A novel POLG gene mutation in a patient with SANDO

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Abstract
The human mitochondrial genome is replicated by DNA polymerase γ, which is encoded by the nuclear-encoded DNA polymerase γ (POLG1) on chromosome 15q25. Patients with POLG1 mutations usually present as Alpers’ syndrome or progressive external ophthalmoplegia.

Our patient was a 48-year-old woman with sensory ataxic neuropathy, dysarthria, ophthalmoplegia, and dysphagia. Sequence analysis revealed that she has two heterozygous missense mutations in the POLG1, a c.1774C>T substitution in exon 10, which results in a p.L591F amino acid change; and a c.3286C>T substitution in exon 21, which results in a p.R1096C amino acid change. The 1774C>T substitution is a novel mutation.

Previously described adult patients with one mutation in exon 10 and the other in exon 21 of POLG1 had presented with progressive external ophthalmoplegia. We now describe a patient with mutations in the same exons but suffering from the more complex clinical syndrome of sensory ataxic neuropathy, dysarthria, ophthalmoplegia.

Key words: Novel mutation; PEO; POLG1; SANDO

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Received: January 21, 2012
Accepted: March 13, 2012
Published online: March 20, 2012
DOI:10.5455/jeim.200312.cr.001

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Introduction
The human mitochondrial genome is replicated by the nuclear-encoded DNA polymerase γ (pol γ) [1]. The catalytic subunit of DNA pol γ, encoded by the polymerase γ gene (POLG1) on chromosome 15q25 [2], is a 140-kDa protein with three functional domains: an exonuclease domain located at the N-terminus, a polymerase domain encompassing the C-terminus, and a linker region connecting the two [3].

The first pathogenic mutations in POLG1 were identified in families with autosomal dominant chronic progressive external ophthalmoplegia (adPEO) and multiple mitochondrial DNA (mtDNA) deletions in muscle [4]. Subsequent reports have shown that POLG1 mutations are associated with at least six different neurodegenerative phenotypes [3] and, to date, about 150 pathogenic mutations have been identified [5].

In this article, we report a patient with two heterozygous missense mutations (one of which is novel) in POLG1 and discuss the relationship between these mutations and the patient’s clinical findings.

Materials and methods

Patient history
This 48-year-old woman was born after a normal pregnancy and delivery. She had normal childhood and young adult life. At the age of 38, she developed difficulty in walking and her work performance deteriorated so rapidly that she had to be retired. At the age of 41, progressive diplopia and ptosis added to the symptoms. Five years later, she gradually had dysarthria and restless leg syndrome. Her current clinical findings include sensory ataxic neuropathy, dysarthria, ophthalmoplegia (SANDO), and dysphagia.

Most of the routine laboratory tests were normal except for a low creatinine (0.34 mg/dL; normal range 0.5-1.2 mg/dL) and increased BUN/creatinine ratio (38; normal range 6-22). At age of 40, a muscle biopsy was performed. It showed many ragged red fibers, which stained intensely with the succinate dehydrogenase and were negative for the cytochrome oxidase reaction. In addition, there was mild to moderate angulated fiber atrophy, predominantly in type 2 fibers, and some fiber-type grouping.
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The patient’s 76-year-old father and 52-year-old sister had also present ptosis. Droopy eyelids in the sister were noted when she was 40 and she also had difficulty in walking.

**DNA sequencing**

Nine (forward and backward) oligonucleotide primers were used to amplify the 22 coding exons of the POLG1. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit. Amplified products were purified using Performa DTR Gel Filtration Cartridges (Edge Biosystem, Gaithersburg, MD, USA) and analyzed on an ABI 3130xl Genetic Analyzer with Sequencing Analyzer Software v5.2 (Applied Biosystems, Foster City, CA, USA). The cDNA sequence corresponding to GenBank accession number NM_002693.2 was used as reference.

**Results**

Sequence analysis revealed two heterozygous missense mutations. The first, a novel mutation, was a c.1774C>T substitution in exon 10 (Fig.1A), which resulted in a p.L591F amino acid change (Fig.1B). The second was a c.3286C>T transition in exon 21 (Fig.1C), resulting in p.R1096C amino acid change (Fig.1D).

**Discussion**

Patients with POLG1 mutations have a wide range of phenotypes, but the genotype-phenotype relationship is not well understood. In 2006, Horvath et al reviewed 38 patients with one or more POLG1 mutations [6], the most common of which was c.1399G>A in exon 7, resulting in a p.A467T substitution in the linker domain. They grouped the patients in four clinical variants characterized by: (1) liver dysfunction and encephalopathy; (2) encephalopathy; (3) PEO; and (4) other features, including ataxia, dysphagia, neuropathy, myopathy, cardiomypathy, cortical blindness, seizures, myoclonus, diabetes, dementia, thyroid dysfunction, hearing loss, and heart block. In this series, most children presented with hepatoencephalopathy while most adults had PEO and limb myopathy, often in association with ataxia and axonal sensorimotor peripheral neuropathy (similar to sensory atactic neuropathy, dysarthria, and ophthalmoplegia; SANDO).

