POLG-related disorders and their neurological manifestations

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Abstract | The *POLG* gene encodes the mitochondrial DNA polymerase that is responsible for replication of the mitochondrial genome. Mutations in *POLG* can cause early childhood mitochondrial DNA (mtDNA) depletion syndromes or later-onset syndromes arising from mtDNA deletions. *POLG* mutations are the most common cause of inherited mitochondrial disorders, with as many as 2% of the population carrying *POLG* mutations. *POLG*-related disorders comprise a continuum of overlapping phenotypes with onset from infancy to late adulthood. The six leading disorders caused by *POLG* mutations are Alpers–Huttenlocher syndrome, which is one of the most severe phenotypes; childhood myocerebrohepatopathy spectrum, which presents within the first 3 years of life; myoclonic epilepsy myopathy sensory ataxia; ataxia neuropathy spectrum; autosomal recessive progressive external ophthalmoplegia; and Autosomal dominant progressive external ophthalmoplegia. This Review describes the clinical features, pathophysiology, natural history and treatment of *POLG*-related disorders, focusing particularly on the neurological manifestations of these conditions.

[H1] Introduction

The development and normal functioning of the CNS depends on a readily available supply of ATP. In neurons, the majority of ATP is generated in the mitochondria by oxidative phosphorylation (OXPHOS) via the electron transport chain (ETC) and ATP synthase. The ETC is composed of complexes I–IV, which set up an electrochemical gradient that drives complex V, the ATP synthase. Of the ~90 proteins that make up the OXPHOS system, 13 are derived from the mitochondrial genome. In humans, the mitochondrial genome is a closed circular DNA molecule of 16,569 bp that also encodes 22 tRNAs and 2 ribosomal RNAs that are required for synthesis of the 13 polypeptides. The mitochondrial DNA (mtDNA) is located in discrete nucleoids localized within the inner matrix of the mitochondrion, each of which contains one or two copies of the mtDNA[1]. The mtDNA is replicated by an assembly of proteins in a replisome consisting of core replication proteins[2] DNA polymerase γ (pol γ), the mitochondrial single-stranded DNA binding protein, and the Twinkle mtDNA helicase, along with topoisomerases and RNase H activities[3].

Human pol γ is composed of POLG, a 140 kDa catalytic subunit that is encoded by *POLG* at chromosomal locus 15q25, and POLG2, a 55 kDa accessory subunit that forms a dimer and is encoded by *POLG2* at chromosomal locus 17q24.1[4-6]. POLG has DNA polymerase, 3' to 5' exonuclease and 5'-deoxyribose phosphate (5'-dRP) lyase activities[7,8]. This subunit contains an amino-terminal exonuclease domain connected by a linker region to the carboxy-terminal polymerase domain. POLG2 enhances polymerase processivity by increasing the affinity of the catalytic subunit for DNA[9-11].

POLG is one of several nuclear genes that are associated with mtDNA depletion or deletion disorders (Table 1). In 2001, Van Goethem *et al.* published a seminal paper describing four mutations in *POLG* that were associated with either autosomal dominant or autosomal recessive progressive external ophthalmoplegia (PEO)[12]. Between 2003 and 2005, several

reports identified *POLG* mutations in patients with ataxia[13-15]. Also in this timeframe, Alpers–Huttenlocher syndrome (AHS) was found to be caused by recessive mutations in *POLG*[16,17]. The high frequency of *POLG* mutations in the Norwegian and Finnish populations led to calls to include *POLG* testing as a first-line diagnostic in ataxia syndromes[15,18]. These reports were the first of many to identify disease-associated mutations in the *POLG* gene. Pathogenic variants in *POLG* are now known to cause a spectrum of overlapping phenotypes, including some that were clinically defined long before their molecular basis was known. This article reviews these clinical disorders and symptoms associated with *POLG*-related disorders, with a focus on the neurological manifestations. The natural history and molecular genetics of *POLG*-related disorders are also reviewed, along with current treatment options for patients with these conditions.

[H1] Epidemiology

Mutations in *POLG* represent the most prevalent single-gene cause of mitochondrial disease, accounting for 10% of adult mitochondrial disease cases in one large Australian cohort[19]. *POLG* mutations are the most frequent cause of mitochondrial epilepsy at all ages[20], and also account for 10–25% of PEO[21] and >10% of ataxia cases[22]. Note that in the text that follows, we refer to these mutations in terms of the resulting amino acid substitution.

Two independent reports identified W748S as a frequent mutation in people with ataxia in the Norwegian population (1:100 with Q497H)[18] and the Finnish population (1:125 in the general population)[15]. In an epidemiological study conducted in North East England, clinically manifesting autosomal recessive *POLG* mutations had a population prevalence of 0.3 per 100,000 adults[23]. As the three most prevalent *POLG* mutations (A467T, W748S and G848S) have a combined carrier frequency of >1% in Northern Europe, application of the Hardy–

Weinberg principle suggests that the frequency of recessive *POLG* disease is likely to be ~1 in 10,000[24]. This discrepancy could be explained by many affected individuals never being diagnosed or dying in childhood.

[H1] Clinical presentations

Age of onset of the *POLG*-related disorders ranges from infancy to late adulthood. *POLG* mutations are now known to account for at least six major syndromes. AHS is characterized by childhood-onset progressive and severe encephalopathy with intractable epilepsy and hepatic failure. Individuals with childhood myocerebrohepatopathy spectrum (MCHS) present with developmental delay, lactic acidosis, myopathy and hepatic impairment. Myoclonic epilepsy myopathy sensory ataxia (MEMSA) encompasses a spectrum of disorders with epilepsy, myopathy and ataxia, typically without ophthalmoplegia, including disorders previously described as spinocerebellar ataxia with epilepsy (SCAE); note that long-term survivors of MEMSA can additionally develop PEO. Ataxia neuropathy spectrum (ANS) includes mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO). Autosomal recessive PEO (arPEO) is characterized by progressive weakness of the extraocular muscles resulting in ptosis and ophthalmoparesis without associated systemic involvement. Autosomal dominant PEO (adPEO) typically includes generalized myopathy and variable degrees of sensorineural hearing loss, axonal neuropathy, ataxia, depression, parkinsonism, hypogonadism and cataracts (Fig. 1; Table 2).

Most patients with *POLG*-related disease, particularly those with adolescent-onset or adult-onset disorders, do not present with a discrete clinical syndrome. Therefore, in this Review, instead of describing each of the above syndromic presentations in detail, we will

take an age-of-onset and symptom-based approach, which we hope will aid the non-mitochondrial expert in recognizing and diagnosing *POLG*-related disease.

[H2] Early-onset phenotypes

[H3] Childhood myocerebrohepatopathy spectrum disorders. Myocerebrohepatopathy is the earliest presentation of biallelic *POLG* mutations[25]. Affected infants usually present in the first few months of life with severe hypotonia, developmental delay and signs of hepatic impairment such as hypoglycaemia. In one study, median age at onset was 4.7 months (range 0.9–7.0 months)[26]. Other clinical features of MCHS include faltering growth, renal dysfunction and cataracts leading to roving eye movements. Seizures are unusual in this group, perhaps because these infants do not survive long enough to develop seizures; death from liver failure typically occurs before the age of 1 year[26].

