The unfolding clinical spectrum of POLG mutations


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The unfolding clinical spectrum of POLG mutations

M J Blok,1 B J van den Bosch,1,2 E Jongen,1 A Hendrickx,1 C E de Die-Smulders,1 J E Hoogendijk,3 E Brusse,4 M de Visser,5 B T Poll-The,6 J Bierau,1 I F de Coo,7 H J Smeets1,2

ABSTRACT

Background: Mutations in the DNA polymerase-γ (POLG) gene are a major cause of clinically heterogeneous mitochondrial diseases, associated with mtDNA depletion and multiple deletions.

Objective: To determine the spectrum of POLG mutations in our Dutch patient cohort, to evaluate the pathogenicity of novel mutations, and to establish genotype–phenotype correlations.

Results: The authors identified 64 predominantly recessive mutations in 37 patients from a total of 232 patients, consisting of 23 different mutations. The substitution p.A467T was most frequently observed (n = 23), but was as frequent in childhood cases as in adult cases. Five new pathogenic recessive mutations, p.Lys925ArgfsX42, p.R275X, p.G426S, p.A804T and p.R869Q were identified. The known dominant chronic progressive external ophthalmoplegia (CPEO) mutation p.R943H was for the first time associated with premature ovarian failure as well. In 19 patients the authors identified only a single recessive mutation, or a sequence variant with unclear clinical significance. The data substantiate earlier observations that in POLG patients a fatal status epilepticus and liver failure can be triggered by sodium valproate. It is therefore important to exclude POLG mutations before administering this treatment.

Conclusion: The clinical features of the patient are the most important features to select putative POLG mutation carriers and not the presence of mtDNA deletions or OXPHOS (oxidative phosphorylation) activity. The authors conclude that POLG mutations are important causes of heterogeneous mitochondrial pathology and that more accurate genotype–phenotype correlations allow a more rapid genetic diagnosis and improved prognosis for mutation carriers.

Maintenance of the mtDNA is critical for the mitochondria to function properly and depends on the replication machinery and nucleotide availability. The most important enzyme involved in mtDNA replication is the polymerase γ (POLG),1 which consists of a polymerase domain, a 3′–5′ exonuclease domain with proofreading activity, connected by a linker region, involved in POLG processing.2 Mutations in POLG are being reported in a growing number of patients with mitochondrial disorders, displaying a broad range of clinical features. Over 100 different dominant or recessive mutations have been described throughout the entire gene, which were familial, sporadic or of unknown origin (http://tools.niehs.nih.gov/polg/), and genotype–phenotype correlations are beginning to emerge.3

The objectives of this study were: (1) to determine the spectrum of mutations in our Dutch patient cohort; (2) to evaluate the pathogenicity of novel mutations; and (3) to establish genotype–phenotype correlations for POLG mutations, including clinical manifestations, activities of the complexes of the respiratory chain, and the presence of mtDNA deletions.

PATIENTS AND METHODS

Patients

A total of 252 patients was screened for POLG mutations. These patients were diagnosed in multiple clinical centres and presented with one or more of the following clinical symptoms: (chronic) progressive external ophthalmoplegia, ptosis, cerebellar ataxia (SCA negative), polynuropathy, Alpers syndrome, liver failure, epilepsy or status epilepticus, intestinal pseudo-obstruction, congenital hypotonia, migraine, premature ovarian failure, failure to thrive, and/or the presence of multiple mtDNA deletions/depletions in muscle. An extensive list of clinical features associated with POLG mutations was recently published.3

Sequence analysis of the POLG gene

Sequence primers were designed to amplify all protein encoding POLG exons and at least 40 nucleotides of the flanking introns (see supplemental tables 1 and 2 for polymerase chain reaction (PCR) primers and reaction conditions). Sequences were analysed using Mutation Surveyor (Softgenetics, Pennsylvania, USA) using NCBI nucleotide NM_002693.1 as reference sequence.