One of the two mutations in our patient was in exon 10 (p.591L>F) and the other one was in exon 21 (p.1096R>C). To our knowledge, only three previously reported adult patients with mutations in both exon 10 (linker domain) and exon 21 (polymerase domain) were reported and all three presented with PEO (Table 1). Our patient had adult-onset SANDO, but also gastrointestinal dysmotility and dysphagia. In addition, she had exercise intolerance, proximal myopathy, diplopia, and restless leg syndrome. Mutation in exons 10 and 21 were previously reported in two children [6, 7]. The first one had infantile hepatocerebral syndrome with mtDNA depletion while the second one had encephalopathy, liver failure, stroke-like episodes, and seizures (Table 1).

![Figure 1](image-url)

**Figure 1. (A)** c.1774C>T substitution in exon 10 was seen in the sequence of POLG1 of our patient; **(B)** this substitution caused a p.L591F amino acid change; **(C)** the second substitution was a c.3286C>T transition in exon 21; and **(D)** This mutation caused a p.R1096C amino acid change.
Table 1. Reported patients with mutations in both exons 10 and 21 of \textit{POLG1}.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age-Sex</th>
<th>Exon 10</th>
<th>Exon 21</th>
<th>Additional mutation/s</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35-F</td>
<td>C1760T</td>
<td>P587L</td>
<td>G3616A V1106I C752T</td>
<td>PEO, myopathy</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(in exon 13)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45-F</td>
<td>G1880A</td>
<td>R627Q</td>
<td>A3428G E1143G G3708T</td>
<td>PEO, ataxia, neuropathy, myoclonus epilepsy</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(in exon 23), T2894G (in exon 18) Q1236H, L965X</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>55-F</td>
<td>C1943G</td>
<td>P648R</td>
<td>C3286T R1096C C752T</td>
<td>PEO, myopathy</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(in exon 13) T251I</td>
<td></td>
</tr>
<tr>
<td>4*</td>
<td>48-F</td>
<td>C1774T</td>
<td>L591F</td>
<td>C3286T R1096C</td>
<td>SANDO, dysphagia, diplophia</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>(in exon 13) T251I</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5 months</td>
<td>C1760T</td>
<td>P587L</td>
<td>G3406A E1136K</td>
<td>Infantile hepatocerebral mtDNA depletion syndrome</td>
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<td>(in exon 13)</td>
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<td>6</td>
<td>7-M</td>
<td>G1880A</td>
<td>R627Q</td>
<td>G3287A R1096H</td>
<td>Encephalopathy</td>
</tr>
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<td></td>
<td></td>
<td>(in exon 13)</td>
<td>Liver failure</td>
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<td></td>
<td></td>
<td></td>
<td>Stroke-like episodes</td>
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<td>Seizures</td>
</tr>
</tbody>
</table>

*Current case; N, nucleotide substitution; AA, amino acid substitution.

The p.1096R>C mutation has been described in Alpers’ syndrome, in arPEO, and in sporadic cases of PEO [8] or PEO-myopathy (in trans with p.648P>R) [6]. In Alpers’ syndrome, the mutation could be either homozygous, associated with the p.1236Q>H polymorphism [3], or compound heterozygous with p.914T>P mutation [9]. Arg1096 is part of the active site of pol γ in a position that can interact with the template DNA and may be involved in DNA binding.

The novel p.591L>F mutation is likely to be pathogenic for the following reasons: first of all, Leu591 is in an area of the protein that is known to interact with the p55 accessory subunit, although the precise role of the Leu residue in this interaction is not clear; secondly, the Leu residue at this position is conserved in animal DNA pol γ sequences and the conservation is consistent with its role in accessory subunit binding (lower eukaryotic DNA pol γ’s do not have an accessory subunit); lastly, because Leu591 makes a close contact with Gln456, its substitution with the larger Phe side chain would predictably clash with the Gln456, pushing away the alpha-helix that makes up the thumb domain and consequently disturbing the interaction of Leu591 with Gln456.

In conclusion, all three previously reported adult patients with mutations in exons 10 and 21 of \textit{POLG1} had presented with PEO. We now report an additional adult patient, who is also compound heterozygous for mutations in these exons but presented with a more complex clinical syndrome, namely SANDO.

Acknowledgements

This work was supported by NIH grant HD-032062 and by the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF).

References