[H3] Alpers–Huttenlocher syndrome. Chronologically, the next presentation of biallelic *POLG* mutations is AHS, which was initially described as a triad of neurodevelopmental regression, intractable seizures and liver failure[27]. In a multinational cohort, 70% of children with *POLG* mutations presented with AHS[26]. Disease onset is typically around the end of the first year of life, with focal motor seizures progressing to generalized status epilepticus. Onset of seizures is frequently explosive, and most patients present with refractory convulsive status epilepticus[26,28]. However, clinical presentation can occur at any time in childhood, and adult onset has even been reported[29-31]. Preceding development is often normal, but some individuals who present with AHS have a history of prior hypotonia and mild developmental delay. A viral prodrome can sometimes be observed, which might arouse clinical suspicion of encephalitis[32], and some evidence indicates that an immunological process contributes to the

pathology of AHS. For example, some individuals were reported to have oligoclonal bands in their cerebrospinal fluid (CSF)[33], whereas others had elevated serum levels of antibodies to voltage gated potassium channels (S.R.) unpublished work). In one case, neuropathology revealed features of acute disseminated encephalomyelitis (ADEM), again suggesting an underlying immune-mediated pathology[33]. The disease course is characterized by recurrent episodes of status epilepticus and epilepsia partialis continua (EPC), leading to death from refractory status, usually in early to mid childhood[26]. Deficient pol γ activity in the skeletal muscle and liver of patients with AHS was first reported by Naviaux *et al.* in 1999[34], but *POLG* mutations were not described until 2004[16].

[H2] Other epilepsy syndromes

Biallelic *POLG* mutations are also associated with a number of other epilepsy phenotypes, and are one of the most frequent genetic causes of mitochondrial epilepsy[20,35,36]. *POLG* mutations were the cause of epilepsy in 3 of 42 (7%) of an adult cohort with mitochondrial epilepsy[35]. More than 80% of paediatric patients with *POLG* mutations have epilepsy at disease onset[26]. A systematic review of 372 patients with *POLG*-related epilepsy revealed a bimodal age distribution at presentation, with an initial large peak in early childhood and a second peak in adolescence[36]. The median age at onset was 2 years, although *POLG*-related epilepsy can begin at any age from the first month of life to the seventh decade[36]. Seizure was the initial clinical manifestation in 50% of cases. Seizure semiology was available for 229 patients: 64% had focal motor seizures, 58% had myoclonus, 49% had generalized status epilepticus and 34% had focal motor status. Therefore, we can conclude that status epilepticus is a frequent occurrence in *POLG*-related disease. Of 37 patients with *POLG*-related status epilepticus

identified in a systematic review, 13 had status epilepticus alone, 7 had only EPC and 17 had both generalized status epilepticus and EPC[36].

POLG-related epilepsies can mimic classic mitochondrial syndromes, including myoclonic epilepsy with ragged-red fibres (MERRF)[37] and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)[38]. MEMSA is a form of syndromic *POLG*-related epilepsy and includes disorders previously referred to as SCAE. In this syndromic presentation, epilepsy is typically associated with myoclonus, myopathy and sensory ataxia. PEO can occur late in the disease.

[H2] Progressive external ophthalmoplegia

POLG mutations can cause adPEO or arPEO. *POLG* mutations contributed to ~25% of PEO cases in a London–Oxford cohort[21] and ~10% of cases in a large Italian cohort[39]. PEO was present in 83% of an Italian cohort of 46 adult patients with *POLG* mutations and was associated with encephalomyopathy in more than half of these cases[39]. In affected individuals, progressive weakness of the extraocular muscles leads to bilateral, usually symmetrical ptosis and limitation of gaze in all directions. The insidious onset means that diplopia is an uncommon symptom. The muscle weakness can extend to the limb-girdle musculature, manifesting as a proximal myopathy that can progress to a generalized myopathy. Other associated clinical features include ataxia, parkinsonism, depression, sensorineural hearing loss, cataracts and premature ovarian failure[40,41].

[H2] Ataxia syndromes

Ataxia in *POLG*-related disease can be sensory or cerebellar. Associated clinical features include dysarthria, encephalopathy with seizures, ophthalmoplegia and peripheral

neuropathy. *POLG* mutations seem to be a fairly frequent cause of cerebellar ataxia, accounting for 9 of 80 cases (11%) in a Central European cohort in whom repeat expansion diseases had been excluded[22]. The umbrella term ANS includes disorders previously referred to MIRAS and SANDO. In one cohort, 6 of 11 patients (55%) with SANDO had *POLG* mutations leading to multiple mtDNA deletions[42].

[H2] Disorders with prominent neuropathy

In an Italian registry of mitochondrial disease, peripheral neuropathy was a feature in 143 of 1,156 patients (12.4%)[43]. Within the mitochondrial neuropathy cohort, 19 patients (13%) had *POLG* mutations (i.e. 13% of the mitochondrial neuropathy cohort). Across the entire cohort, 19 of 45 (42%) of patients with *POLG* mutations had neuropathy. In a UK–Australian cohort, 7 of 27 children (26%) with genetically confirmed mitochondrial neuropathy had *POLG* mutations[44]. *POLG* mutations are usually associated with an axonal or mixed, predominantly sensory neuropathy, although some patients have a demyelinating neuropathy[43,44]. More than one-third of the Italian patients with *POLG*-related neuropathy had neuropathic pain, which is an unusual feature of mitochondrial neuropathy[43].

[H2] MNGIE-like disorder

Five patients with *POLG* mutations have been reported to have a clinical phenotype closely resembling mitochondrial neurogastrointestinal encephalopathy (MNGIE) syndrome[45-48]. These patients were aged between 7 and 50 years and had prominent symptoms of persistent diarrhoea and cachexia related to gastrointestinal dysmotility, as well as ptosis, proximal myopathy and sensory neuropathy. Patients with classic MNGIE caused by thymidine

phosphorylase deficiency have striking white matter changes, but leukoencephalopathy was notably absent in the patients with POLG-related MNGIE reported to date[46].

[H2] Movement disorder syndromes

Parkinsonism is the most frequently observed extrapyramidal movement disorder in patients with *POLG* mutations, and has been associated with both dominant and recessive mutations[40,41,49]. In a cohort of adult patients with mitochondrial movement disorders, 5 of 42 (12%) had *POLG* mutations[50]. These five patients all had parkinsonism, and three also had restless legs syndrome[50]. *POLG*-related parkinsonism has an earlier onset than idiopathic Parkinson disease — typically ~40 years but as early as the third decade in some families[51] — and is associated with initially asymmetric clinical and imaging features and a good response to levodopa[50]. Palatal tremor also seems to be a characteristic feature in some patients with *POLG* mutations, occurring together with facial dyskinesia and progressive ataxia in the so-called progressive ataxia palatal tremor (PAPT) syndrome[52,53]. Dystonia, the most frequent movement disorder in other mitochondrial disorders such as Leigh syndrome, is rarely observed in patients with *POLG* mutations[54,55].

[H2] Other phenotypes

The clinical spectrum of *POLG*-related disease is extremely wide (Fig. 1; Table 2). Other less frequently reported phenotypes include distal myopathy[56], premature menopause[40,41,57] and cataracts[40,45,56]. Neuropsychiatric manifestations, including recurrent major depression, are well known[15]. Prenatal onset of *POLG*-related disease, characterized by fatal congenital myopathy and gastrointestinal pseudo-obstruction, has also been described[58].

[H1] Environmental triggers and toxins

Antiviral drugs that are used to treat HIV infection, such as zidovudine, didanosine, zalcitabine, stavudine, lamivudine, carbovir and tenofovir, have long been known to cause an induced mitochondrial toxicity, leading to peripheral neuropathy and/or myopathy due to inhibition of pol γ and consequent reduction in mtDNA copy number[59,60]. In 1990, Dalakas et al. first described a mitochondrial myopathy, characterized by ragged-red fibres in muscle and reduced amounts of mtDNA, in patients receiving zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI) that inhibits the HIV life cycle[61,62]. NRTI-induced mitochondrial dysfunction, also termed mitochondrial toxicity, occurs in as many as 20% of patients undergoing NRTI therapy. This mitochondrial toxicity mimics mitochondrial genetic diseases and induces similar clinical syndromes, including ragged-red muscle fibres, lactic acidosis, myopathies, cardiomyopathies, hepatic steatosis, lipodystrophy and neuropathy[59,60,63,64].