Criteria to determine pathogenicity of new POLG mutations

A mutation was considered new if it was neither protein encoding POLG exons and at least 40 nucleotides of the flanking introns (see supplemental tables 1 and 2 for polymerase chain reaction (PCR) primers and reaction conditions). Sequences were analysed using Mutation Surveyor (Softgenetics, Pennsylvania, USA) using NCBI nucleotide NM_002693.1 as reference sequence.
variants were found in combination with a known recessive mutation in two of our patients (patient 15 and 22, table 1), these two being girls. The p.A467T mutation was as common in the childhood cases, four girls and one boy were compound heterozygotes for this variant. Homozygous p.A467T mutations were detected in five patients, compound heterozygous in 10 patients, and as a single mutation in five patients. They cannot by themselves explain the clinical features observed. In four additional patients we found the p.G517V mutation that was previously reported as a dominant mutation, but its pathogenicity is questionable.

**Novel POLG mutations and variants**

Seven previously unreported variants were identified in six patients (table 1, patients 1–6)—that is, p.Lys925ArgfsX42, p.R275X, p.G426S, p.A804T, p.R869Q, and p.G426S in combination with p.D156E. None of the variants was found in a control panel of 100 individuals and they all affect evolutionary conserved amino acids, except for the p.D156E variant (fig 2). The pathogenicity of p.Q45R and p.D156E is unclear, whereas p.Lys925ArgfsX42, p.R275X, p.G426S, p.A804T and p.R869Q are considered pathogenic mutations. The mutations p.Lys925ArgfsX42 and p.R275X were found in compound with a known recessive pathogenic mutation, whereas for the other novel mutations no second POLG mutation was found. To check for a digenic cause, all POLG patients with a single mutation were screened for mutations in *Twinkle*, *DGuok*, *Tk2* and *Tp*, but no mutations were found. Variants p.S505R and p.S1095R have been reported in patients as the only POLG variant present making the clinical relevance unconfirmed.

In two of our patients (patient 15 and 22, table 1) these two variants were found in combination with a known recessive pathogenic mutation.

**Frequency and phenotypic characteristics of POLG mutations**

The p.A467T mutation is the most common mutation in our patient cohort, representing 40% of all alleles with a sequence variant. Homozygous p.A467T mutations were detected in five patients, compound heterozygous in 10 patients, and as a single mutation in three patients. The most common compound heterozygous combination was with the p.W748S mutation (n = 3). Of the 18 index patients for whom the clinical features are explained by the presence of two recessive or one dominant pathogenic mutation (table 1, patients 6–23), 12 patients were adults and six were children (<16 years). Of the childhood cases, four girls and one boy were compound heterozygotes for the p.A467T mutation and died at a young age, except for one girl. The p.A467T mutation was as common in the childhood onset cases (83%, five out of six) as in adult onset cases (85%, 10 out of 12). All index patients with homozygous p.A467T mutations were in the group of adult cases. The second most common mutation is the syntenic combination p.[T251I; P587L]. It was detected seven times (11% of all mutations)—that is, four times as compound heterozygous and three times as a single mutation. Only one known dominant mutation, p.R943H, was detected in a family with premature ovarian failure (POF) and chronic progressive external ophthalmoplegia (CPEO). In the group of patients with only one mutation, the p.G517V mutation was detected most frequently (n = 4), but the pathogenicity is questionable based on segregation and population data.

**RESULTS**

**General overview POLG mutations**

For the 252 patients in this study, 64 mutations were identified in 37 index patients (table 1), consisting of 23 different mutations. These mutations explained the clinical features in 18 patients (patients 6 to 23, table 1), 17 of whom had two known recessive mutations and one a dominant mutation. Most common was p.A467T, which was compound heterozygous in 10 patients and homozygous in five patients. In 200 Dutch controls we did not detect this mutation, so the carrier frequency seems <0.5%. For the remaining 19 patients, only one single known recessive mutation was found in 10 patients and a single new mutation in five patients. They cannot by themselves explain the clinical features observed. In four additional patients we found the p.G517V mutation that was previously reported as a dominant mutation, but its pathogenicity is questionable.

