Association of Novel POLG Mutations and Multiple Mitochondrial DNA Deletions With Variable Clinical Phenotypes in a Spanish Population

Emiliano González-Vioque, BSc; Alberto Blázquez, BSc; Daniel Fernández-Moreira, PharmB; Belén Bornstein, MD, PhD; Juan Bautista, MD; Javier Arpa, MD; Carmen Navarro, MD, PhD; Yolanda Campos, PhD; Miguel A. Fernández-Moreno, PhD; Rafael Garces, PhD; Joaquin Arenas, PhD; Miguel A. Martín, PhD

Background: Both dominant and recessive mutations were reported in the gene encoding the mitochondrial (mt) DNA polymerase γ (POLG) in patients with progressive external ophthalmoplegia (PEO). Phenotypes other than PEO were recently documented in patients with mutations in the POLG gene.

Objective: To screen patients with mitochondrial disease and multiple mtDNA deletions in muscle for mutations in the coding regions of the POLG, PEO1, and SLC25A4 genes.

Design: To identify the underlying molecular defect in a group of patients with multiple mtDNA deletions comparing their molecular genetic findings with those of healthy controls.

Patients: Twenty-four patients (16 men and 8 women) diagnosed with mitochondrial disease and having multiple mtDNA deletions in muscle by Southern blot analysis. Thirteen patients had PEO; 2 had PEO alone, 4 had PEO and myopathy, and 5 had PEO and multisystem involvement. Four patients had multisystem disease without PEO. The remaining 9 patients had isolated myopathy. DNA from 100 healthy individuals was also studied.

Results: No mutation was identified in the PEO1 or SLC25A4 genes. Nine POLG mutations were observed in 6 of 24 patients. Four novel mutations were detected and mapped in the linker region (M603L) and in the pol domain of the enzyme (R853W; D1184N; R1146C). Five patients with PEO had mutations: 2 were compound heterozygotes, 1 was homozygous, and another showed a mutation in a single allele. The remaining patient also showed a sole mutation and had an unusual phenotype lacking ocular involvement.

Conclusions: POLG molecular defects were found in 25% of our patients with multiple mtDNA deletions and mitochondrial disease. The uncommon phenotype found in 1 of these patients stresses the clinical variability of patients harboring POLG mutations. Molecular studies in the POLG gene should be addressed in patients with mitochondrial disease, particularly those with PEO, and multiple mtDNA deletions.

Arch Neurol. 2006;63:107-111

In this study, we screened 24 patients with mitochondrial disease and multiple mtDNA deletions in muscle for mutations in the coding regions of the POLG, PEO1, and SLC25A4 genes.

METHODS

PATIENTS AND CONTROLS

We studied 24 patients (16 men and 8 women) diagnosed with mitochondrial disease by clinical, morphological, and biochemical criteria. All 24 patients displayed multiple mtDNA deletions in muscle by Southern blot analysis. The clinical picture of these patients was heterogeneous. Thirteen patients had PEO. Of them, 2 had PEO alone, 4 had PEO and myopathy, and 5 had PEO and multisystem involvement. Four patients had multisystem disease without PEO. The remaining 9 patients had isolated myopathy. These patients were not
obtained by a population-based genetic survey. In addition, we studied DNA from 100 healthy individuals.

An appropriate institutional review board approved this work, and informed consent was obtained from the patients and controls included in this study. A summary of clinical, biochemical, and morphological findings of the 6 patients carrying POLG mutations is given in Table 1 and Table 2.