As highlighted below, valproic acid (VPA) is contra-indicated in all patients with *POLG* mutations. VPA can precipitate liver failure in AHS, and sensitivity to this drug has been reported in adolescent and early-adulthood patients with status epilepticus[65]. VPA is a histone deacetylase inhibitor but is also known to inhibit fatty acid β-oxidation, which primarily occurs in the liver. In contrast to antivirals, VPA further compromises mitochondrial function in *POLG*-related disease without directly inhibiting pol γ or acting on the DNA replication pathway.

Patients with *POLG*-related disorders, similarly to those with other mitochondrial disorders, are hypersensitive to several pharmaceuticals that are known to inhibit mitochondrial function, including antibiotics, statins, anaesthetics and chemotherapeutics[66]. These drugs do not usually cause symptoms in healthy individuals but can aggravate or trigger disease in patients with a genetic ('primary') mitochondrial disorder. Thus, drugs that

compromise mitochondrial function should be used with caution in patients with mitochondrial disease, although treating a potentially life-threatening infection with antibiotics clearly outweighs any theoretical risks of mitochondrial inhibition. Physical stressors such as infection, fever, dehydration and anorexia can result in sudden deterioration in patients with *POLG*-related disorders and should be avoided if possible.

In the context of mitochondrial toxins, the energetic threshold of mitochondria in different tissues should be taken into account. When ATP production cannot serve the respiratory demands of a certain tissue owing to a decline in mitochondrial function, resulting from a genetic defect and/or environmental exposure, tissue death can ensue. As discussed later in this article, some therapies can upregulate mitochondrial biogenesis and have the potential to overcome mild genetic defects or environmental insults.

[H1] Pathophysiology

More than 300 pathogenic mutations of *POLG* have been reported, as presented in the Human DNA Polymerase Gamma Mutation Database and in Fig. 2. The consequences of *POLG* mutations can be divided into two broad groups: multiple mtDNA deletions and mtDNA depletion. No direct genotype–phenotype correlations are evident for *POLG* mutations: the same mutation can often lead to mtDNA deletions, mtDNA depletion or both, making it difficult to predict the phenotype on the basis of observed mutations. For example, homozygosity for the most common *POLG* mutation, A467T, has been associated with a range of phenotypes, from childhood-onset fatal AHS to MEMSA, ANS and SANDO[67,68]. It has been suggested that depletion of mtDNA in neurons is the trigger for development of epilepsy, and preliminary data suggest a relationship between the mtDNA phenotype caused by the *POLG* mutation and the clinical phenotype. For example, we observed AHS in a child with profound

mtDNA depletion, but SANDO in a patient with multiple mtDNA deletions; both individuals had the homozygous A467T *POLG* genotype[68].

Modelling of mutations on the pol γ structure has revealed that mutations cluster into five distinct regions[69,70,71]. The AHS presentation seems to require a combination of mutations affecting two different conserved regions, whereas ataxia phenotypes were associated with regions affecting the intrinsic processivity domain, possibly affecting the interaction with the accessory subunit encoded by *POLG2*[71]. On the basis of these five distinct clusters, a POLG Pathogenicity Prediction Server was generated to help predict the clinical outcomes of known mutations[72]. However, the onset age and progression of *POLG*-related disease in patients with the same *POLG* mutations can span several decades, making predictions difficult. For example, a review of patients with T251I–P587L in *trans* with G848S showed that the presentation of disease spans >70 years[73], and another review revealed that disease related to homozygosity for A467T spans at least four decades of life[65,68]. This enigma of presentation suggests that other factors modify the *POLG* disease phenotype, including genetic modifiers (nuclear DNA or mtDNA), immune dysfunction, and environmental effects such as viral infection and mitochondrial toxins[74,75].

In addition to mitochondrial disease, mtDNA mutations have been implicated in the ageing process[76,77], and several lines of evidence suggest that DNA polymerase errors have a prominent role in mtDNA mutation. Mutations in mtDNA can arise through spontaneous errors of DNA replication or through unrepaired damage to mtDNA that introduces miscoding lesions. Owing to its high nucleotide selectivity and exonucleolytic proofreading, POLG exhibits exceptionally high fidelity of DNA replication, with nucleotide misinsertion events occurring only once per 500,000 nucleotides synthesized[78]. The intrinsic 3' to 5' exonuclease activity that contributes to replication fidelity can be completely eliminated by substituting

alanine for Asp198 and Glu200 in the conserved Exo I motif of POLG[79]. Comparison of the *in vivo* mutational spectrum to the error spectrum generated by human pol γ *in vitro* strongly implicated biosynthetic errors by pol γ as the main driver of point mutations in mtDNA[80]. Furthermore, analysis of age-specific mtDNA sequences revealed mutation signatures more consistent with polymerase errors than with the effects of oxidative damage[81]. Thus, spontaneous replication errors by pol γ account for the majority of base substitution mutations in mtDNA and are likely to be responsible for the accumulation of point mutations and deletions in mtDNA during ageing[77,82-84].

No mouse model of *POLG*-related disease is available that recapitulates human disease phenotypes. In the absence such a model, two independent groups created mice with mutations that disrupted the exonuclease function of the mouse Polg protein[85,86]. Mice that were homozygous for these mutations exhibited premature ageing between 6 and 9 months of age, characterized by greying hair, loss of hair and hearing, curvature of the spine, enlarged hearts, and decreased body weight and bone density[85,86]. In one of these models, the frequency of mutations was found to be 500-fold higher in heterozygous mice and 2,500-fold higher in homozygous mice than in aged wild-type mice[87]. The heterozygotes were asymptomatic, indicating that a 500-fold increase in mutation frequency was not sufficient to cause phenotypes associated with ageing. Further analysis demonstrated a 90-fold increase in mtDNA deletions in homozygous *Polg* exonuclease-deficient mice compared with age-matched wild-type or heterozygous mice[88]. Thus, the high frequency of mtDNA deletions in the homozygous mice is thought to be the main driver of the premature ageing phenotype. This mouse model has some relevance to humans in that accumulation of mtDNA deletions seems to be the driving force for mitochondrial dysfunction in adult-onset *POLG*-related disorders.

[H1] Diagnosis

POLG mutations should be considered not only in patients presenting with one of the classic *POLG* syndromes (MCHS, AHS, MEMSA, ANS and PEO), but also in patients with epilepsy (especially drug-resistant seizures, myoclonus, EPC or convulsive status epilepticus), ataxia, neuropathy, myopathy or other symptoms suggestive of an underlying mitochondrial disorder. Full clinical assessment should encompass a multisystem evaluation, including vision and hearing, and cardiac, hepatic, renal, gastrointestinal and respiratory function.

[H2] EEG

In patients presenting with seizures, EEG might provide a diagnostic clue. Children with AHS usually have rhythmic high-amplitude delta with superimposed polyspikes (RHADS) on EEG performed early in the disease course, although later EEG can be nonspecifically abnormal[28]. An occipital lobe predilection for EEG abnormalities is typically observed in *POLG*-related disease[89]. A systematic review of EEG findings in 72 patients with *POLG*-related epilepsy revealed focal changes in most cases. Changes included epileptiform discharges, RHADS and focal slowing, and involved posterior (occipital), frontal or temporal regions (61%, 6% and 2% of cases, respectively) or were multifocal (23% of cases)[36].