**Novel pathogenic POLG mutations**

p.Lys925ArgfsX42

Patient 6 (table 1) was a compound heterozygote for the new mutation p.Lys925ArgfsX42 in the polymerase domain and the p.A467T mutation. The patient died of status epilepticus and liver failure at the age of one and a half. The healthy parents are heterozygous carriers (fig 1). The p.Lys925ArgfsX42 mutation results in a premature stop codon at position 966 and is pathogenic by either removing part of the catalytic domain of polymerase γ and/or leading to nonsense mediated mRNA decay. In general, these mutations are recessive (http://tools.niehs.nih.gov/polg/) as demonstrated for the p.L965X mutation.

p.R275X

The novel p.R275X mutation was compound heterozygous with the known recessive combination p.[T251I; P587L] in a patient with CPEO and mental retardation (table 1, patient 2). The p.R275X is located within the second highly conserved motif (exo II) in the exonuclease domain and is predicted to remove a large part of the downstream functional POLG domains and/or leads to nonsense mediated mRNA decay. The sister and two daughters of the patient only carry the p.R275X mutation (fig 1). The sister displays similar, albeit less severe, clinical symptoms including CPEO, and one of the daughters presented with fatigue and the other with fatigue and muscle pain. Two other daughters carried only the p.[T251I; P587L] mutations, one unaffected and one diagnosed with multiple sclerosis and muscle pain. Parents of the index patient were not available for testing. Most likely the p.R275X mutation is recessive and the complaints of the carriers should have a different cause, although clinical symptoms in carriers cannot be completely excluded.

