Clinical and Genetic Heterogeneity in Progressive External Ophthalmoplegia Due to Mutations in Polymerase γ

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Background: The mendelian forms of progressive external ophthalmoplegia (PEO) associated with multiple mitochondrial DNA deletions are clinically heterogeneous disorders transmitted as dominant or recessive traits. Autosomal dominant PEO is caused by mutations in at least 3 genes: adenine nucleotide translocator-1 (ANT1), encoding the muscle-specific adenine nucleotide translocator; chromosome 10 open reading frame 2 (C10orf2), encoding Twinkle helicase; and polymerase γ (POLG), encoding the α subunit of polymerase γ. Mutations in POLG can also cause autosomal recessive PEO, which is often associated with multisystemic disorders.

Objective and Methods: To further investigate the frequency and genotype-phenotype correlations of mutations in the POLG gene, we used single-stranded conformational polymorphism analysis and direct sequencing to screen 30 patients with familial or sporadic PEO and multiple mitochondrial DNA deletions in muscle but without mutations in ANT1 and C10orf2.

Results: Four unrelated patients had novel POLG mutations. A woman with PEO and mental retardation had a heterozygous Gly1076Val mutation. Two patients, one with PEO, exercise intolerance, and gastrointestinal dysmotility and the other with PEO, neuropathy, deafness, and hypogonadism, both had a Pro587Leu change. The fourth patient, who was compound heterozygous for Ala889Thr and Arg579Trp mutations, had PEO, gastrointestinal dysmotility, and neuropathy. These mutations were not detected in 120 healthy control alleles.

Conclusions: Our results demonstrate that POLG mutations account for a substantial proportion of patients (13%) with PEO and multiple mitochondrial DNA deletions and cause both clinically and genetically heterogeneous disorders.

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Pedigrees of 4 families with polymerase γ (POLG) mutations. Affected individuals are indicated by solid symbols. Stippled symbols indicate subjects without progressive external ophthalmoplegia (PEO) but with other symptoms. Asymptomatic individuals are indicated by open symbols. Triangles indicate individuals whose sex was unknown. Arrows indicate index cases. Slashed symbols indicate deceased subjects. Heterozygotes are indicated by +/-, healthy individuals by -/-, and compound heterozygotes by +/-.

Patient 1 (Figure 1A), a 51-year-old Ukrainian woman, was born to nonconsanguineous parents. She was healthy until age 4 years, when she was noted to have a learning disability. Mental retardation was later diagnosed, and she required special schooling throughout childhood. At age 41 years, she had difficulty moving her eyes, and she had to turn her head to fixate on objects. She also developed progressive bilateral ptosis. She did not have dysarthria, dysphagia, dyspnea, or limb weakness. Both her father, who died at age 72 years, and her paternal grandmother, who died at age 93 years, had long-standing histories of bilaterally impaired eye movements. The patient’s brother and her 7-year-old daughter are healthy. Neurological examination results showed bilateral ptosis, despite previous blepharoplasties, and minimal lateral and convergence eye movements. No other neurological abnormalities were noted. Endocrinological study results, including parathyroid and thyroid function test results, were normal. Magnetic resonance imaging of the brain demonstrated mild dilatation of the subarachnoid spaces, prominence of the cerebellar folia, and a retention cyst along the posterior wall of the sphenoid sinus.

Patient 2 (Figure 1B), a 61-year-old man, noted numbness in his legs at age 47 years. Later, he developed PEO, generalized muscle weakness, exercise intolerance, unsteadiness (especially in the dark or with eyes closed), abdominal cramping, and gastrointestinal dysmotility with alternating diarrhea and constipation. A 63-year-old sister had an 18-year history of PEO, exercise intolerance, distal limb weakness, and diabetes mellitus. She also had peripheral neuropathy with stocking-glove numbness. Her 44-year-old son and a 42-year-old daughter have diabetes mellitus. The proband’s parents are described as healthy, although the mother apparently had mood swings. A maternal aunt had PEO.

Patient 3 (Figure 1C) was a 43-year-old man, who, at age 7 years, developed progressive hearing loss. At age 10 years, he had difficulty with his balance, and at age 25 years, he had dysconjugate gaze. At age 31 years, he had hypogonadism with low testosterone levels and normal pituitary function. Neurological examination results showed impaired communication and speech because of severe deafness and oropharyngeal muscle weakness. In addition, he had bilateral ptosis and ophthalmoplegias, with restriction of eye movement in all directions to 60% of normal. There was also proximal and distal symmetrical limb weakness, more severe in the legs than in the arms, and impaired vibration sensation in the feet. Electromyograms showed mixed neuropathic and myogenic features. His 33-year-old brother has similar but more severe symptoms and is wheelchair-bound. Another brother, 2 sisters, their children, and both parents are healthy.

Patient 4 (Figure 1D), a 58-year-old woman, at age 30 years noted slowly progressive ptosis and limitation of eye movements. Later, she developed ataxia, orthostatic dizziness, cataracts, and gastrointestinal dysmotility with diarrhea and constipation. Neurological examination results showed normal mental status, ptosis with almost complete PEO, vibration sensory loss, sensory ataxia, and areflexia. Her sister, who had died in a car accident, reportedly had PEO. Her parents and her 4 children are healthy.