[H2] Neuroimaging

A systematic review of neuroimaging findings in 136 patients with *POLG*-related epilepsy revealed that stroke-like lesions were the most prevalent abnormality, being present in 43% of cases[36]. These lesions affected the occipital lobes in half the cases, but could also involve the parietal, temporal or frontal lobes. Other neuroradiological abnormalities observed in

patients with *POLG* mutations include thalamic (37%), basal ganglia (14%) and cerebellar (17%) lesions, generalized brain atrophy (28%) and involvement of the cerebral white matter (7%)[36]. Importantly, imaging results can be normal in some patients presenting with seizures, so a normal brain MRI scan does not exclude *POLG* disease. Dopamine transporter imaging demonstrated a bilateral nigrostriatal dopaminergic defect in patients with dominant and recessive *POLG* mutations[50,90]. This defect seemed be universal in patients aged >25 years and was not dependent on the presence of clinically overt parkinsonism[91]. Hypertrophic degeneration of the inferior olives seems to be a characteristic feature of the PAPT syndrome[52].

[H2] Biomarkers

No blood or urine biomarkers are known to be specific for *POLG*-related disease, although peripheral blood levels of lactate can be modestly elevated, and plasma alanine levels might be increased in cases of persistent lactic acidemia. Other metabolites that have been suggested to be biomarkers of mitochondrial disease include fibroblast growth factor 21 (FGF21)[92] and growth and differentiation factor 15 (GDF15)[93]. FGF21, which has been widely reported as a marker for mitochondrial disease manifesting in muscle, is rarely elevated in patients with *POLG*-related disease, in whom muscle pathology is frequently mild or even absent. Absolute levels of FGF21 have been reported in 17 patients with *POLG* mutations and ranged from normal (25 pg/ml) to extremely elevated (>4000 pg/ml)[92]. The highest values were seen in patients with AHS and in a patient with MIRAS and terminal status epilepticus. The observation of normal values in several patients with *POLG* mutations implies that FGF21 is not a useful marker to screen for *POLG* disease, although its levels do seem to correlate with disease severity. Absolute GDF15 values have not been reported in patients with *POLG*-

related disease. Cerebral folate deficiency has been noted in some patients with AHS[94], and CSF oligoclonal bands have been observed in both children and adults with *POLG*-related epilepsy, leading to suspicion of ADEM or multiple sclerosis[33,95].

[H2] Histopathology

In patients with *POLG*-related disorders, muscle histology might reveal classic mitochondrial features such as ragged-red or cytochrome *c* oxidase-negative fibres or nonspecific abnormalities such as increased lipid deposition, or can be normal[26]. Some infants with normal muscle biopsies had severely abnormal liver histology[96]. The characteristic liver pathology of AHS, which can be triggered by VPA exposure, includes microvesicular steatosis, bile duct proliferation, hepatocellular necrosis, bridging fibrosis or cirrhosis, and disorganization of the normal architecture[27]. Macroscopic examination of the brain in AHS shows patchy grey matter pathology in the occipital lobes, particularly the striate cortex, and lesions have also been observed in the basal ganglia and thalamus in some cases. Histological features include neuronal depletion, associated with spongiosis and gliosis, progressing through the cortical layers, but affecting the calcarine cortex most severely[27]. ADEM-like neuropathological changes were reported in a 4-year-old boy with recessive *POLG* mutations[33]. Neuropathological studies in MIRAS revealed subtotal loss of large myelinated fibres in the sural nerve, with severe axonal neuropathy; atrophy of the posterior columns of the spinal cord, posterior spinocerebellar tracts and dentate nuclei; and patchy dropout of cerebellar Purkinje cells[97]. Neuronal loss was also observed in the inferior olives, substantia nigra and mediodorsal thalamic nuclei, with and, in addition, the parieto-occipital subcortical white matter showed neuronal loss, gliosis and spongiosis. Distinctive neuropathology was reported in a patient with SANDO, who had multisystem neurodegeneration (pronounced

gliosis and neuronal loss) predominantly affecting the brainstem, cerebellum and dentate nuclei, with less severe changes in the basal ganglia and thalamus[98].

[H2] Respiratory chain enzymology

In a subset of individuals with *POLG*-related disease, respiratory chain enzyme (RCE) assays demonstrate isolated deficiency of complex I or IV or combined deficiencies of multiple enzymes. Interestingly, a review of a multinational cohort revealed normal RCE activities or isolated deficiency of a single enzyme complex (I or IV) in children with AHS but multiple RCE deficiencies in children with MCHS, implying a more severe *POLG* defect in the latter group[26]. In children with normal muscle RCE activities, RCE deficiencies might be restricted to a clinically affected tissue such as the brain or the liver.

[H2] Molecular genetics

If a diagnosis of *POLG*-related disease is suspected clinically, the most appropriate investigation is direct sequence analysis of the *POLG* gene. Traditionally, Sanger sequencing has been used, but *POLG* is increasingly being included in next-generation sequencing (NGS) gene panels (for epilepsy, ataxia or mitochondrial disease, for example), and NGS is likely to be the diagnostic modality of choice going forwards. In some regions where the founder mutations A467T, W748S and G848S are particularly prevalent[15,99], screening for these common mutations could continue as a first-line investigation. Although hundreds of different disease-causing *POLG* mutations have been reported, new potentially pathogenic variants continue to be identified (Fig. 2). Determining the pathogenicity of these variants of uncertain significance can be challenging, and a pathogenicity prediction server was recently reported[72]. In some patients with a probable recessive phenotype such as AHS, only one

mutation was identified despite an extensive search for a second mutation. Whole-genome DNA and/or RNA sequencing might reveal deep intronic or regulatory sequence variants in such patients in the future[100,101].

The majority of *POLG*-related disorders have been associated with one of four common mutations: A467T, W748S, G848S and the T251I–P587L allelic pair (Table 3). In one study, these mutations accounted for ~50% of all mutations identified in patients with *POLG*-related disease, and ~75% of patients carried at least one of these four mutations[102]. A467T is considered to be the most common pathogenic variant of *POLG* (although its prevalence varies by country and population group (Table 3)), and is estimated to occur in 36% of all alleles associated with *POLG*-related disease[25,103-106]. This mutation is present in 0.2–1.0% of asymptomatic European individuals[12,18,105,107]. The A467T mutation severely reduces pol γ activity (4% of wild-type activity) by reducing the affinity of the enzyme for deoxynucleotide triphosphates (dNTPs) and lowering catalytic activity[108]. In addition, the POLG subunit containing the A467T variant fails to associate with the POLG2 accessory subunit, which is critical for highly processive DNA synthesis (defined as the number of nucleotides incorporated per DNA-binding event). The combined defects lead to stalling at the replication fork and depletion of mtDNA over time.

The second most common *POLG* mutation is W748S, which causes a reduction in DNA polymerase activity, low processivity and a severe DNA-binding defect, but normal POLG2 interactions [109]. W748S is nearly always found in *cis* with the E1143G mutation, and is a frequent cause of ANS[15]. The W748S mutant protein has reduced polymerase activity and a decreased affinity for DNA[110]. The E1143G substitution results from a single nucleotide polymorphism that is found in 4% of European populations. The phenotypic effects of W748S are modulated when in *cis* with E1143G, which is considered a benign variant[110].