p.G426S

The novel POLG mutation p.G426S was identified in a patient with cerebellar ataxia, epilepsy, myoclonus, cognitive delay and juvenile cataract (table 1, patient 3). Her brother and sister, both carrying the same mutation, also have epilepsy and additionally juvenile cataract and asthma (fig 1). Parents of the index patient were unavailable. This mutation is likely pathogenic (table 2). The mode of inheritance is unclear, although the mutation is not located in the region where most dominant mutations are present. An unaffected brother carries the same mutation as well as his daughter, who has a history of epileptic seizures, most likely stress related. She remains to be neurologically examined in more detail. Family history further revealed juvenile cataract in the offspring of a maternal aunt. The available data do not provide a conclusive answer regarding its mode of inheritance.
<table>
<thead>
<tr>
<th>Index patient</th>
<th>Mutation</th>
<th>Nucleotide change</th>
<th>Predicted amino acid substitution</th>
<th>Previously reported?</th>
<th>Mode of inheritance</th>
<th>Domain</th>
<th>Index</th>
<th>Prominent clinical features</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>New mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>c.134A&gt;G</td>
<td>p.Q45R</td>
<td>N</td>
<td>AR?</td>
<td>PolyQ-tract</td>
<td>Exo</td>
<td></td>
<td>Epilepsy</td>
<td>Father epilepsy, carrier variant c.134A&gt;G, mother unaffected, carrier variant c.408C&gt;G, brother with epilepsy has both variants (fig 1)</td>
</tr>
<tr>
<td></td>
<td>c.408C&gt;G</td>
<td>p.D136E</td>
<td>N</td>
<td>AR?</td>
<td>+</td>
<td>Link</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>c.823C&gt;T</td>
<td>p.R275K</td>
<td>N</td>
<td>AR?</td>
<td>+</td>
<td>Pol</td>
<td></td>
<td>Ataxia, epilepsy, myoclonus, cognitive delay, juvenile cataract, MRI showed atypical white matter lesions</td>
<td>Brother and sister with epilepsy and cataract, other sister with epilepsy (fig 1)</td>
</tr>
<tr>
<td></td>
<td>c.[752C&gt;T; 1760C&gt;T]</td>
<td>p.[T251I; P587L]</td>
<td>Y&lt;sup&gt;13&lt;/sup&gt;</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>c.1276G&gt;A</td>
<td>p.G426S</td>
<td>N</td>
<td>AR?</td>
<td>+</td>
<td></td>
<td></td>
<td>Epilepsy, CPEO and mental retardation</td>
<td>No further family details known</td>
</tr>
<tr>
<td>4</td>
<td>c.2410G&gt;A</td>
<td>p.A804T</td>
<td>N</td>
<td>AR?</td>
<td>+</td>
<td></td>
<td></td>
<td>General fatigue muscle complaints, bulbar dysarthria</td>
<td>No further family details known</td>
</tr>
<tr>
<td>5</td>
<td>c.2606G&gt;A</td>
<td>p.R869Q</td>
<td>N</td>
<td>AR?</td>
<td>+</td>
<td></td>
<td></td>
<td>Myopathy with extensive neurogenic atrophy; severe sensomotor axonal polyneuropathy; ataxia and ptosis</td>
<td>Mutation status other family members not known; Father “ptosis” (not clinically confirmed), no known further family history</td>
</tr>
<tr>
<td>6</td>
<td>c.2772_2773delG</td>
<td>p.Lys925ArgX42</td>
<td>N</td>
<td>AR?</td>
<td>+</td>
<td></td>
<td></td>
<td>Prematurity, epilepsy, deafness, retinitis pigmentosa, status epilepticus, hemiparesis, liver failure</td>
<td>Healthy parents both carrier (fig 1)</td>
</tr>
<tr>
<td>Homozygous p.A467T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Migraine, sensory ataxia, seizures, status epilepticus, liver insufficiency</td>
<td>Sister with cerebellar ataxia, axonal neuropathy, migraine, initial external ophthalmoplegia, epilepsy, myoclonic seizures, mild mental retardation</td>
</tr>
<tr>
<td>8</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Focal seizures, Alpers syndrome</td>
<td>Healthy parents both carriers; two healthy brothers carrier status unknown</td>
</tr>
<tr>
<td>9</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Alpers syndrome</td>
<td>Healthy parents both carriers</td>
</tr>
<tr>
<td>10</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Alpers syndrome (no use of sodium valproate)</td>
<td>Healthy parents both carriers</td>
</tr>
<tr>
<td>11</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Mental retardation, encephalopathy, ataxia, neuropathy, myopathy, CPEO, polyneuropathy</td>
<td>Brother died of mitochondrial myopathy, not tested for mutations</td>
</tr>
<tr>
<td>Compound heterozygous for p.A467T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Failure to thrive, died after status epilepticus</td>
<td>Brother failure to thrive, died due to encephalopathy and hepatopathy at the age of one</td>
</tr>
<tr>
<td></td>
<td>c.680G&gt;C</td>
<td>p.R227P</td>
<td>Y</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Three other affected sisters with ptosis, external ophthalmoplegia, proximal muscle weakness, polyneuropathy. Mother with ataxia and diabetes, died from cardiac arrest without diagnosis</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Exercise intolerance, CPEO, retinitis pigmentosa, diabetes, limb girdle weakness</td>
<td>No further family details known</td>
</tr>
<tr>
<td></td>
<td>c.[752C&gt;T; 1760C&gt;T]</td>
<td>p.[T251I; P587L]</td>
<td>Y&lt;sup&gt;13&lt;/sup&gt;</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Cataract, myopathy</td>
<td>No further family details known</td>
</tr>
<tr>
<td></td>
<td>c.[752C&gt;T; 1760C&gt;T]</td>
<td>p.[T251I; P587L]</td>
<td>Y&lt;sup&gt;13&lt;/sup&gt;</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Alpers syndrome</td>
<td>Sister also died of Alpers syndrome, not tested for mutations (fig 1)</td>
</tr>
<tr>
<td>16</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td></td>
<td></td>
<td>Ptosis, CPEO, polyneuropathy, cerebellar ataxia, dysarthria, also SNP p.E1143G present</td>
<td>No further family details known</td>
</tr>
</tbody>
</table>