**RESULTS**

Muscle biopsy results in all 30 patients revealed ragged red fibers and cytochrome c oxidase–negative fibers (data
not shown). Biochemical analysis of muscle extracts showed decreased activities of multiple respiratory chain enzymes; values in patients with POLG mutations are listed in Table 3. All patients had multiple mtDNA deletions detected with Southern blot analysis (data not shown).

We found POLG mutations in 4 patients (13% of all cases; 17% of familial cases). In agreement with results from a previous report,7 we considered the A of the AUG chain-initiation codon of the POLG messenger RNA as nucleotide position +1 and the corresponding methionine as the first amino acid residue of the protein sequence.

Patient 1 was heterozygous for a G3227T transition in exon 10, which resulted in a Gly1076Val amino acid change. Analysis of DNA in her healthy brother did not show any POLG mutation (Figure 2A).

Patient 2 and his affected sister were both heterozygous for a C1760T transition in exon 10, which resulted in a Pro587Leu amino acid change (Figure 2B). Patient 3 was heterozygous for the same C1760T mutation identified in patient 2 (Figure 2C).

Patient 4 was compound heterozygous for a G2665A mutation in exon 17, which resulted in an Ala889Thr amino acid change (Figure 2D), and for a C1735T transition in exon 10, which resulted in an Arg579Trp amino acid change (Figure 2E). None of these mutations was detected with Southern blot analysis (data not shown).

In these 4 patients with POLG mutations, we also found a Gln1236His change already described as a polymorphism7 and 2 heterozygous nucleotide transitions, G2178A and C2254T, that do not alter amino acid residues.

**COMMENT**

Mutations in 3 nuclear genes, ANT1, C10orf2, and POLG, have been associated with adPEO.4–7,12–14 Adenine nucleotide translocator-1, the gene responsible for the chromosome 15q22-linked form of adPEO,15 encodes an isofrom of the adenosine triphosphate–adenosine diphosphate translocator common to muscle, heart, and brain. Adenine nucleotide translocator-1 regulates the adenine nucleotide pool within mitochondria and is a structural element of the mitochondrial permeability transition pore, thus playing an important role in apoptosis mediated by mitochondria.5 Twinkle, the product of the C10orf2 gene, appears to be an adenine nucleotide–dependent mtDNA helicase.4 Twinkle alterations may enhance dNTP breakdown and impair mtDNA replication through nucleotide pool imbalances.4

The α subunit of polymerase γ, the product of the POLG gene, is responsible for the chromosome 19q22-26–linked form of adPEO.8 Polymerase γ is a heterodimeric complex composed of a 140-kDa α subunit and an accessory protein of about 41 kDa (β subunit).10 It is part of a multienzymatic complex located within the inner mitochondrial membrane and required for mtDNA replication.11 The α subunit is catalytic and contains both polymerase and exonuclease activities, whereas the β subunit seems to enhance DNA binding and promote DNA synthesis.12 Mutations in the β subunit have not been de-
The human enzyme has weak homology with family A of DNA polymerases. Sequences in higher eukaryotes differ from those in lower eukaryotes, and similarities are confined mainly to the amino-terminal exonuclease domain involved in proofreading and to the COOH-terminal polymerase domain. A unique characteristic of human POLG is the presence of 10 glutamic acid or threonine residues, which are conserved throughout evolution and are located near the DNA terminus at the downstream template sequence. The heterozygous POLG mutations identified in patients 2 and 3 (Figure 2B and E) may produce a similar effect on the protein secondary structure because proline is structurally different from other amino acid residues that have charged lateral groups, while tryptophan has an aromatic side group. Neither mutation produces clinical effects in heterozygosity, but when both mutations coexist in compound heterozygotes, they appear to have deleterious effects on POLG function.

Most mutations described in POLG affect functional exonuclease or polymerase domains. Two of the 4 novel mutations described here are located in exon 10, outside functional domains, which shows that the protein region encoded by exon 10 and adjacent to the exonuclease domain is important for the stability and correct conformation of the human protein.

Table 2. Polymerase Chain Reaction–Restriction Fragment Length Polymorphism Analysis

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Oligonucleotide Sequences</th>
<th>Annealing Temperature, °C</th>
<th>Restriction Endonuclease</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3227T</td>
<td>F 5′-GGGTGCGAGTGGAGACACTCC R 5′-GGGCTGGGCTGTAGCGAGAAC mm</td>
<td>64</td>
<td>BstI</td>
</tr>
<tr>
<td>C1760T</td>
<td>F 5′-TGAGAGAGGAGAAGACCTTCC R 5′-AGGCTGGGCTGTAGCGAGAACCA</td>
<td>56</td>
<td>Smal</td>
</tr>
<tr>
<td>G2655A</td>
<td>F 5′-TAAATCTGCTGGCAGCAGAAAT R 5′-TTATGCTACCTGAGGCTGCG</td>
<td>60</td>
<td>Csp6</td>
</tr>
<tr>
<td>C1735T</td>
<td>F 5′-CCCTGGGCTAGGGATCTGCTT R 5′-CTCCTGACGGGTCCTGTCAGC</td>
<td>55</td>
<td>Nci</td>
</tr>
</tbody>
</table>