The G848S variant is the third most common *POLG* mutation in the *POLG* mutation database. This pathogenic variant results in <1% of normal polymerase activity and a defect in DNA-binding function[111]. Gly848 is located in the thumb subdomain of the polymerase active site in a cluster of mutations associated with AHS[111]. An in vitro study showed that mutations in the most conserved sites in this cluster, including G848S, T851A, R852C and R853Q, decreased the activity of pol γ to <1% of the wild-type level[111].

The T251I and P587L amino acid substitutions, which are usually found in *cis* and occur in up to 1% of the Italian population, have been implicated in PEO[103]. Individually, these mutations cause a ~30% reduction in DNA polymerase activity, together they act synergistically to functionally impair polymerase function to levels ~5% of normal owing to a combination of loss of enzyme stability, decreased DNA-binding affinity and reduced catalytic efficiency[112].

The Y955C variant is the most common autosomal dominant mutation in *POLG* and causes PEO. The symptoms can progress to include parkinsonism or premature ovarian failure[40,41]. The alteration to cysteine at position 955 of POLG causes severe reduction in pol γ (<1% of wild-type)[113,114].

[H2] Differential diagnosis

In addition to *POLG*, mutations in many genes encoding proteins that regulate mtRNA stability have been shown to cause conditions resembling *POLG*-related disease (Table 1). Mutations in *POLG2*, *TWNK*, *RRM2B*, *SLC25A4*, *MGME1*, *DNA2*, *RNASEH1*, *TK2*, *DGUOK*, *MPV17*, *SPG7* and *AFG3L2* have been implicated in adPEO and/or arPEO[115], and *TWNK* mutations can also lead to SANDO[42]. A disorder closely resembling classic AHS syndrome can be caused by mutations of *TWNK*, and of *FARS2, NARS2* and *PARS2*, which encode the tRNA aminoacyl

synthetases for phenylalanine, asparagine and proline, respectively. Mutations in the latter three genes result in global impairment of mitochondrial translation. Other mitochondrial epilepsies associated with mtDNA depletion have been attributed to mutations in *TWNK*, *RRM2B*, *DGUOK*, *TK2*, *SUCLA2*, *SUCLG1, TYMP*, *MPV17*, *ABAT* and *FBXL4*[115].

The differential diagnosis of treatment-resistant convulsive status epilepticus in childhood includes febrile infection-related epilepsy syndrome, for which a genetic basis has not yet been established[116]. Other causes of acute liver failure in infancy include mtDNA depletion due to *DGUOK* and *MPV17* mutations, and impaired mitochondrial translation caused by *TRMU* mutations[96,117]. However, the differential diagnosis is wide and includes other inborn errors of metabolism, for example, recurrent acute liver failure caused by biallelic mutations in the *NBAS* gene[118], and viral infections. Liver failure of later onset might be attributable to drug toxicity.

[H1] Natural history

The epileptic phenotypes of *POLG*-related disease are associated with high morbidity and mortality, especially AHS, which is usually characterized by relentless disease progression leading to death from status epilepticus in early childhood[26]. One study of *POLG*-related epilepsy suggested that homozygous mutations in the linker region of the enzyme were associated with later onset and longer survival compared with compound heterozygous mutations affecting the same domain[36,65]. Another study suggested that the presence of anemia correlates with worse outcomes in *POLG*-related disease[45]. Liver failure in *POLG*-related disorders is usually fatal, although one report describes a patient who recovered spontaneously from acute liver failure and remained well 6 years later[96].

[H1] Treatment

Evidence-based therapies for *POLG*-related disorders are currently lacking. No randomized controlled clinical trials have been performed for these conditions, and symptomatic therapies are the mainstay of treatment.

[H2] Management of epilepsy

No antiepileptic drug (AED) has been shown to be particularly efficacious for *POLG*-related seizures, which are frequently resistant to multiple AEDs. A systematic review revealed that the mean number of AEDs used in *POLG*-related epilepsy was three, and some patients received as many as ten drugs[36]. The best options for POLG-related epilepsy seem to be a sodium channel blocker (for example, lamotrigine) together with a benzodiazepine (for example, clobazam) and levetiracetam or topiramate as needed[89,119]. However, randomized clinical trials of AEDs have not been performed in *POLG*-related disease, and trial design is likely to be extremely challenging in view of the clinical heterogeneity of affected patients and unpredictable disease course.

VPA is absolutely contra-indicated in patients with *POLG*-related disease as it can precipitate liver failure (although some patients might develop liver failure without prior VPA exposure, and VPA hepatotoxicity is occasionally reversible[120]). It is recommended that the *POLG* gene should be sequenced before prescribing VPA to patients with status epilepticus[121].

Management of status epilepticus is particularly challenging in patients with *POLG* mutations. Many therapeutic modalities have been tried, including anaesthetic agents such as ketamine[122], magnesium infusion[123], high-dose steroids and intravenous immunoglobulin[124], and even palliative functional hemispherectomy in one individual with AHS[125], but an effective therapeutic regimen remains elusive.

[H2] Other supportive therapies

Other important symptomatic therapies for patients with *POLG*-related disease include removal of cataracts where necessary, brow suspension surgery for PEO, levodopa for individuals with symptoms of parkinsonism, antidepressants, and psychological support for affected patients and their carriers. Mitochondrial 'cocktails' of various combinations of vitamin supplements and/or antioxidants are frequently prescribed for patients with *POLG* mutations in an attempt to support mitochondrial function, but no evidence-based rationale exists for their use[126].

[H2] Liver transplantation

Since 1992, orthotopic liver transplantation has been reported in >40 individuals with presumed or genetically confirmed *POLG*-related disease, including patients with VPA-associated acute liver failure (VPA-ALF), and has shown life-saving potential for adolescents and adults (Table 4)[25,127,128]. However, in younger patients, death from progressive neurological decline has frequently occurred within 1 year of the transplant[117,129,130]. This experience has led to the suggestion that VPA-ALF is an absolute contraindication to liver transplantation in children aged <10 years, in whom neurological progression post-transplant seems almost inevitable, but that transplantation might be considered in carefully screened teenagers or adults[130]. In patients who are not considered suitable for liver transplantation, supportive therapy including carnitine should continue, as spontaneous resolution of VPA-ALF has occasionally been reported in patients with confirmed *POLG* mutations[120,131].

[H2] Experimental approaches

The ketogenic diet — a high fat, low carbohydrate diet — has been proposed as a therapy for various mitochondrial diseases on the basis of observations in cell and animal models[132,133]. Treatment with a ketogenic or low glycemic index diet has been reported in only a handful of patients with *POLG* mutations[49,134,135], without clear evidence of efficacy.

Decanoic acid, a fatty acid that is elevated in the blood of individuals on a ketogenic diet, has been implicated as an effective anticonvulsant agent. This compound seems to have pleiotropic roles, including stimulation of mitochondrial biogenesis and inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors[136,137]. Preliminary preclinical data in complex I-deficient patient fibroblasts were promising[138], but cells with *POLG* mutations have not yet been studied.

A high-throughput drug screen in *Saccharomyces cerevisiae* identified clofilium tosylate, a potassium channel blocker that functions as an anti-arrhythmic agent, as a potential mtDNA stabilizer in POLG deficiency, although the precise mechanism of action was unclear[139]. Further studies in a *Caenorhabditis elegans* model and fibroblasts from a single POLG-deficient patient with MCHS showed promise[139]. However, additional work is needed to assess the clinical applicability of clofilium tosylate and related compounds.

