A Novel POLG Gene Mutation in 4 Children With Alpers-like Hepatocerebral Syndromes

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Objective: To describe a novel POLG missense mutation (c.3218C>T; p.P1073L) that, in association with 2 previously described mutations, caused an Alpers-like hepatocerebral syndrome in 4 children.

Design: Genotype-phenotype correlation.

Setting: Tertiary care universities.

Patients: Four children, 2 related and 2 unrelated, with the novel p.P1073L mutation (all patients) and either the p.A467T (2 patients), p.G848S (1 patient), or p.W748S (1 patient) mutation presented with psychomotor delay, encephalopathy, and liver failure.

Interventions: Detailed clinical and laboratory examinations including brain magnetic resonance imaging, muscle biopsy, measurement of mitochondrial DNA, and sequencing of the POLG gene.

Main Outcome Measures: Definition of clinical variability.

Results: All 4 patients had psychomotor delay, seizures, and liver disease. Three patients had severe gastrointestinal dysmotility, which may be associated with the new p.P1073L mutation.

Conclusions: The heterozygous presence of the novel p.P1073L mutation in trans with another recessive POLG mutation causes a hepatocerebral disorder identical or very similar to Alpers syndrome. This adds to the already striking clinical heterogeneity of POLG mutations. In the Belgian patients, the familial occurrence without consanguinity is related to the high frequency of the recessive p.A467T and p.W748S mutations in northwestern Europe and reveals a pitfall for diagnosis and genetic counseling.

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POLYMERASE γ (pol-γ) is the only DNA polymerase found in animal cell mitochondria and thus bears the sole responsibility of DNA synthesis in all replication and repair transactions involving mitochondrial DNA (mtDNA). Mature pol-γ is a 140-kDa protein composed of 3 functional domains: an exonuclease domain located at the N-terminal, a polymerase domain encompassing the C-terminal, and a linker region connecting the two. In human mitochondria, pol-γ is part of an enzyme complex that contains an accessory subunit of 55 kDa, p55, that is encoded by the POLG2 gene.

The gene encoding human pol-γ (POLG; RefSeq NM_002693.2) was identified in 1996 and is located on chromosome 15q25; its complementary DNA comprises 4465 base pairs (bp) including a 282-bp 5' untranslated region and a 463-bp 3' untranslated region. The gene contains 23 exons, and the translation initiator methionine codon is located in exon 2. The p55 subunit is required for processive DNA synthesis and tight binding of the pol-γ complex to DNA. Thus, mutations in the POLG or POLG2 genes affect some of the functions of pol-γ and cause either quantitative or qualitative alterations of mtDNA (depletion or multiple deletions). The first incidence of mutation of POLG causing disease was described in 2001. To date, about 150 mutations have been described (http://tools.niehs.nih.gov/polg) that have been associated with at least 6 phenotypes of neurodegenerative disease.

In this article we describe a novel recessive POLG missense mutation in 4 children who were compound heterozygotes and discuss the relationship between clinical findings and the genotype.

METHODS

PATIENTS

Patient 1 was the 9-year-old daughter of non-consanguineous parents. There was no family history of neurodegenerative disease. She was hypotonic at birth and showed developmental delay. Over time, she developed short stat-
ure, ptosis, neurosensory hearing loss, celiac disease, gastrointestinal pseudoobstruction, headache, and seizures. At 9 years of age, her clinical picture was dominated by seizures, liver dysfunction, developmental delay, and intestinal dysmotility. She had elevated blood lactate (37.8-62.2 mg/dL venous, 39.6-72.1 mg/dL arterial; to convert to millimoles per liter, multiply by 0.111), pyruvate (1.91 mg/dL venous; to convert to micromoles per liter, multiply by 113.56), and cerebrospinal fluid protein and lactate levels. A cranial magnetic resonance image (MRI) showed signal abnormalities in the basal ganglia and thalami. A liver biopsy at 10 years of age showed nodular lobular architecture with areas of duct proliferation. She had portal and focal lobular inflammation, patchy cholestatic changes, and ballooned hepatocytes, together with macrovesicular and microvesicular fatty changes. Trichrome and reticulin stains showed portal fibrous expansion with bridging and pericellular fibrosis. After prolonged hospitalization complicated by worsening seizures, apneic episodes, mechanical ventilation, and tracheostomy placement, she died at home at 9 years of age from an apparent aspiration event associated with vomiting. The clinical impression was Alpers syndrome, and frozen liver tissue was sent to us to confirm this diagnosis.

