in which features predominate. Based on our age groups, we can see clear clinical patterns and these will dictate which investigations are appropriate and useful. For example, in the older age category, PEO and ataxia dominate the clinical spectrum and in these cases one can choose either to screen the known genes or to take a muscle biopsy which give both structural clues (COX negative fibres) and the possibility to examine mtDNA (for multiple deletions). We also see that the typical occipital epilepsy occurs in the younger two categories and it is in these that MRI imaging also provides important clues. Peripheral neuropathy occurs in all age groups. In earlier studies, we showed that elevated CSF protein can be helpful, for example in a child with epilepsy and focal MRI changes it can be an important indicator of poor prognosis. a: Direct *POLG* gene sequence analysis is recommended to confirm the diagnosis in a case of strong clinical suspicion. b: Absence of these findings does not exclude the diagnosis of POLG disease. c: targeted variant analysis for the most common variants (p. Ala467Thr and p.Trp748Ser) can be performed first in juvenile and late onset disease, whole *POLG* gene sequence analysis is recommended for all early onset disease and those with strong clinical suspicion of POLG disease regardless of the age of onset. CSF, cerebrospinal fluid; RRF, ragged-red fibres; PEO, progressive external ophthalmoplegia; P. neuropathy, peripheral neuropathy; SLE, stroke- like episodes

and, when treatments become available, for use in clinical trials.

We provide a robust and simplified clinical classification based on data from the largest cohort of patients with POLG disease published to date. This classification highlights three distinct age groups and within these groups the major clinical features. Earlier classifications of POLG disease have focused primarily on phenotypic elements; for example, the presence of ataxia with or without myoclonus or epilepsy has variously been referred to as SANDO, ANS, or MIRAS/MSCAE. Early onset diseases have been separated into Alpers or MCHS or Leigh-like syndromes. The presence of mtDNA depletion has also been used to define POLG related disease although the presence of this is known to be tissue dependent and depletion in brain and liver is found in both young and older patients. We feel that these phenotypic labels create an unnecessarily complicated classification. Age alone appears robust enough to delineate the important features of POLG disease such that we would recommend simplifying classification to early onset, juvenile onset and late onset POLG disease. The algorithm (Figure 3) shows how recognition of these key clinical features could be used to direct clinical investigation in the different age groups.

ACKNOWLEDGMENTS

This work was supported by grants from the Western Norway Regional Health Authority (Helse-Vest, grant no. 911944). P.I. is supported by grant from the special governmental subsidy for health sciences research of the Helsinki University Hospital. S.R. is supported by research grant funding from Great Ormond Street Hospital Children's Charity, the NIHR Great Ormond Street Hospital Biomedical Research Centre, and the Lily Foundation. I.d.C. was supported by the NeMO foundation (no.17_P19). We would also thank professor Geir Egil Eide, Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway for his help with some of the statistical analysis.

CONFLICT OF INTEREST

All declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

O.H. and L.B. designed the study, were responsible for data collection, analysed the data, and drafted the initial manuscript, and approved the final manuscript as submitted. K.N., M. E., C. K., M.R., C.M.E.T., E.B., T.F., E.O., I.F.M.D., L.P., P.I., J.U., N.D., and S.R., were responsible for data acquisition and analysis, revising the manuscript critically, and approving the final manuscript as submitted. All authors are responsible for accuracy and integrity of the work.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Ethical approval for the study was obtained from the Regional Committee for Medical and Health Research Ethics, Western Norway (REK 2014/1783-4). Each participating country has obtained approval from their local ethical committee. The study was registered as an audit at Great Ormond Street Hospital, London, UK (Registration Number 1675). This article does not contain any studies with animal subjects performed by any of the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Hikmat O, Naess K, Engvall M, et al. Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset; studies using a cohort of 155 cases. *J Inherit Metab Dis*. 2020;1–11. https://doi.org/10.1002/jimd.12211