Nucleotides and nucleosides have been suggested as potential therapies for mtDNA depletion disorders arising from deficient intramitochondrial nucleoside salvage[140,141]. However as might be expected given that impaired nucleoside supply is not thought to be the primary disease mechanism in *POLG* disorders, nucleotide supplementation did not correct mtDNA depletion in POLG-deficient patient fibroblasts[142]. Gene therapy has been reported to be successful in animal models of other mitochondrial disorders, including MNGIE and ethylmalonic encephalopathy[143,144], and might be a future therapeutic approach for *POLG*-related disease.

[H1] Conclusions

Mutations in *POLG*, which encodes the catalytic subunit of pol γ, are associated with numerous clinically heterogeneous syndromes characterized by a quantitative and/or qualitative mtDNA defect. Seizures dominate the clinical picture, not only in childhood-onset cases, but also in *POLG*-related disease presenting in early adult life and in the adult ataxic forms of the disease, indicating a poor prognosis. Other disease manifestations include ataxia, movement disorders, PEO, myopathy and peripheral neuropathy, as well as multisystem features such as cataracts, cardiomyopathy, premature menopause and gastrointestinal pseudo-obstruction. Despite tremendous advances in mitochondrial disease diagnostics in recent years, effective disease-modifying therapies are still lacking, although some promising candidates are beginning to emerge.

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Author contributions

Both authors contributed to all aspects of manuscript preparation.

Competing interests

The authors declare no competing interests.

Publisher's note

DATABASES

Human DNA Polymerase Gamma Mutation Database: https://tools.niehs.nih.gov/polg/

dbSNP rs113994098: https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=113994098

Key points

- *POLG* encodes the catalytic subunit of DNA polymerase γ, the enzyme responsible for replicating the mitochondrial DNA (mtDNA)
- Mutations in *POLG* are associated with a clinical continuum of heterogeneous syndromes, ranging from infantile-onset epilepsies and liver failure to late-onset ophthalmoplegia and muscle weakness
- *POLG* mutations are a frequent cause of mitochondrial disease, particularly mitochondrial epilepsy, polyneuropathy, ataxia and progressive external ophthalmoplegia
- *POLG* mutations can lead to depletion of the mtDNA and/or accumulation of multiple mtDNA deletions
- To a limited extent, clinical phenotypes correlate with the mtDNA phenotype (depletion or deletions)
- No effective disease-modifying therapies are currently available for *POLG*-related disease, and symptomatic therapies are the mainstay of treatment

Figure 1 | The clinical spectrum of *POLG*-related disease. Clinical spectrum of *POLG*-related disease according to age of onset, and the defects (mitochondrial DNA (mtDNA) depletion or deletions) associated with the diseases. AHS, Alpers–Huttenlocher syndrome; ANS, ataxia neuropathy spectrum; MCHS, myocerebrohepatopathy spectrum; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MEMSA, myoclonic epilepsy myopathy sensory ataxia; MNGIE, mitochondrial neurogastrointestinal encephalopathy; PEO, progressive external ophthalmoplegia; SANDO, sensory ataxia neuropathy dysarthria and ophthalmoplegia; SCAE, spinocerebellar ataxia with epilepsy.

Figure 2 | *POLG* mutations. Mutation map depicting disease-associated amino acid substitutions on the primary structure of POLG. In each panel, the top line depicts the 23 exons of the cDNA and the lower line represents the linear polypeptide with the functional domains (exonuclease and polymerase) indicated. The polymerase active site is subdivided into thumb, palm and fingers subdomains. A full list of disease-related substitutions can be found in the Human DNA Polymerase Gamma Mutation Database. Asterisks indicate mutations that have also been identified as frequent single nucleotide polymorphisms.a | Mutations associated with Alpers–Huttenlocher syndrome and other infantile hepatocerebral syndromes that cause mitochondrial DNA depletion. b | Mutations associated with progressive external ophthalmoplegia (PEO). c | Other mutations linked to *POLG*-related disease. ANS, ataxia neuropathy spectrum; MIRAS, mitochondrial recessive ataxia syndrome; NRTI, nucleoside reverse transcriptase inhibitor; SANDO, sensory ataxia neuropathy dysarthria and ophthalmoplegia; SCAE, spinocerebellar ataxia with epilepsy.

Table 1 | Genes associated with disorders of mitochondrial DNA stability

Pathway	Gene	Chromosomal locus	Protein function	Disorders
mtDNA replication	*POLG*	15q25	Pol γ catalytic subunit	Alpers–Huttenlocher syndrome, polyneuropathy, ataxia, PEO[12,14,16]
	POLG2	17q23–24	Pol γ accessory subunit	PEO[146]
	TWNK	10q24	Mitochondrial DNA helicase	PEO, mtDNA depletion, IOSCA[147]
	RNASEH1	2p25	RNA–DNA hybrid endoribonuclease	Encephalomyopathy, mtDNA deletions[148]
	TFAM	10q21.1	Transcription factor	mtDNA depletion[149]
	TOP3A	17p11.2	Topoisomerase	PEO, mtDNA deletions[150]
mtDNA repair	*DNA2*	10q21.3–22.1	Flap endonuclease	mtDNA deletions, PEO[151]
	MGME1	20p11.23	Single-strand DNA nuclease	PEO, mtDNA depletion[152]
dNTP metabolism	*SLC25A4*	4q35	Adenine nucleotide translocator	PEO[153]
	TYMP	22q13.32	Thymidine phosphorylase	MNGIE, mtDNA deletions and depletion[154]
	TK2	16q22–23.1	Mitochondrial thymidine kinase	PEO, mtDNA depletion[155]
	DGUOK	2p13	Deoxyguanosine kinase	mtDNA depletion[156]
	RRM2B	8q23.1	p53-inducible small subunit of ribonucleotide reductase	PEO, mtDNA depletion[157]
	SUCLA2	13q14.2	ATP-dependent succinate CoA ligase	mtDNA depletion[158]
	SUCLG1	2p11.2	GTP-dependent succinate CoA ligase	mtDNA depletion[159]
	MPV17	2p23.2	Mitochondrial inner membrane protein	mtDNA deletions and depletion[160]
	ABAT	16p13.2	4-aminobutyrate aminotransferase	mtDNA deletions and depletion[161]
Mitochondrial dynamics	*OPA1*	3q28–29	Dynamin-related GTPase	DOA, mtDNA deletions, ataxia[162]
	MFN2	1p36.22	Mitofusin 2	DOA, mtDNA deletions[163]
	FBXL4	6q16.1–3	Mitochondrial leucine-rich repeat F-box protein	mtDNA depletion, encephalopathy[164]

	AFG3L2	18p11.21	Mitochondrial inner membrane metalloprotease	Spinocerebellar ataxia, mtDNA deletions[165]
	SPG7	16q24.3	Mitochondrial inner membrane metalloprotease component	PEO, ataxia, spastic paraplegia[166]
	GFER	16p13.3	Protein import to the intermembrane space	mtDNA deletions, myopathy[167]

The table is adapted and updated from ref. 145. dNTP, deoxynucleotide triphosphate; DOA, autosomal dominant optic atrophy; IOSCA, infantile-onset spinocerebellar ataxia; MNGIE, mitochondrial neurogastrointestinal encephalopathy; mtDNA, mitochondrial DNA; PEO, progressive external ophthalmoplegia; pol γ, DNA polymerase γ.