### Table 1. Main Clinical and Morphological Features in Patients With POLG Mutations

<table>
<thead>
<tr>
<th>Patient/ Sex</th>
<th>Age at Onset, y</th>
<th>Age at Diagnosis, y</th>
<th>Clinical Findings</th>
<th>Family History</th>
<th>Serum CK Level</th>
<th>Serum Lactate Level</th>
<th>Muscle Morphological Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F</td>
<td>49</td>
<td>53</td>
<td>PEO, ptosis</td>
<td>Yes*</td>
<td>N</td>
<td>N</td>
<td>RRF-COX negative</td>
</tr>
<tr>
<td>2/M</td>
<td>61</td>
<td>65</td>
<td>PEO, ptosis, mild atrial hypertrophy</td>
<td>Yes†</td>
<td>N</td>
<td>N</td>
<td>RRF-COX negative</td>
</tr>
<tr>
<td>3/F</td>
<td>32</td>
<td>54</td>
<td>PEO, tetraparesis</td>
<td>No</td>
<td>E</td>
<td>N</td>
<td>RRF-COX negative</td>
</tr>
<tr>
<td>4/M</td>
<td>17</td>
<td>24</td>
<td>PEO, sensorimotor neuropathy</td>
<td>No</td>
<td>E</td>
<td>N</td>
<td>RRF-COX negative</td>
</tr>
<tr>
<td>5/M</td>
<td>18</td>
<td>22</td>
<td>Muscle atrophy in calf muscle</td>
<td>No</td>
<td>N</td>
<td>N</td>
<td>Enlarged mitochondria by electron microscopy</td>
</tr>
<tr>
<td>6/M</td>
<td>33</td>
<td>55</td>
<td>PEO, ptosis, muscle weakness, sensorimotor neuropathy, hearing loss</td>
<td>Yes‡</td>
<td>N</td>
<td>N</td>
<td>RRF-COX negative</td>
</tr>
</tbody>
</table>

Abbreviations: CK, creatine kinase; COX, cytochrome-c oxidase; E, elevated; N, normal; PEO, progressive external ophthalmoplegia; RRF, ragged red fibers.
*Patient’s sister was diagnosed with myoclonic epilepsy.
†Proband’s sister had PEO.
‡Proband’s sister (died at age 38 y) had PEO, muscle weakness, and seizures.

### Table 2. Muscle Mitochondrial Respiratory Chain Enzyme Activities in Patients With POLG Mutations

<table>
<thead>
<tr>
<th>Patient†</th>
<th>Controls</th>
<th>Mean (Range)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>11.5</td>
<td>9.1</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>18.6</td>
<td>9.0</td>
</tr>
<tr>
<td>6</td>
<td>106</td>
<td>185</td>
</tr>
</tbody>
</table>

Abbreviations: CoQ1, coenzyme Q1; NADH, nicotinamide adenine dinucleotide hydrogenase.
*Enzyme activities expressed as percentage of citrate synthase activity in nanomoles minute⁻¹milligram protein⁻¹.
†Muscle biopsy specimen from patient 4 was not available for biochemical analysis.
‡Control range denotes the 2.5th and 97.5th percentile (n = 50).
§Citrate synthase activity indicated as nanomoles minute⁻¹milligram protein⁻¹.

### Table 3. Genotype of Patients With Mutant Alleles in the POLG Gene

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>P587L</td>
<td>R853W</td>
</tr>
<tr>
<td>2</td>
<td>T251I+P587L</td>
<td>M603L</td>
</tr>
<tr>
<td>3</td>
<td>D1184N</td>
<td>N468D</td>
</tr>
<tr>
<td>4</td>
<td>G268A</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>R1146C</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>A467T</td>
<td>A467T</td>
</tr>
</tbody>
</table>

*Novel mutations described in this study.

### METHODS

DNA from skeletal muscle or blood was isolated by standard methods. The entire coding region for the POLG, SLC25A4, and PEO1 genes was amplified and sequenced as reported. Sequence analysis of DNA was carried out as described. Muscle histochemical and biochemical tests were performed as previously reported. Southern blot analysis of mtDNA was carried out as described. Polymerase chain reactions and restriction fragment length polymorphism were used to confirm the mutations.

Sequencing of the SLC25A4 and PEO1 genes failed to detect the presence of mutations in all patients. We found 9 POLG mutations in 6 (25%) of 24 patients, in agreement with previous reports (Table 3). The proportion of patients with PEO and mutations in the POLG gene (5 [45%] of 11) was significantly higher than in those without PEO (1 [8%] of 13) (P<.001, χ² test). Four of the POLG mutations are novel (Figure A) and were not detected in 100 healthy control subjects.
Patient 1 was compound heterozygous for an already described P587L (c.1760C>T) mutation and for a novel c.2557C>T mutation in exon 16, which results in a R853W missense change. This mutation abolishes an Nci I recognition site (not shown).

Patient 2 was compound heterozygous for the previously described mutations T251I (c.752C>T) and P587L and for a novel c.1807A>T mutation in exon 10, which yields an M603L amino acid change. This mutation eliminates an Nla III recognition site (not shown). The T251I and P587L mutations were found in cis in 3 previous reports.  