<sup>1</sup>Exo: exon; Link: link; Pol: polymorphism; AR: autosomal recessive; Y: yes; N: no; +: present; **: repeated case; polyQ-tract: poly-Q tract length; CPEO: cerebro-oculomotor syndrome; MRI: magnetic resonance imaging.
<table>
<thead>
<tr>
<th>Index patient</th>
<th>Nucleotide change</th>
<th>Predicted amino acid substitution</th>
<th>Previously reported?</th>
<th>Mode of inheritance</th>
<th>Domain*</th>
<th>Index</th>
<th>Gender</th>
<th>Age</th>
<th>Prominent clinical features</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>40</td>
<td>Occipital lobe epilepsy, myoclonus, cognitive delay, polyneuropathy, cerebellar ataxia</td>
<td>Two deceased sisters and one affected brother with similar features, all not tested for the mutations</td>
<td></td>
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<tr>
<td>18</td>
<td>c.2243G&gt;C</td>
<td>p.W748S</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>19</td>
<td>MELAS-like features, including occipital epilepsy</td>
<td>No further family details known</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>1</td>
<td>Status epilepticus, myoclony, developmental delay</td>
<td>Healthy parents both carriers</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>c.2740A&gt;C</td>
<td>p.T914P</td>
<td>Y</td>
<td>+</td>
<td></td>
<td>F</td>
<td>1*</td>
<td>Growth retardation, occipital strokes, focal epilepsy, liver failure, died of heart failure</td>
<td>Healthy parents carriers; healthy sister carrier p.A957P mutation</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>c.752C&gt;T; 1760C&gt;T</td>
<td>p.[T251I; P587L]</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>51</td>
<td>Ptosis</td>
<td>No further family details known</td>
<td></td>
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<tr>
<td>23</td>
<td>c.2828G&gt;A</td>
<td>p.R943H</td>
<td>Y</td>
<td>AD</td>
<td>+</td>
<td>F</td>
<td>71</td>
<td>POF and CPEO</td>
<td>Fig 3</td>
<td></td>
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<tr>
<td>24</td>
<td>c.752C&gt;T; 1760C&gt;T</td>
<td>p.[T251I; P587L]</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>M</td>
<td>10</td>
<td>Mental deterioration, treatment resistant epilepsy</td>
<td>Healthy parents, father also carrier of mutations</td>
<td></td>
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<tr>
<td>25</td>
<td>c.752C&gt;T; 1760C&gt;T</td>
<td>p.[T251I; P587L]</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>44</td>
<td>Cataract, cerebellar syndrome, polyneuropathy, myopathy, ataxia</td>
<td>Three brothers with a mild polyneuropathy, healthy parents</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>c.752C&gt;T; 1760C&gt;T</td>
<td>p.[T251I; P587L]</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>4</td>
<td>CPEO, ptosis, motor development delay</td>
<td>No further family details known</td>
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<tr>
<td>27</td>
<td>c.852C&gt;T</td>
<td>p.G268A</td>
<td>Y*</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>7</td>
<td>Parkinsonism, chorea, dystonia, mental retardation</td>
<td>Consanguineous parents</td>
<td></td>
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<tr>
<td>28</td>
<td>c.852C&gt;T</td>
<td>p.G268A</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>M</td>
<td>3</td>
<td>Feeding problems, hepatocerebral syndrome</td>
<td>Consanguineous parents</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>c.852C&gt;T</td>
<td>p.G268A</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>M</td>
<td>16</td>
<td>Severe progressive neuropathy, ptosis, intestinal problems</td>
<td>No further family details known</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>F</td>
<td>48</td>
<td>Neurodegeneration, impaired cognitive functions</td>
<td>Deceased sister with similar clinical features</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>c.1399G&gt;A</td>
<td>p.A467T</td>
<td>Y</td>
<td>AR</td>
<td>+</td>
<td>M</td>
<td>19</td>
<td>Spino cerebellar ataxia</td>
<td>No further family details known</td>
<td></td>
</tr>
</tbody>
</table>

**Continued**
In patient 4 (table 1), who presented with general fatigue, muscle complaints and dysarthria, we only identified the novel p.A804T mutation. Based on amino acid and structural alignment with T7 DNA polymerase, the late linker region, including the p.A804 residue, is located in the predicted thumb domain known to interact with the DNA. Two other recessive pathogenic mutations, p.R807P and p.R807C, in this thumb domain were reported as compound heterozygous in patients with PEO910 and SANDO (sensory ataxic neuropathy dysarthria ophthalmoplegia syndrome). Polyphen and SIFT prediction results indicate that the substitution is of little significance and neutral, but the for this domain relevant protein–DNA interactions are not fully taken into account by the in silico tools. Based on the genetic data of the patient only it is likely that the p.A804T mutation is a recessive pathogenic mutation.