Table 2 | Common *POLG*-related disorders

Age of onset	Syndrome	Mitochondrial DNA defect
Neonatal or Infancy	Myocerebrohepatopathy spectrum (MCHS)	Depletion
Infancy or childhood	Alpers–Huttenlocher syndrome (AHS)	Depletion
Adolescent or young adult	Ataxia neuropathy spectrum (ANS) including mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO)	Deletions
	Myoclonic epilepsy myopathy sensory ataxia (MEMSA) including mitochondrial spinocerebellar ataxia with epilepsy (SCAE)	Deletions
	Progressive external ophthalmoplegia (PEO) with or without sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO)	Deletions

Table 3 | Common *POLG* pathogenic variants

POLG pathogenic variant	Prevalence	Reference
A467T	Europe: 0.17–0.69%	[105]
	Belgium: 0.6%	[12]
	UK: 0.69%	[168]
	Italy: 0%	[168]
W748S	Finland: 0.8%	[15]
	Italy: 0%	[168]
G848S	0.05–0.10%	dbSNP rs113994098
T251I–P587L	0.05–1.00%	[103]

Table 4 | Orthotopic liver transplantation: outcomes in suspected and proven *POLG*-related disease

Report	Number of recipients	Diagnosis	Age at transplantation	Outcome
Bicknese *et al*. (1992)[169]	1	VPA-ALF (AHS)	3 years 9 months	Died from relentlessly progressive neurological deterioration 3 months post-transplant
Bell *et al*. (1992)[127]	1	VPA-ALF	23 years	Long-term survival reported
Thomson *et al*. (2000)[129]	5	VPA-ALF (AHS)	15 months, 3 years 6 months, 3 years 8 months, 3 years 11 months and 6 years 6 months	All died of progressive neurological disease within 1 year of transplantation (1–11 months)
Delarue *et al*. (2000)[170]	1	VPA-ALF (AHS)	3 years	Seizures recurred immediately after transplantation and patient died from neurological progression 4.5 months post-transplant
Kayihan *et al.* (2000)[171]	1	VPA-ALF (AHS)	12 years	Rapid neurological deterioration (severe ataxia, tremor and generalized epilepsy) 6 weeks after transplantation; died 6 months post-transplant
Tzoulis *et al.* (2006)[65]	2	*POLG*-related disease	20 and 28 years	One died immediately after transplantation; second alive 5 years post-transplant
Wolf *et al*. (2009)[28]	1	*POLG*-related disease (AHS)	6 years 9 months	Progressive neurological deterioration leading to death 10 months post-transplant
Wong *et al*. (2008)[25]	1	*POLG*-related disease	19 years	Alive 9 years post-transplant
Saneto *et al*. (2010)[121]	1	*POLG*-related disease	21 years	Died 2 days post-transplant
Mindikoglu *et al*. (2011)[131]	17	VPA-ALF	1–16 years (15 cases <8 years)	14 died within 1 year of transplantation (median survival for whole group: 2.8 months post-transplant); no long-term survivors
Hynynen *et al*. (2014)[128]	4	*POLG*-related disease	20 , 21, 14 and 36 years	All had only occasional seizures post-transplant; three long-term survivors (4, 4 and 19 years); fourth patient (aged 36 years at transplantation) died suddenly 2 years post-transplant
Parikh *et al.* (2016)[172]	6	*POLG*-related disease	Not specified	Direct worsening of mitochondrial disease symptoms post-transplant in three patients, with two dying shortly after transplantation; two patients

				with *POLG*-related AHS had no complications or symptom progression (ages not given)

AHS, Alpers–Huttenlocher syndrome (clinical and/or neuropathological diagnosis); VPA-ALF valproic acid-associated acute liver failure.