Supplementary table 1: Summary of the diagnostic investigations

Diagnostic investigations	Number of patients with pathological findings	
	At onset	Later
1-Blood		
Raised lactate	29/84(35%)	63/98(64%)
Raised creatine kinase	5/88(6%)	21/73(29%)
Low albumin	18/83(22%)	66/95(69%)
Raised aspartate aminotransferase	33/107(31%)	83/124(67%)
Raised alanine aminotransferase	29/120(24%)	89/137(65%)
2-Cerebrospinal fluid		
Raised lactate	19/47(40%)	26/39(67%)
Raised albumin	14/23(61%)	12/18(67%)
Raised protein	44/65(68%)	22/30(73%)
	Number of patients with pathological findings during the disease course	
3- Muscle biopsy		
Pathological finding in general	61/103(59%)	
Ragged-Red fibres	22/85(26%)	
COX-negative fibres	37/87(45%)	
Excessive lipid accumulation	22/82(27%)	
Abnormal respiratory chain activities	25/54(46%)	
4-EEG findings -epileptiform activities		
Frontal lobe	18/107(17%)	
Parietal lobe	25/107(23%)	
Temporal lobe	33/107(31%)	
Occipital lobe	58/107(54%)	
Multifocal	15/107(14%)	
5- EMG		
Pathological findings in general	25/55(45%)	
Myopathic	6/25(24%)	
Neuropathic	18/25(72%)	
Non-specified	1/25(4%)	
6-Nerve conduction study		
Pathological findings in general	43/61(70%)	
Axonal	35/43(81%)	
Demyelinating	0/43(0%)	
Combined	5/43(12%)	
Non-specified	3/43(7%)	

Supplementary table 2: Neuroimaging findings

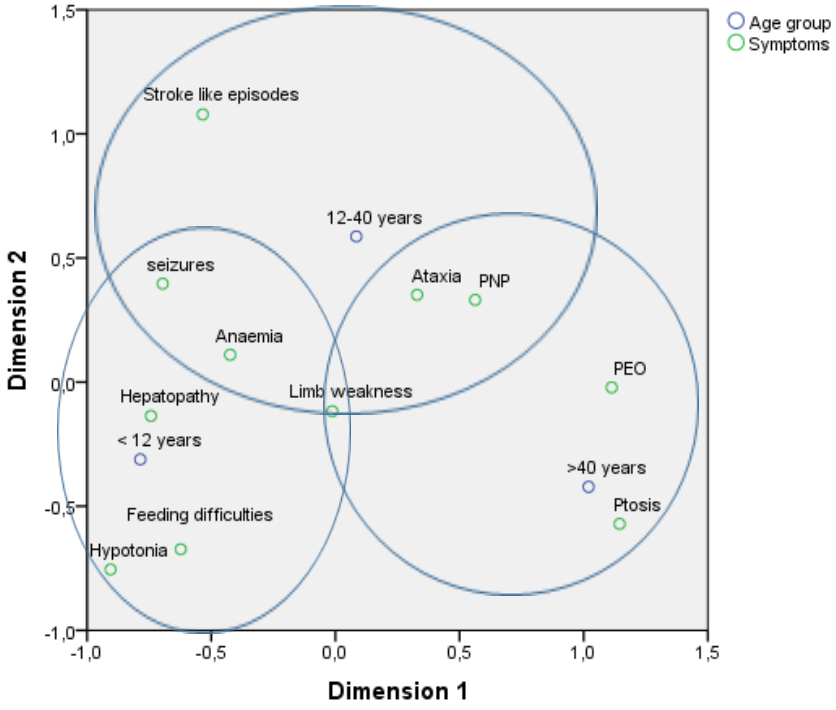
Cerebral MRI findings	General	Epilepsy+	Epilepsy -
Cortical focal lesions	59/108(54%)	54/80(68%)	2/29(7%)
Generalised cerebral atrophy	35/59(59%)	14/27(52%)	3/8(38%)
Putamen lesions	4/106(4%)	3/77(4%)	1/29(3%)
Caudate nucleus lesions	6/104(6%)	6/75(8%)	0/29(0%)
Thalamus lesions	44/109(4%)	39/80(49%)	5/29(17%)
Pons lesions	10/105(10%)	4/76(5%)	6/29(21%)
White matter lesions	8/105(8%)	6/79(8%)	2/29(7%)
Vermis lesions	4/103(4%)	3/74(4%)	1/29(3%)
Dentate nucleus lesions	5/103(5%)	3/74(4%)	2/29(7%)

MRI: Magnetic Resonance Imaging, Epilepsy+: Subjects with epilepsy. Epilepsy -: subjects without epilepsy

Supplementary table 3: The frequency of Homozygous variant *c.1399G>C, p.(Ala467Thr, Hetrozygous variants c.1399G>C, p.(Ala467Thr)/ c.2243G>C, p.(Trp748Ser) and Homozygous variant c.2243G>C, p.(Trp748Ser)* according to 3 age groups.