Patient 3 was compound heterozygous for a previously reported c.1402A>G mutation in exon 7, yielding an N468D change, and for a novel c.3550G>A mutation in exon 22, which gives rise to a D1184N amino acid substitution. This mutation abolishes a TaqI recognition site (not shown).

Patient 4 was heterozygous for the already described G268A mutation (c.803G>C).  

Patient 5 was heterozygous for a novel c.3436C>T mutation in exon 21, which results in a missense R1146C change. This mutation abolishes a Cfr13 I restriction sequence (not shown).

Patient 6 was homozygous for the well-known A467T (c.1398G>A) mutation in exon 7.

We also found the following polymorphisms in the POLG gene: the neutral polymorphism c.2256C>T (L752L) in 1 patient, the change c.3428 C>G (E1143G) in exon 23 (National Center for Biotechnology Information single-nucleotide polymorphism E1143G cluster ID rs2307441) in 1 patient, and a novel nonsynonymous change c.391C>T (Y131H) in another patient; the microdeletion 3770delG in the 3’ untranslated region and several CAG repeats in exon 2 in 3 patients; and the substitution c.3708G>T (Q1236H) in exon 23 (National Center for Biotechnology Information single-nucleotide polymorphism Q1236H cluster ID rs3087374) in 5 patients.  

We studied 24 patients whose clinical findings varied from PEO alone or in combination with other symptoms and signs of mitochondrial myopathy to limb muscle myopathy with no ocular muscle involvement. All patients showed morphological or biochemical findings, or both, of mitochondrial dysfunction. Moreover, Southern blot analysis of mtDNA revealed multiple mtDNA deletions. All these data prompted us to look for alterations in the SLC25A4, PEO1, and POLG genes as the underlying cause of mtDNA rearrangements.  

Molecular analysis of the SLC25A4 and PEO1 genes failed to reveal mutations, but we found alterations in the POLG gene in 25% (6/24) of the patients, accounting for 20.8% (10/48) of alleles. These data are consistent with those documented by other authors. Indirect evidence for the pathogenicity of these mutations comes from the following criteria: (1) They were associated with mitochondrial dysfunction and multiple mtDNA deletions. (2) They were the only significant nucleotide alterations in the POLG gene (besides polymorphisms), as well as in the SLC25A4 and PEO1 genes. (3) The mutations cause substitution in conserved amino acid residues of the POLG protein (Figure B). (4) They were absent in more than 100 healthy controls from the same ethnic background.

**Figure.** POLG mutations in our patients (A) and multiple alignments of the protein regions containing the novel mutations in different species (B). A, Novel mutations (open boxes) and reported mutations (shaded boxes) are indicated on the exo, linker, and pol domains of the protein. B, Protein sequences aligned on ClustalW Multiple Sequence Alignment (European Bioinformatics Institute, Cambridge, England) for DNA or proteins (version 1.8). Black shading shows amino acid identity; gray shading, similarity. Arrows indicate the position of the amino acid changes. Human indicates Homo sapiens; Mouse, Mus musculus; Xenia, Xenopus laevis; Drome, Drosophila melanogaster; Schpo, Schizosaccharomyces pombe.
Three of our patients had allelic mutations, and 1 had the same mutation in both alleles, suggesting a recessive mode of inheritance. We were able to identify mutations in a single allele in the remaining 2 patients. The novel mutations described herein map in the linker region (M603L) and in the pol domain of the enzyme (R853W; D1184N; R1146C).

We found the already-documented P587L mutation in 2 patients. Although the P587L change is usually associated with the T251I substitution within the same allele, we observed that 1 of our patients had the P587L mutation alone, whereas another showed the usual association of both mutations.

The clinical phenotypes of our patients with POLG mutations were heterogeneous, expanding the spectrum of mitochondrial disorders associated with POLG mutations. The 3 patients who were compound heterozygous for POLG mutations had adult-onset PEO, sensorimotor neuropathy, and ragged red fibers, and mitochondrial respiratory chain defects. Of the 2 patients with mutations in a single allele, 1 had early-onset PEO, sensorimotor neuropathy, and ragged red fibers, and another showed an unusual phenotype with lack of ocular muscle involvement. Given the early onset, PEO and other symptoms and signs usually associated with POLG mutations may develop later. The only mutation we have detected (c.3718C>T, which produces the amino acid change R1146C) has been reported as a single-nucleotide polymorphism. However, the only data available, to our knowledge, come from studies using the National Institutes of Health Polymorphism Discovery Resource, and the c.3718C>T nucleotide change has been found by 2 independent groups in the same sample (rs230744015). It is tempting to propose that the R1146C mutation may alter moderately the biochemical behavior of the enzyme, resulting in the mild clinical phenotype observed in our patient. In this context, the biochemical defects associated with several POLG mutations have been recently characterized in vitro, and there is a good correlation between biochemical and clinical findings.

