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.

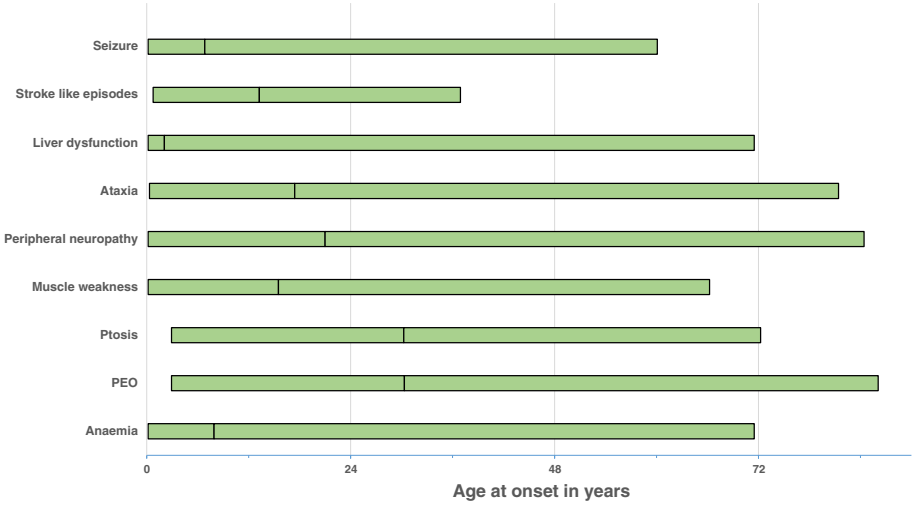

FIGURE 1 Age of onset of each individual symptom in patients with POLG disease. Range I, Median. PEO, progressive external ophthalmoplegia

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%

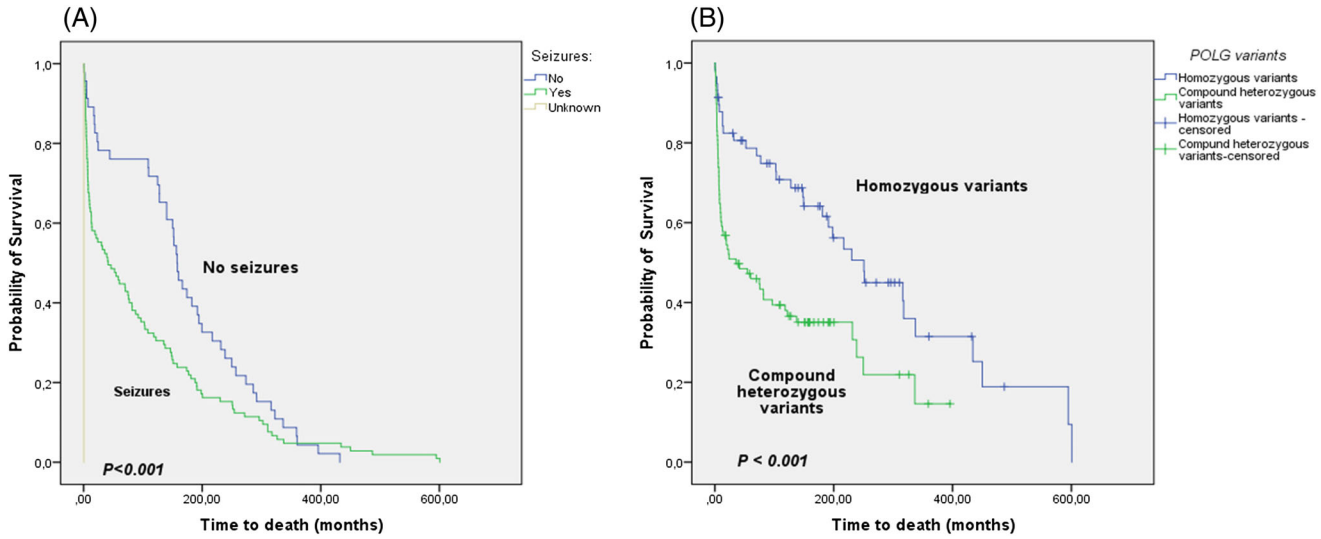

FIGURE 2 Survival analysis. A, Kaplan-Meier curve comparing survival of those with seizures and those without seizures and showed those with seizures carried significantly worse survival. B, Kaplan-Meier curve comparing survival of those with homozygous variants and those with compound heterozygous *POLG*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