POLG variants	Early onset (< 12 years)	Juvenile/adult onset (12-40 years)	Late onset (> 40 years)
Homozygous variant *c.1399G>C, p.(Ala467Thr)*	7 % (no.6 /83)	13%(no. 7/52)	5% (no.1/19)
Hetrozygous variants *c.1399G>C, p.(Ala467Thr)/ c.2243G>C, p.(Trp748Ser)*	6% (no.5/83)	23%(no.12/52)	5% (no. 1/19)
Homozygous variant *c.2243G>C, p.(Trp748Ser)*	13%(no.11/83)	51(no.27/52)	5%(no.1/19)

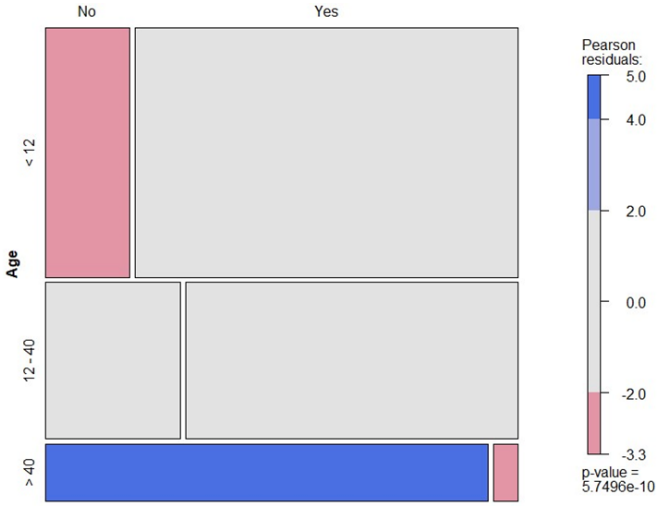
Supplementary Figure 1: Correspondence analysis showed clustering of variable symptoms around the three different age groups (onset prior to the age of 12 years, onset between the age of 12-40 years and onset after the age of 40 years).



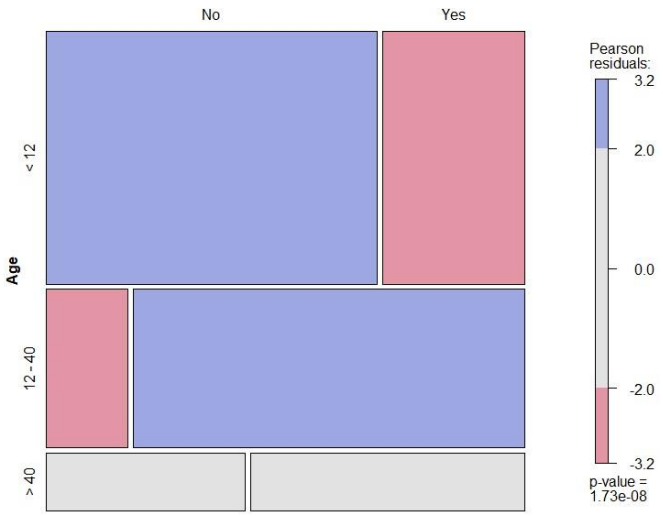
PEO: Progressive external ophthalmoplegia

Supplementary Figure 2 (A-K): Moisac plots shows there are a statistically significant differences between the 3 age groups (those with onset before 12 years, onset 12-40 years and onset after 40 years of age).