The clinical picture of the patient homozygous for the A467T mutation was consistent with those documented by others and associated with the same mutation, namely PEO, sensorimotor polyneuropathy, and deafness. We did not find the substitution in Spanish controls, but Van Goethem et al. reported that 0.6% of a Belgian population harbored this change.

Of the 6 patients with POLG mutations, 5 had PEO and other clinical features such as muscle weakness, neuropathy, atrial hypertrophy, or hearing loss. Remarkably, 1 patient had a less typical phenotype characterized by early-onset thinness of the left calf muscle. This point bolsters the clinical variability of patients with POLG mutations.

Moreover, 45% of patients with PEO (with or without multisystem involvement) and multiple mtDNA deletions showed mutations in the POLG gene. By contrast, only 8% of patients with no ocular paresis had mutations in the POLG gene. Our data indicate that in patients with multiple mtDNA deletions in muscle those with PEO are more likely to harbor POLG mutations than those without PEO.

Although we did not observe parkinsonism, we were not able to perform positron emission tomographic studies, and it is therefore possible, as already suggested, that muscle weakness and neuropathy might have masked symptoms of parkinsonism in our patients. In addition, we failed to demonstrate early menopause in our female patients, which is not consistent with recent findings by Luoma et al.

The phenotype of our patients stresses the clinical variability of patients harboring POLG mutations and indicates that molecular studies in the POLG gene should be addressed in patients with mitochondrial disease, particularly in those with ocular myopathy and multiple mtDNA deletions.

Accepted for Publication: May 24, 2005.

Authors Affiliations: Departamento de Bioquimica, Instituto de Investigaciones Biomédicas “Alberto Sol” CSIC-UAM, Facultad de Medicina, Universidad Autónoma de Madrid (Mr González-Vioque and Drs Bornstein, Fernández-Moreno, and Garesse), Centro de Investigación, Hospital Universitario 12 de Octubre (Messrs Blázquez and Fernández-Moreira and Drs Campos, Arenas, and Martín), and Servicio de Neurología, Hospital Universitario La Paz (Dr Arpa), Madrid, Spain; Servicio de Neurología, Hospital Virgen del Rocío, Sevilla, Spain (Dr Bautista); Servicio de Anatomía Patológica, Hospital Do Meixoeiro, Vigo, Spain (Dr Navarro). Correspondence: Miguel A. Martín, PhD, Centro de Investigación, Hospital Universitario 12 de Octubre, Avda de Córdoba s/n, 28041 Madrid, Spain (mamcasanueva@h12o.es).

Author Contributions: Messrs Blázquez and Fernández-Moreira and Drs Campos, Arenas, and Martín. Acquisition of data: González-Vioque, Blázquez, Bornstein, Garesse, Arenas, and Martin. Analysis and interpretation of data: González-Vioque, Blázquez, Fernandez-Moreira, Bornstein, Bautista, Arpa, Navarro, Campos, Fernández-Moreno, and Martin. Critical revision of the manuscript for important intellectual content: González-Vioque, Blázquez, Fernandez-Moreira, Garesse, Arenas, and Martin. Drafting of the manuscript: González-Vioque, Blázquez, Fernandez-Moreira, Garesse, Arenas, and Martin. Administrative, technical, and material support: González-Vioque and Blázquez. Study supervision: Garesse, Arenas, and Martin.

Funding/Support: This study was supported by grant PI030224 from Fondo de Investigación Sanitaria (FIS), Ministerio de Sanidad y Consumo, grant BMC01-1525 from Ministerio de Ciencia y Tecnología, grant GR/SAL/0333/2004 from Consejería de Educación, Comunidad de Madrid, and Instituto de Salud Carlos III, Madrid, Redes de centros (grant C03/08, Metabolism and Nutrition Network Coordination Center, Barcelona, Spain) and...
Acknowledgment: We are grateful to Pilar del Hoyo, PharmB, and Sara Jiménez, BSc, the technicians working on the respiratory chain activities.

REFERENCES