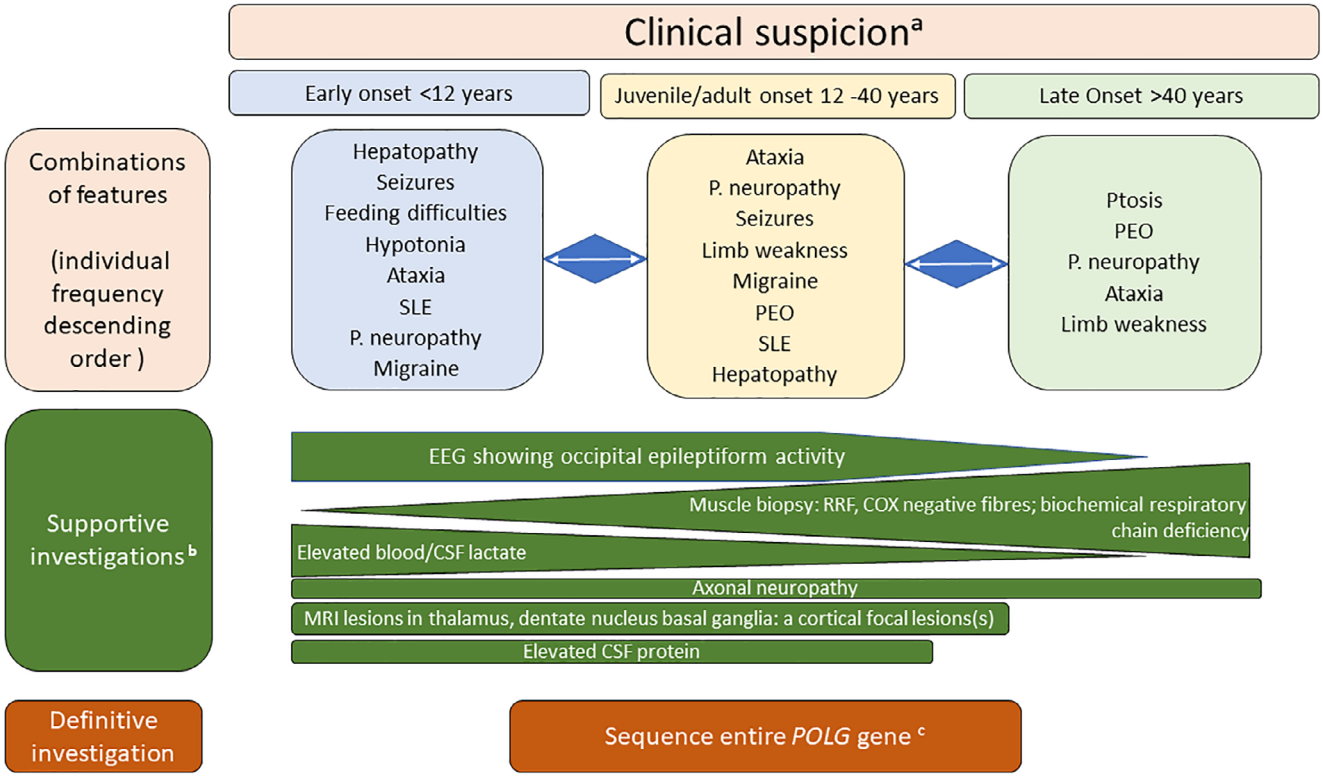

FIGURE 3 Diagnosing POLG disease; clinical suspicion and relevant investigations according to the age of onset. While we have shown that POLG clinical features form a continuum, but it is also clear that age plays a role in which features predominate. Based on our age groups, we can see clear clinical 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