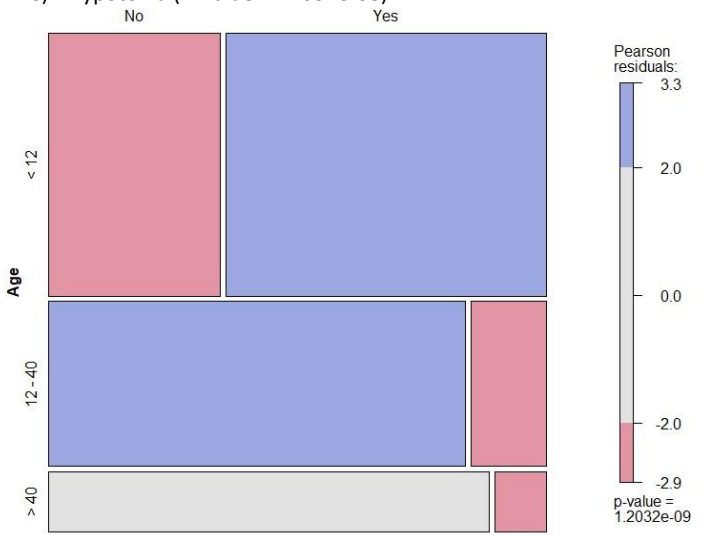
A) Seizures (P-value= 5,7496e-10)



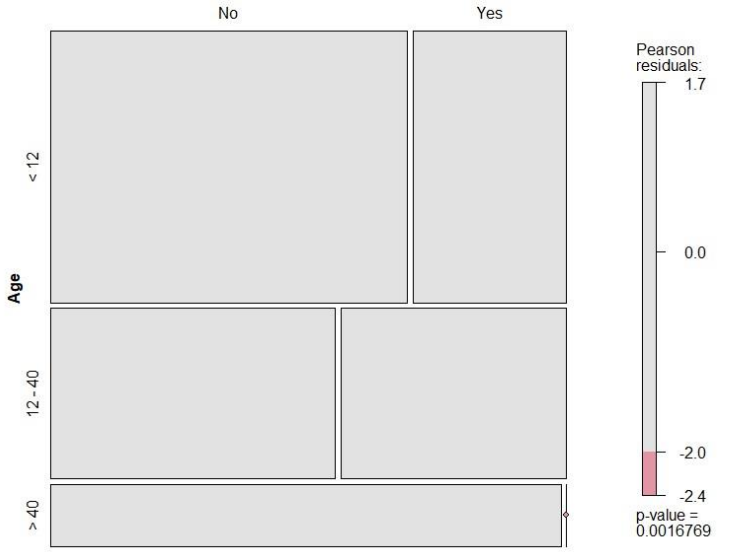
B) Ataxia (p-Value= 1,73e-08)



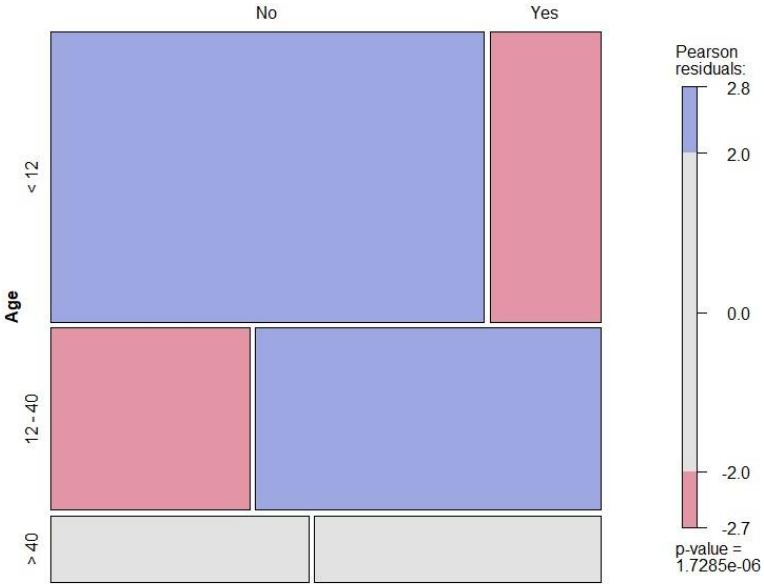
C) Hypotonia (P-Value= 1.2032e-08)