Patient 2 was the 6-month-old son of nonconsanguineous Hispanic parents. He had hypotonia, developmental delay, and persistent vomiting since 5 months of age. Pregnancy and delivery had been normal. Physical examination at 6 months showed a nondysmorphic, emaciated infant. His head circumference, weight, and height were below the 5th percentile. He showed only mild enlargement of the lateral ventricles. Laboratory studies showed lactic acidosis (lactate level, 31.5 mg/dL; reference range, <18 mg/dL) and low free and total carnitine levels. Results of liver function tests were initially within the reference range but became indicative of cholestasis. Gastroscopy showed reflux esophagitis grade 4, hyperplastic gastropathy, and a gastric ulcer. Ophthalmoscopy revealed optic atrophy. An electroencephalogram showed slow rhythm but no epileptic features. A computed tomographic scan of the brain showed only mild enlargement of the lateral ventricles. The child died at 3 years, 4 months of age. Postmortem examination showed confluent centrolobular necrosis of the liver with microvesicular/macrovesicular steatosis and extensive chronic fibrosis of the pancreas. Results of muscle histochemistry, including cytochrome C oxidase staining, were normal.

Patient 4 was a first paternal cousin of patient 3 (Figure 1; patient III-2). He also had delayed psychomotor development (he sat unsupported at 1 year and walked at 2 years of age). His gait was unsteady, with frequent falls. At 3 years of age, he developed status epilepticus following an episode of vomiting and a minor fall. His eyes were deviated to the left, and he had visual hallucinations and myoclonic jerks of the left arm and leg. He went into a coma and showed hyperreflexia in all 4 limbs.

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**mtDNA QUANTIFICATION**

Mitochondrial DNA was quantified by real-time polymerase chain reaction using an ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, California) as previously described. The primers for mtDNA were: forward, 5'-CCACGGAAAACAGCATGATT-3' and reverse, 5'-
CTATTGACTTGGGTAAATCGTGTGA-3'; the probe was FAM-TGCAAGCCACCGG-G-MGB. TaqMan RNAse P Control Reagents Kit (catalogue number 4316844; Applied Biosystems) was used as primers and probes for nuclear DNA. The mtDNA levels were compared with those of nuclear DNA, and the data were expressed in percentages.

DNA SEQUENCING

Nine (forward and backward) oligonucleotide primers were used to amplify the 22 exons of the POLG gene. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Amplified products were purified using preparative DTR Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, Maryland) and analyzed on an ABI3130XL Genetic Analyzer with sequencing analyzer software v5.2 (Applied Biosystems). The complementary DNA sequence corresponding to RefSeq number NM_002693.2 was used as reference.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

The primers designed to confirm the G1399A mutation were: forward, 5'-ACCTGTCCGTCAACCAGAAC-3' and reverse, 5'-AAGGCTTGGCTACTCTCTC-3'. These primers amplify a 146-bp fragment of the POLG gene. The wild-type has 1 MwoI restriction site, whereas the mutant had no MwoI restriction sites. Thus, on digestion with MwoI, the wild-type yields 2 fragments (99 bp and 47 bp) while the mutant yields only 1 (146 bp).

The primers designed to confirm C3218T mutation were: forward, 5'-GGAAGAAGTGGGAGGTGGTT-3' and reverse, 5'-CCATGCTCTAAAGGTTGCA-3'. A 186-bp fragment of the POLG gene was amplified. In the wild-type, there was 1 MspI restriction site but the mutant type had no MspI restriction sites. Thus, the wild-type had 2 fragments (105 bp and 81 bp) whereas the mutant had an additional fragment (186 bp, 105 bp, and 81 bp).

The fragments were separated in 12% acrylamide gel and visualized under UV light.

RESULTS

Real-time polymerase chain reaction of the liver specimen in patient 1 showed severe reduction of the mtDNA to nuclear DNA ratio, corresponding to 72.1% depletion. Depletion of mtDNA was also shown in muscle (64%) from patient 2.

Sequencing analysis revealed 2 heteroplasmic missense mutations in all 4 patients. In patients 1 and 4, the first mutation was a c.1399G>A transversion in exon 7 that resulted in a p.A467T amino acid change (Figure 2A and B). In patient 2, the first mutation was a c.342G>A transversion, resulting in p.G114R. In patient 3, the first mutation was a c.2243G>C transversion resulting in p.W748S. The second mutation, common to all 4 patients, was a c.3218C>T transversion in exon 20, resulting in a p.P1073L amino acid acid change (Figure 2D and E). This latter mutation has not been previously reported. Parental studies confirmed biallelic inheritance in all patients.