A single new p.R869Q mutation was identified in a patient with myopathy, severe sensomotor axonal polyneuropathy, ataxia and ptosis (table 1, patient 5). The deceased father of the patient might have had ptosis as well. All three prediction programs indicate a possibly damaging effect on protein function (table 2) and it is the highest conserved site of all our novel variants identified (fig 2). The p.N864S mutation, which is present in the same conserved thumb domain, shows a comparable high level of conservation and is associated with MNGIE (mitochondrial neurogastrointestinal encephalopathy)-like syndrome clinical symptoms. The mode of inheritance remains unclear.

Novel unclassified POLG variants

The novel variants p.D136E and p.Q45R were identified as compound heterozygous in a patient who presented with epilepsy during fever at the age of 1.5 years (table 1, patient 1). His affected brother also carried both variants. The father with the p.Q45R substitution had cataract, while the healthy mother carried the p.D136E substitution. The D at this position is not highly conserved, and E is also present in Xenopus and Drosophila, making a pathogenic role questionable (table 2). The p.Q45R substitution interrupts a polyglutamine tract (polyQ), encoded by a CAG repeat in exon 2, at the most conserved position. Another variant in the same conserved domain—that is, p.Q43R—was also unclassified. The identified variants were not present in a Dutch control panel, but the patient is not Caucasian and an ethnically matched control panel was not available. Therefore, due to lack of further information, the clinical significance of both variants remains currently unclear.

Recessive mutations previously reported as the only variant detected in POLG

In patient 15 (table 1), the p.S305R mutation was detected together with the p.A467T mutation (table 1 and 2) in a patient with Alpers syndrome. Recently, this mutation has been reported as the only mutation present in a 1-year-old patient with Alpers syndrome. It is located within a conserved region of the POLG gene, which also includes the known pathogenic mutation p.L304R. In patient 22, we identified the p.S1095R mutation in combination with the known recessive pathogenic p.D1184N mutation. Her healthy parents are both carrier of one of the mutations (fig 1). Recently, the p.S1095R mutation has been identified in a patient with CPEO, but as the only mutation in POLG. Our data confirm both mutations as recessive and pathogenic.
**Figure 1**  Family trees with segregation pattern of (new) mutations. +, mutation carrier; −, mutation not detected; CPEO, chronic progressive external ophthalmoplegia; n.d., not determined.
Homo sapiens
Pan troglodytes
Mus musculus
Rattus norvegicus
Xenopus laevis
D. melanogaster
S. cerevisiae
S. pombe
Neurospora crassa
Consensus


**Cases with one recessive mutation**

In 19 other patients we identified either sequence variants of unclear clinical relevance, or only a single known or new, recessive mutation (patients 1–5, 24–27, table 1). In these cases it is possible that the detection of the POLG mutation is just a coincidence and that the mutation causing the disease is located in another (unknown) gene. Based on the reported carrier frequencies for the p.A467T mutation we estimated the likelihood that a second mutation would be present. The allele frequency in the neighboring countries Belgium and Germany is, respectively, 0.6% and 0.19% and the mutation was not found in 400 Dutch control alleles, which indicates the carrier frequency is not higher than in Germany. In 214 unresolved cases (423 chromosomes) the p.A467T was detected three times, which is an enrichment compared to the control population, supporting the likelihood of a second POLG mutation in an intron or promoter or a large rearrangement, which is currently not being tested. For the detection of these kind of defects, other assays should be developed and implemented in the standard approach. For the detection of these kind of defects, other assays should be developed and implemented in the standard approach.