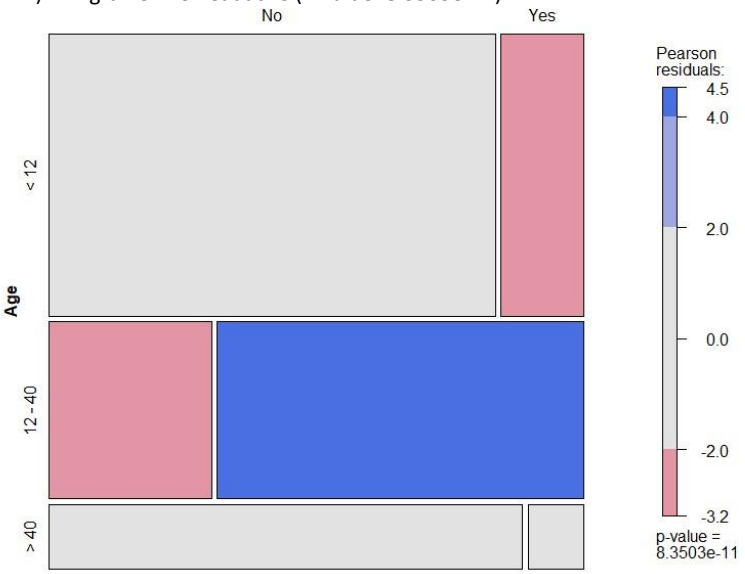
D) Stroke-like episodes (P-Value = 0.0016769)



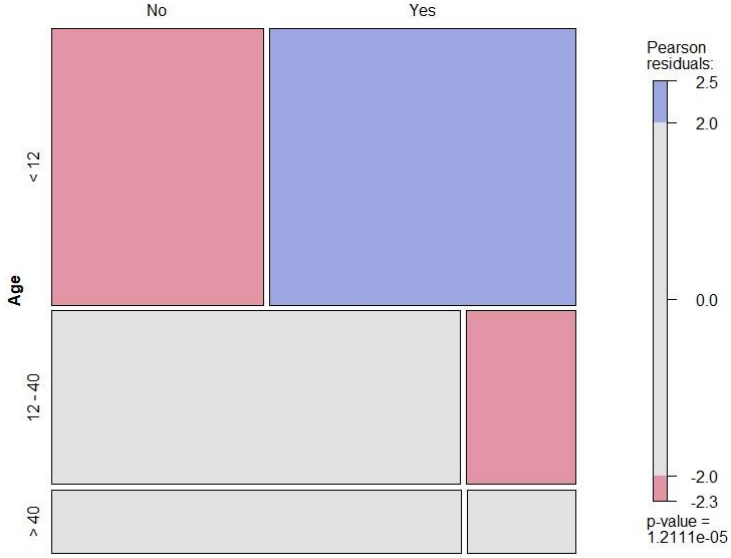
E) Peripheral neuropathy (P-value = 1.7285e-06)



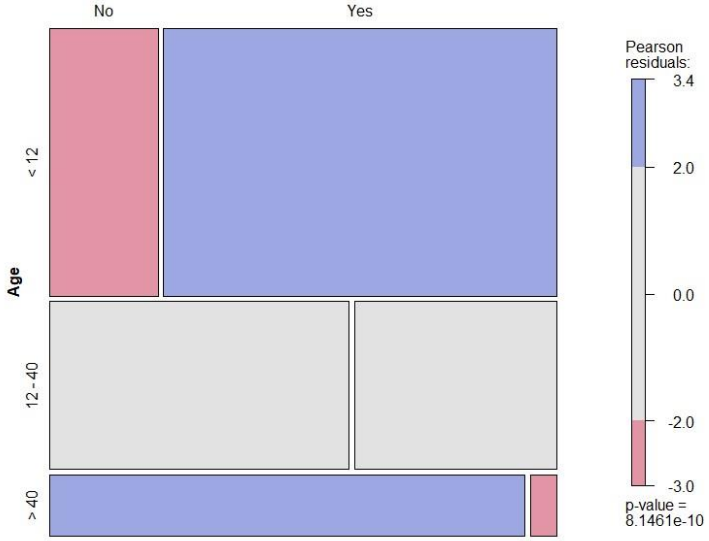
F) Migraine-like headache (P-value=8.3503e-11)