To confirm these mutations, we carried out restriction fragment length polymorphism in patient 1. The first mutation was revealed by the presence of 3 fragments (146 bp, 99 bp, and 47 bp) in the patient's DNA, while only 2 (99 bp and 47 bp) were seen on digestion of control DNA with MspI (Figure 2C). For the second mutation, the control DNA had 2 (105 bp and 81 bp) fragments but the patient had an additional (mutant) fragment (186 bp, 105 bp, and 81 bp) (Figure 2F). Sequencing of DGUOK in all patients did not reveal a mutation and screening of the 22 transfer RNA genes of mtDNA in patient 3 was normal.

Biochemical analyses revealed multiple respiratory chain enzyme defects involving complexes I, III, and IV in the liver biopsy of patient 1 and in postmortem livers of patients 3 and 4. Biochemical studies in skeletal muscle and cultured skin fibroblasts from patient 4 yielded normal results.

Table. Clinical and Laboratory Features

<table>
<thead>
<tr>
<th>Patient No./Sex</th>
<th>Age at Onset</th>
<th>Age at Death</th>
<th>Mutation</th>
<th>PM Delay</th>
<th>Seizures</th>
<th>Status Epilepticus</th>
<th>Liver Disease</th>
<th>GI Dysmotility</th>
<th>Lactic Acidosis</th>
<th>Other</th>
</tr>
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<tbody>
<tr>
<td>1/F</td>
<td>Birth</td>
<td>9 y</td>
<td>A467T/P1073L</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Ptosis, hearing loss</td>
</tr>
<tr>
<td>2/M</td>
<td>5 mo</td>
<td>10 mo</td>
<td>G684S/P1073L</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3/M</td>
<td>13 y</td>
<td></td>
<td>W748S/P1073L</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ADHD, MR</td>
</tr>
<tr>
<td>4/M</td>
<td>3 y</td>
<td></td>
<td>A467T/P1073L</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>OA</td>
</tr>
</tbody>
</table>

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; GI, gastrointestinal; MR, mental retardation; OA, optic atrophy; PM, psychomotor.

Mutations in the POLG gene have been linked to a wide variety of mitochondrial diseases. A recent review of 60 patients suggested 6 major clinical phenotypes, some transmitted as dominant, others as recessive traits. They include (1) a rather ill-defined childhood myocerebrohepatopathy spectrum disorder with hypotonia, developmental delay or regression, and inconsistent hepatopathy; (2) Alpers syndrome, dominated by refractory seizures and liver disease; (3) ataxia neuropathy spectrum, a mixed bag of disorders sharing ataxia and sensory neuropathy; (4) myoclonus epilepsy myopathy sensory ataxia, another heterogeneous group of disorders with seizures, neuropathy, and myopathy sparing the extracocular muscles; (5) autosomal recessive progressive external ophthalmoplegia (PEO)–plus; and (6) autosomal dominant PEO–plus. The most common POLG gene mutation is c.1399G>A in exon 7, which results in a p.A467T substitution in the linker domain. This substitution is most commonly seen in Alpers syndrome, autosomal recessive PEO, and juvenile spinocerebellar ataxia-epilepsy syndrome. The p.A467T change behaves as an autosomal recessive mutation in PEO, and heterozygotes are asymp-
tomatic. Similarly in Alpers syndrome, the p.A467T mutation is either homozygous or paired with other mutations in compound heterozygotes. Two of our patients are compound heterozygous for the common p.A467T substitution and for an unreported p.P1073L change. This new mutation is in the polymerase domain of POLG. Although detailed structural or biochemical information about this residue is lacking, the proline at residue 1073 is invariant from yeast to humans and is located within the polymerase active site region.