**Genotype–phenotype correlations**

The p.A467T mutation and childhood versus adult cases

The p.A467T mutation is the most common mutation in the POLG gene (http://tools.ncbi.nlm.nih.gov/polg/). Biochemical studies showed that the mutated protein possesses only 4% of wild-type DNA polymerase activity, while it failed to interact with and was not stimulated by the accessory subunit POLG2. The late onset ptosis in carriers of an Austrian family, was not present in carriers of our families. Our data do not confirm the reported male gender bias for children, although the number of severely affected children is low in our study. We detected the p.A467T mutation as frequent in the childhood onset as in adult cases (83%), which is in contrast to the reported higher frequency in children (60% vs 20%). Moreover, all index patients with homozygous p.A467T mutations were in the group of adult cases. This confirms previous observations that homozygous p.A467T mutations are correlated with a later onset form of Alpers syndrome, or a more chronic spectrum of disorders referred to as the ataxia neuropathy spectrum.

**Compound heterozygous p.A467T mutations**

The finding that the majority of the severe clinical cases involve one mutation in the linker domain—for example, the p.A467T mutation—and one mutation in the polymerase domain, does not apply to our severely affected patients. In concordance with this observation are the two families 6 and 20, in which the p.A467T mutation was detected together with the p.R227P mutation, which is located in the exonuclease domain, or the first 2/3 of the linker domain. The finding that the majority of the severe clinical cases involve one mutation in the linker domain—for example, the p.A467T mutation—and one mutation in the polymerase domain, does not apply to our severely affected patients. In concordance with this observation are the two families 6 and 20, in which the p.A467T mutation was detected together with the p.R227P mutation, which is located in the exonuclease domain, or the first 2/3 of the linker domain.
given the highly reduced POLG activity for the p.A467T mutation. An additional dominant negative effect for the heterozygous alleles, through a possible quaternary interaction between catalytic subunits in different heterotrimers composed of POLG and POLG2 subunits, has been postulated. In particular, symptoms of Alpers syndrome appear to be triggered in p.A467T homozygotes by treatment with the widely used drug sodium valproate for epileptic seizures (patients 7, 8 and 9; table 1). This can also be the case for heterozygous p.A467T or p.W748S carriers. The reported mean age of onset in these patients is 7 years later than for p.A467T or p.W748S homozygotes, but the median survival after onset is reported to be only 6 years versus 50 and 26 years, respectively. The deceased patients in their study suffered from epilepsy and developed liver failure, and were all treated with sodium valproate. The surviving patients were all over age 40, did not have epilepsy, and were never treated with sodium valproate. Patient 16, 17 and 18 of our cohort were compound heterozygotes for the p.A467T and p.W748S mutations and showed severe clinical symptoms, including epilepsy (patient 17 and 18), but were never treated with sodium valproate and have no liver failure until now. Remarkably, one sister of patient 17 died at the age of 34 years of toxic liver failure, although sodium valproate had been replaced by phenytoin and carbamazepine 2 years before. The presence of POLG mutations in this sister was not determined, however. A detrimental effect of sodium valproate use in POLG mutation carriers has been reported before and our data substantiate these findings. Sodium valproate is known to inhibit mitochondrial fatty acid oxidation and a mitochondrial deficiency should therefore exclude its use.

OXPHOS complex activity and POLG mutations

POLG mutations may cause deletions and/or single base point mutations in the mtDNA and possibly a reduction in copy number. This can result in a measurable deficiency of the OXPHOS (oxidative phosphorylation) system, mostly detected in muscle. However, this is not always the case. For example, patient 22 showed normal biochemistry in muscle, while in liver reduced complex I, III and IV activity was found. Also patient 7, who suffered from multiorgan failure, including liver failure, showed normal muscle biochemistry, but reduced complex II activity in liver and reduced complex III activity in brain. Finally, patient 9, who died at the age of 18 years of Alpers syndrome, also did not show a complex deficiency in muscle. Unfortunately, a liver biopsy was not available for testing. These findings corroborate with previous case reports in which POLG mutations are not necessarily correlated with decreased OXPHOS activity both in children and adults. We therefore conclude that the absence of a complex deficiency in a muscle biopsy does not exclude the presence of a POLG mutation. Complex activity in other affected tissues, such as liver and brain, is more likely to be reduced.