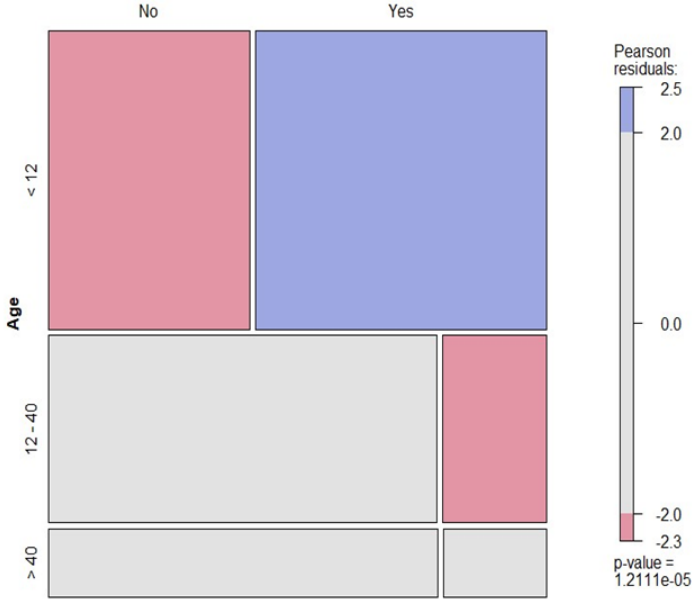
G) Feeding difficulties (P-value = 1.2111e-05



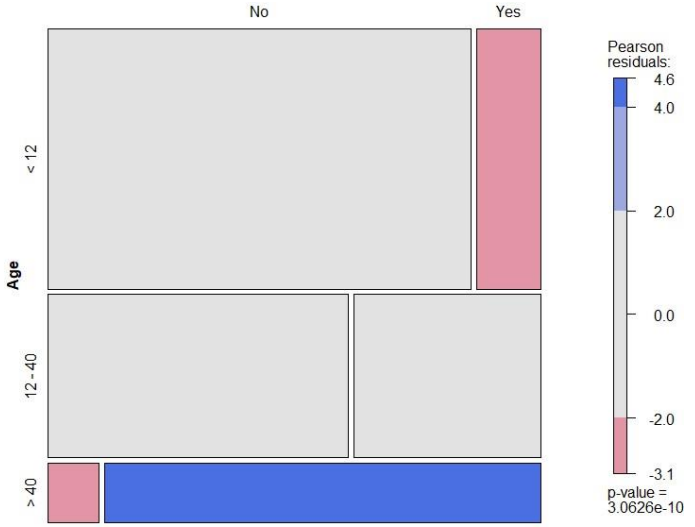
H) Liver involvement (P-value= 8.1461e-10)



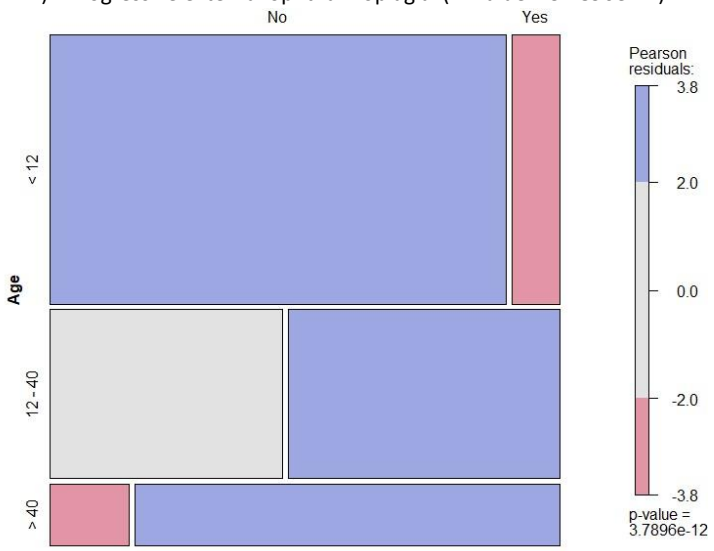
I) Anaemia (p-Value=1.2111e-05)