Table 3. Enzyme Values* in Patients With Polymerase γ Mutations

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Control Alleles†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome c oxidase</td>
<td>0.598 (35)</td>
<td>0.196 (69)</td>
<td>0.110 (39)</td>
<td>0.283 ± 0.052</td>
</tr>
<tr>
<td>Succinate cytochrome c reductase</td>
<td>0.031 (73)</td>
<td>0.033 (47)</td>
<td>0.018 (26)</td>
<td>0.070 ± 0.023</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase</td>
<td>0.033 (29)</td>
<td>0.040 (35)</td>
<td>0.044 (39)</td>
<td>0.115 ± 0.038</td>
</tr>
<tr>
<td>NADH dehydrogenase</td>
<td>0.910 (24)</td>
<td>1.261 (34)</td>
<td>1.261 (34)</td>
<td>3.760 ± 0.715</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>0.066 (66)</td>
<td>0.096 (95)</td>
<td>0.041 (40)</td>
<td>0.101 ± 0.053</td>
</tr>
</tbody>
</table>

Abbreviations: F, forward; mm, mismatched; R, reverse.

Abbreviation: NADH, reduced nicotinamide adenine dinucleotide.

*Data are expressed as micromoles per minute or fresh tissue; data in parentheses are the percentage of normal.
†Data are the mean plus or minus SD.

In the middle of motif A of the polymerase domain, although the alanine-to-threonine amino acid change is mild, its pathogenicity is probably enhanced by the association with the Arg579Trp change. Arginine 579 is relatively well conserved through evolution, although in some species it has been replaced by a lysine residue. Both arginine and lysine contain positively charged lateral groups, while tryptophan has an aromatic side group. Neither mutation produces clinical effects in heterozygosity, but when both mutations coexist in compound heterozygotes, they appear to have deleterious effects on POLG function.

The results of this study can be used to confirm the genetic and clinical heterogeneity of diseases caused by POLG mutations. While mutations in ANT1 and C10orf2 have been associated with dominant or sporadic cases of PEO, mutations in POLG can cause either adPEO
The clinical phenotypes of patients with POLG mutations appeared to be both more heterogeneous and more severe than those of patients with ANT1 and C10orf2 mutations. In addition to PEO, symptoms included...
psychiatric disorders, dysphagia, dysphonia, facial diplegia, neuropathy, ataxia, extrapyramidal syndromes, and profound muscle weakness.1,2

In contrast, psychiatric disorders, neuropathy, and a motor neuron disease–like scenario were described in some cases of C10orf2 mutations,3,4,12 whereas mutations in ANT1 caused a relatively homogeneous phenotype, usually limited to adPEO, ptosis, and muscle weakness;5,6,11,12 only 1 family with a bipolar affective disorder and ANT1 mutation has been recently described.20 In our patients with POLG mutations, the gastrointestinal symptoms resembled those described in mitochondrial neurogastrointestinal encephalomyopathy, an autosomal recessive disorder with multiple deletions and depletion of mtDNA caused by mutations in the gene encoding thymidine phosphate.27

Hypogonadism, another clinical feature recently associated with POLG dysfunction,28 was present in patient 3. There was both interfamiliar and intrafamilial heterogeneity in severity and age at onset of symptoms. For example, in families 2 and 3, the parents were reportedly healthy (Figure 1B and C), but a maternal aunt was affected in family 2 (Figure 1B), which suggests that the Pro587Leu mutation has variable penetrance. Our data agree with those of a previous report that also showed variable penetrance, evidence of anticipation, and variable clinical expression of POLG mutations.7

We show that POLG mutations account for a substantial proportion of patients with PEO and multiple mtDNA deletions (13% of all cases; 17% of familial cases). However, the proportion of patients with familial autosomal PEO with POLG mutations in our series is lower than that in a previous study from Europe (46% [13 of 28].8) This smaller number may be because of differences in the ethnic backgrounds of the 2 series of patients.

We suggest screening of POLG in patients with familial PEO with multiple mtDNA deletions, especially in patients with multisystemic involvement. However, it is also important to note that the genetic abnormalities underlying many cases of adPEO and arPEO remain unknown, which indicates that other genes are involved in the cause of these syndromes and remain to be identified.

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Author contributions: Study concept and design (Drs Filostro, Mancuso, Shanske, Hirano, and DiMauro); acquisition of data (Drs Filostro, Mancuso, Nishigaki, Harati, Gooch, Mankodi, Bayne, and Bonilla and Ms Pancrudo); analysis and interpretation of data (Drs Filostro, Mancuso, Shanske, Hirano, and DiMauro); drafting of the manuscript (Drs Filostro and DiMauro and Ms Pancrudo); critical revision of the manuscript for important intellectual content (Drs Mancuso, Nishigaki, Harati, Gooch, Mankodi, Bayne, Bonilla, Shanske, Hirano, and DiMauro); study supervision (Drs Filostro, Hirano, and DiMauro).

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REFERENCES


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