POLG mutations and mtDNA deletions

The screening for mtDNA deletions in muscle also appears to be of limited value for the selection of those patients eligible for POLG mutation analysis. For example, patient 12, 22 and the sister of patient 7 did not show mtDNA deletions in muscle, although being severely affected, even though patient 12 did show decreased OXPHOS activity in muscle. On the contrary, in four other adults (patients 11, 14, 16, 21, and 25), all aged over 47 years, multiple mtDNA deletions were detected in...
muscle using PCR and confirmed by Southern blotting, except for patient 11. This is in agreement with the observation that a sensitive PCR assay will detect deleted mtDNA in POLG patients presenting in adult life. However, it should be noted that the presence of mtDNA deletions in adult patients can be a secondary effect due to, for example, ageing.

Therefore, detection of deleted mtDNA in young children could be more indicative for a POLG mutation, but absence does not exclude it.

POLG mutations and POF

The clinical spectrum of POLG defects includes premature ovarian failure (POF) for the dominant POLG mutation p.Y955C in combination with PEO. Previous structure-function studies showed that the p.Y955C mutation leads to a severe decrease (<1%) in polymerase activity compared to wild-type. This is also the case for the dominant p.R943H mutation, which we report for the first time in a family with POF and CPEO and/or cataract (patient 23, table 1 and fig 3). This indicates that similar biochemical consequences of mutations based on functional studies can predict genotype-phenotype correlations.

Reclassification of the dominant p.G517V mutation to an unclassified variant

In four patients (34–37) we found the p.G517V mutation, which was previously reported as a dominant mutation in a single family, but its pathogenicity and causal relation to the clinical features is questionable. We detected this mutation in a consanguineous family in three affected brothers, but also in unaffected family members (fig 4). In three additional patients, the p.G517V mutation was detected but there was no clear family history suggesting a dominant inheritance pattern. Altogether, the p.G517V should be considered as an unclassified sequence variant for which the clinical relevance is currently unclear. This is in agreement with recent findings reported by Wong et al and Sarzi et al who suggested that the p.G517V mutation either shows a recessive inheritance pattern or it is a rare polymorphism or modifier.

CONCLUSIONS

Mutations in the POLG gene are a common cause of OXPHOS disease and result in a broad spectrum of clinical features. The detection of five new mutations and the establishment of pathogenicity in our study for two recessive mutations previously reported as single variants, further expands the spectrum of POLG mutations. There is a clear need for more functional studies to confirm the pathogenicity of POLG sequence variants. The genotype-phenotype correlations for mutations in sporadic mitochondrial diseases with multiple mtDNA deletions.

Figure 4 Family with p.G517V sequence variant, including index patient 34. +, mutation carrier; −, mutation not detected; MR, mental retardation; n.d., not determined.

POLG mutations are unfolding, particularly for the p.A467T mutation, since most data are available for this commonly detected mutation. The p.A467T mutation is often detected in patients with severe clinical features including status epilepticus and liver failure. It is therefore important that the use of sodium valproate to treat epileptic seizures in patients with POLG mutations should be avoided since this drug appears to have strong adverse effects in these patients. Finally, since OXPHOS activity measurements and mtDNA deletion analysis both appear to be of limited value for the selection of putative POLG mutation carriers, the inclusion of patients for mutation screening should be largely driven by clinical features, of which CPEO, ataxia (preferably including epilepsy) and liver failure (particularly in young children), are important features.

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Competing interests: None.

Provenance and peer review: Not commissioned; externally peer reviewed.

REFERENCES

5. Horvath R, Hudson G, Ferrari G, Futterer N, Ahola S, Lamantea E, Prokisch H, Lochmuller H, McFarland R, Ramesh V, Kloostop

**Mutation report**

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**Figure 3** p.R943H mutation in a family with premature ovarian failure (POF) and chronic progressive external ophthalmoplegia (CPEO), including index patient 23. +, mutation carrier; n.d., not determined.

**Figure 4** Family with p.G517V sequence variant, including index patient 34. +, mutation carrier; −, mutation not detected; MR, mental retardation; n.d., not determined.

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