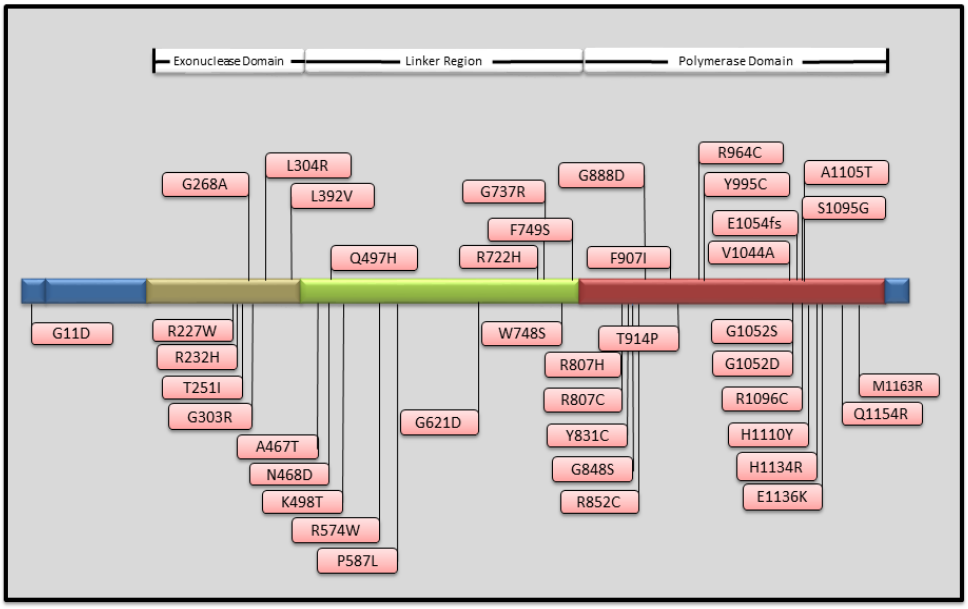
J) Ptosis (P-Value= 3.062e-10)



K) Progressive external ophthalmoplagia (P-value = 3.7896e-12)

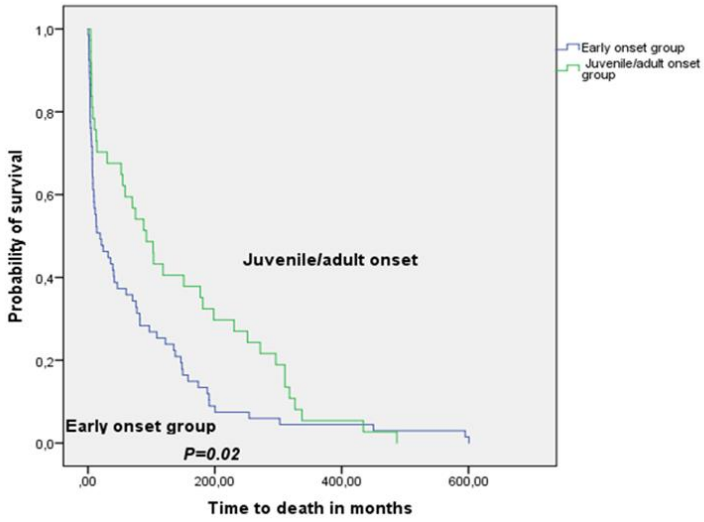


Supplementary figure 3: Genetic findings (variants) of patients with POLG disease reported in this study

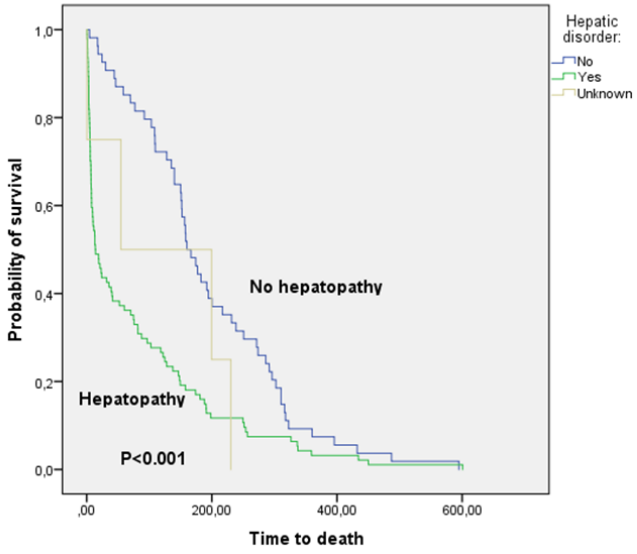


Supplementary figure 4 (A-B): Survival analysis.

3- A: Kaplan-Meier curve comparing survival after the onset of seizure in those with early onset disease and those with juvenile/ adult onset ,and showed those with early onset had significantly worse survival



3-B: Kaplan-Meier curve comparing survival of those with hepatopathy and those without, and showed those with hepatopathy had significantly worse survival



Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230845752 (print)
9788230850718 (PDF)