Figure 2. Electrophoretograms (A and D) and amino acid sequences (B and E) of the 2 mutations in patient 1, confirmed by restriction fragment length polymorphism (C and F). A and B, G1399A substitution (arrow) is shown. D and E, C3218T substitution (arrow) is shown. C, Wild-type DNA is cut into fragments by the Mwo restriction enzyme, while mutant DNA is not. In the patient’s sample, an additional 146–base pair (bp) band is seen. F, Wild-type DNA is cut into fragments by the Mps restriction enzyme, while mutant DNA is not. In the patient’s sample, an additional 186-bp band is seen. G, Alignment of the pol-γ fragment containing the novel p.P1073L mutation across 9 species shows the high conservation of proline 1073. S c indicates Saccharomyces cerevisiae; S pombe, Saccharomyces pombe.
The high conservation of the proline 1073 suggests that such structural rigidity is needed at this residue in pol-γ and that substitution by any amino acid would disrupt the local structure. The p.P1073L mutation is close to p.G1051R associated with PEO and ataxia,8 p.G1076V associated with autosomal dominant PEO,9 and p.I1079L associated with autosomal dominant PEO1. These residues lie between 2 highly conserved motifs, the B motif that forms the fingers domain (residues 946-957) and the C motif (residues 1134-1140). The fingers domain is responsible for proper recognition and binding of the incoming nucleotide triphosphate, whereas the C motif contains the invariant aspartic acid and glutamic acid residues, 2 of the 3 carboxylic residues that are critical for binding the 2 catalytic Mg2+ ions. Mutations within the B motif are associated with autosomal dominant PEO and cause severe defects of polymerase activity,10 while mutations in any of the conserved carboxylic residues in B motif completely inactivate the polymerase,11 cause mtDNA depletion,12 and have been associated with infantile hepatocerebral mtDNA depletion syndromes.13

Children with the p.A467T mutation and mutations in the polymerase domain of pol-γ usually have Alpers syndrome.14,15 Alpers syndrome is characterized by (1) refractory, mixed-type seizures; (2) psychomotor regression, often episodic and triggered by intercurrent infection; and (3) hepatopathy with or without acute liver failure. Our patients 1 and 2 had the full triad plus other clinical signs such as headache, gastrointestinal problems, ptosis, and hearing loss, all of which have been described in Alpers syndrome.14,16,17 Patients 3 and 4, who were first cousins and who both had status epilepticus, psychomotor delay, and hepatopathy, although their course was slower than in patient 1. Also, although both cousins developed terminal status epilepticus, they had not previously presented with seizures, and the electroencephalogram for patient 4 showed slowing but not an epileptogenic pattern.

The morphological changes in the liver biopsies of patients 1 and 2 were typical of Alpers syndrome, including at least 3 of the 8 features required by Nguyen et al14 for this diagnosis, namely, (1) microvesicular steatosis, (2) bile duct proliferation, (3) hepatocyte dropout or focal necrosis with or without portal inflammation, (4) collapse of liver cell plates, (5) parenchymal disarray or disorganization of the normal lobular architecture, (6) bridging fibrosis or cirrhosis, (7) regenerative nodules, and (8) oncocytic change (mitochondrial proliferation associated with intensely eosinophilic cytoplas) in scattered hepatocytes not affected by steatosis. Postmortem studies of the livers of patients 3 and 4 showed microvesicular steatosis and, in patient 4, ultrastructural alterations of the mitochondria, including pale matrix and sparse cristae.

Although patient 2 had a hepatocerebral syndrome, seizures were the terminal features. On the other hand, the prominent gastrointestinal dysmotility that characterized both his clinical presentation and that of patient 1, together with the reflux esophagitis of patient 3, suggest the possibility that the common, novel p.P1073L may especially affect the gastrointestinal tract.

Another clinical presentation associated with the coexistence of the p.A467T transversion and a mutation in the polymerase domain is autosomal recessive PEO.1,18 These patients have bilateral ptosis, severe limitation of ocular motility, and reveal mosaic distribution of ragged-red and cytochrome C oxidase-negative fibers in muscle biopsy.

There are limited studies of the relationship between molecular change in POLG, biochemical consequences, and clinical phenotypes. It has been reported that the A647T mutation reduces the overall efficiency of DNA synthesis to less than 4% of normal and reduces the processivity of the mutant DNA polymerase by the accessory subunit.1 The p.G848S mutation found in patient 2 has recently been shown to reduce polymerase activity to less than 1% of normal, with a marked decrease in DNA binding.9 This is the third most common mutation associated with Alpers syndrome.1 Patient 3 had a p.W748S mutation, also commonly found in Alpers syndrome.10

In conclusion, patients heterozygous for one mutation in the spacer region and a second mutation in the polymerase domain of POLG usually have Alpers syndrome. We describe 4 such patients with an unreported recessive p.P1073L mutation in the polymerase domain.

The simultaneous occurrence of p.W748S and p.A467T in trans in the Belgian family is misleading because the pedigree analysis at first sight may suggest a dominant disorder, whereas molecular analysis shows that these first cousins have a recessive disorder (Figure 1). This phenomenon, which is owing to the high frequency of the p.A467T mutation in northwestern Europe (0.6% allele T frequency in Belgium),10 and of the p.W748S mutation in the general population,19 complicate clinical diagnosis and genetic counseling.

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REFERENCES


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