Fig 1

POLG mutations – clinical spectrum

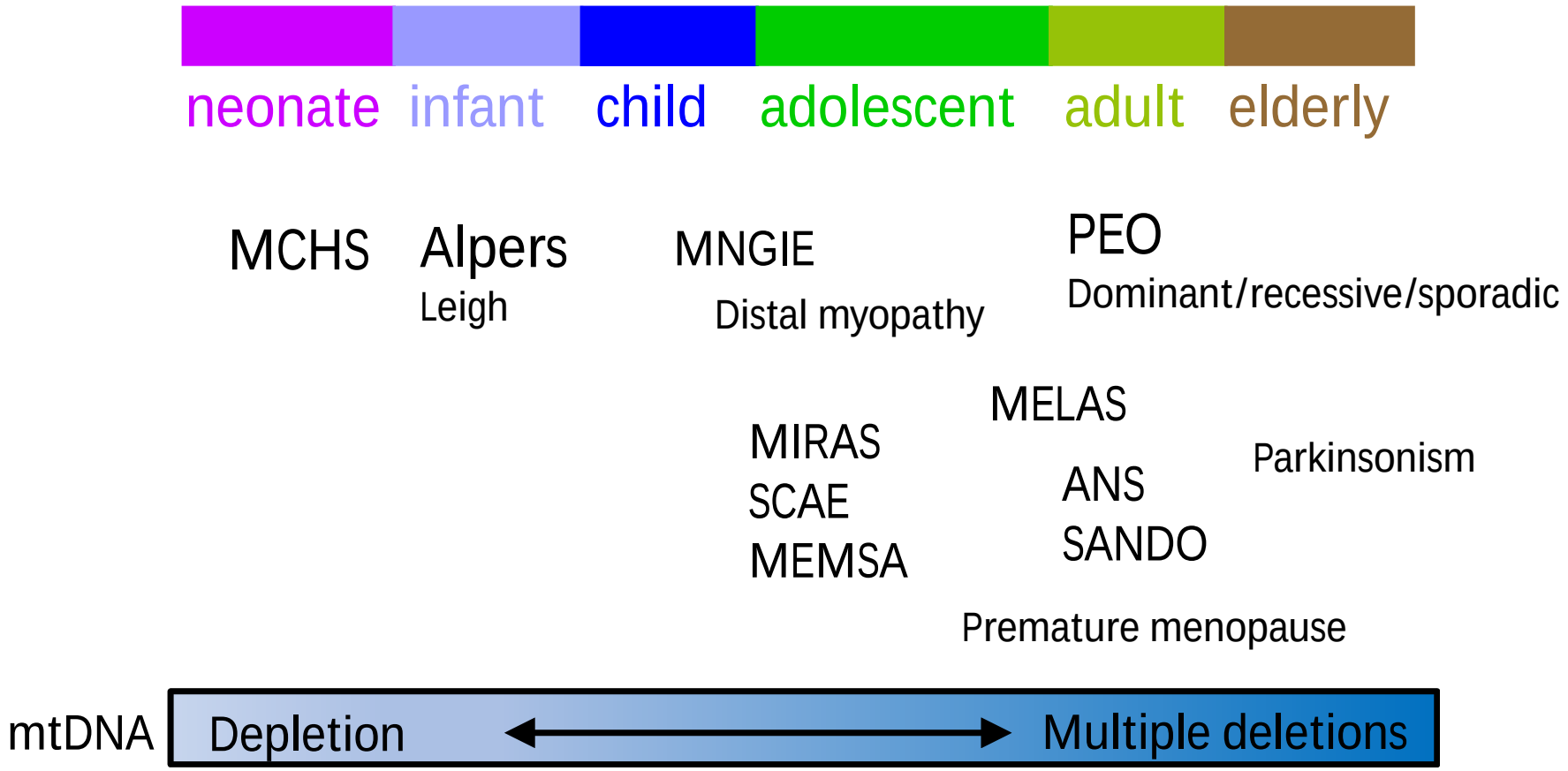

Fig 2a

Alpers mutations in human DNA polymerase γ, *POLG*

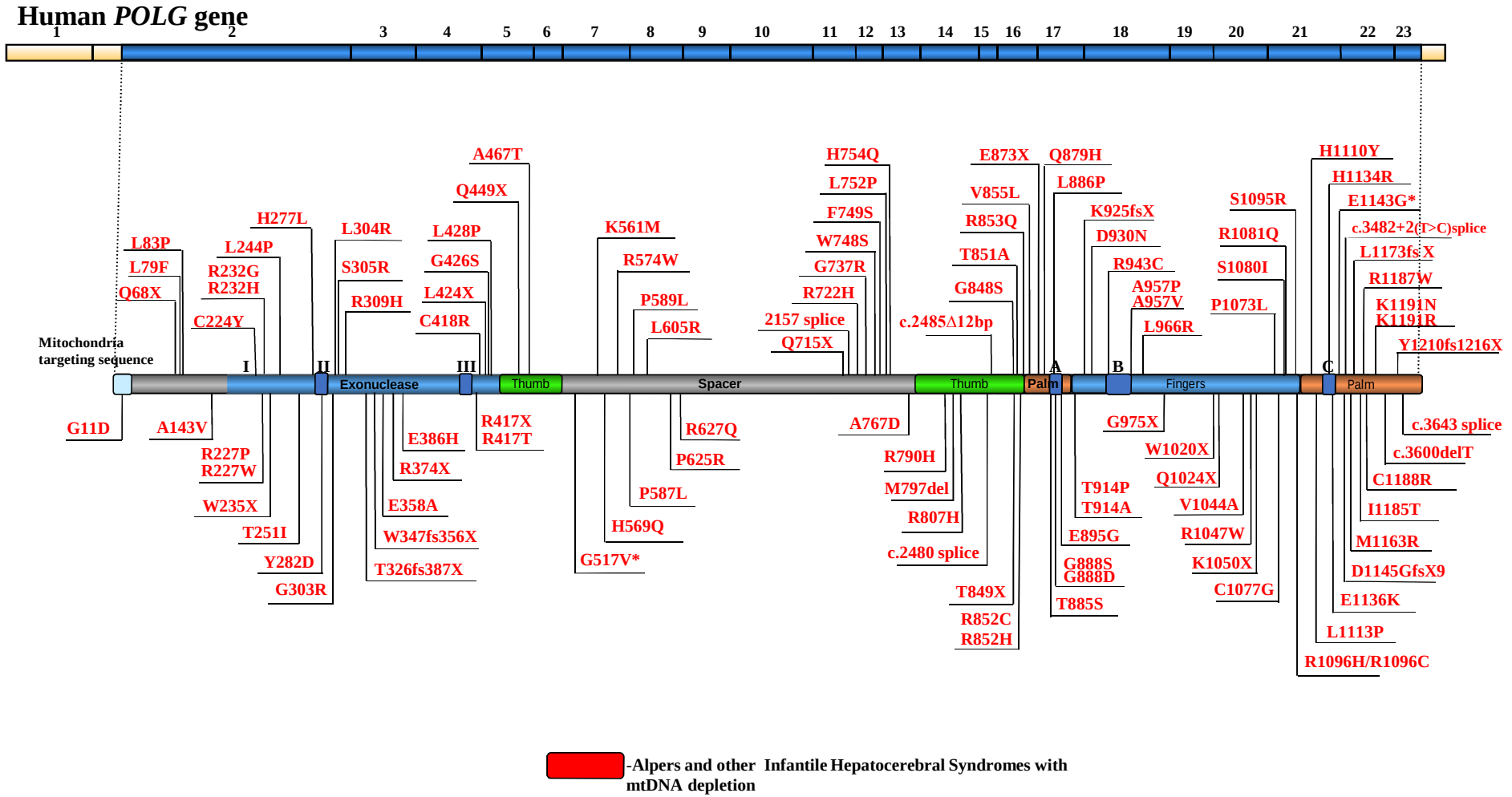

Fig 2b

PEO mutations in human DNA polymerase γ, *POLG*

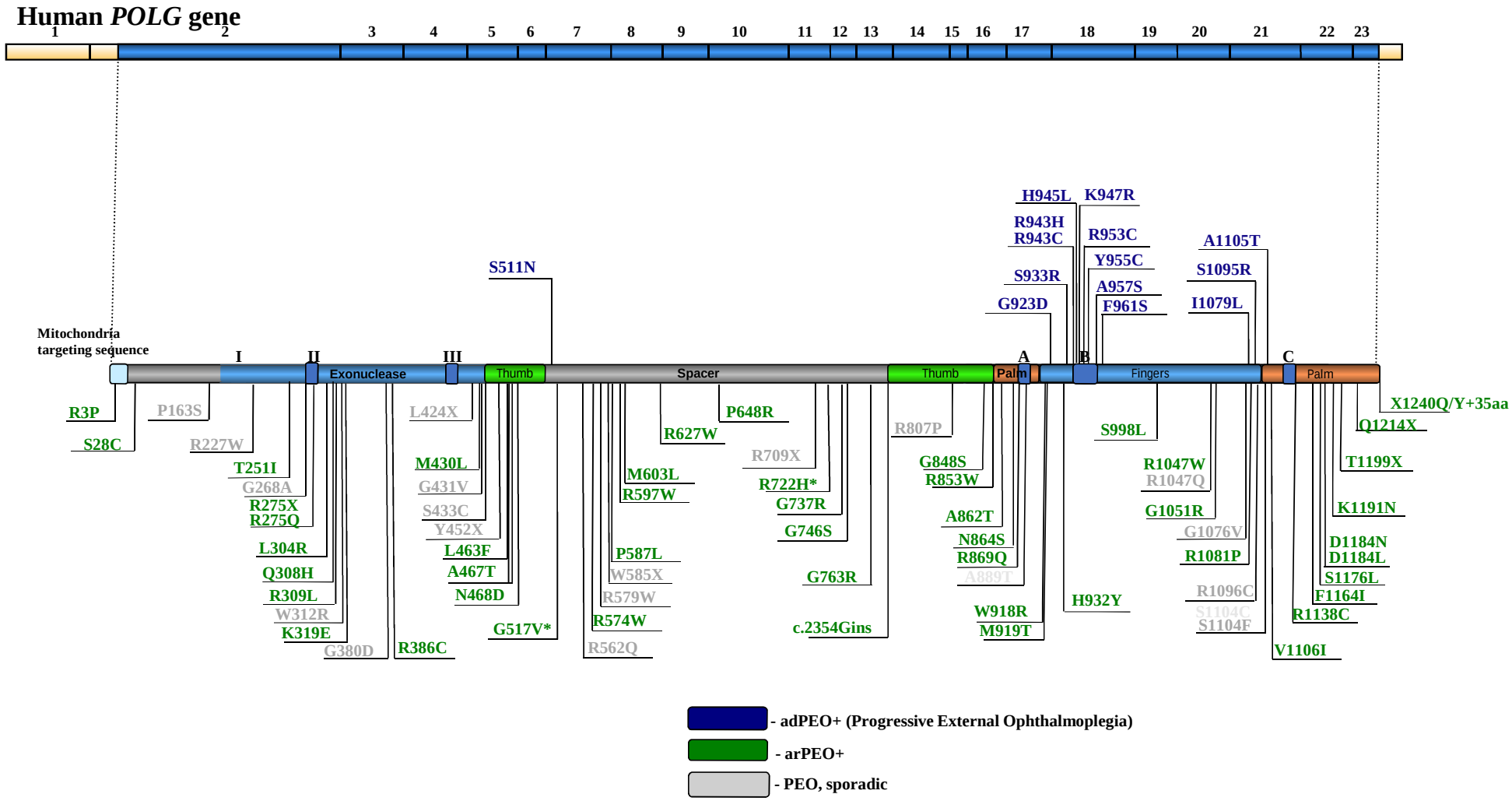

Fig 2c

Other mutations in human DNA polymerase γ, *POLG*