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

Bulent Kurt, MD; Jaak Jaeken, MD, PhD; Johan Van Hove, MD; Lieven Lagae, MD, PhD; Ann Løfgren, MSc; David B. Everman, MD; Parul Jayakar, MD; Ali Naini, PhD; Klaas J. Wierenga, MD, MSc; Gert Van Goethem, MD, PhD; William C. Copeland, PhD; Salvatore DiMauro, MD

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.

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.

Arch Neurol. 2010;67(2):239-244

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.¹ 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 1996² 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.¹ The gene contains 23 exons, and the translation initiator methionine codon is located in exon 2.¹ 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 2001.³ 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.¹

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-
brospinal fluid analysis showed increased total protein content, increased fumarate level, consistent with malnutrition. Cerebrospinal fluid analysis showed only mild enlargement of the lateral ventricles. An electroencephalogram showed reflux esophagitis grade 4, hyperplastic gastritis, 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/mediovesicular 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 II-3). 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.

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. Gas troscopy showed reflux esophagitis grade 4, hyperplastic gastritis, 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 microvesicular steatosis of the liver with ultrastructural evidence of excessive lipid droplets and abnormal mitochondria. Muscle histology results were normal but ultrastructural studies showed increased lipid droplets. The clinical features of the 4 patients are summarized in the Table.

**Table**

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′- CCACGGAAACAGCAGATT-3′ and reverse, 5′-
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 Performa 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’-ACCTGCTCTGACGACAGACG-3’ and reverse, 5’-CAAGGCTGGTCTACCTCTCTC-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’-GGAAAGTGGGGAGTGTT-3’ and reverse, 5’-CCATGCTCTAAAAGGTAGCAA-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 transition in exon 7 that resulted in a p.A467T amino acid change (Figure 2, A and B). In patient 2, the first mutation was a c.2542G>A transition, resulting in p.G848S. 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 change (Figure 2, D 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.

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 extracranial 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 asym-
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. The cy-
The cristae usually have a rugose or sparse cristae. 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. In conclusion, patients heterozygous for one 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), and of the p.W748S mutation in the general population, complicate clinical diagnosis and genetic counseling.

Accepted for Publication: September 2, 2009.

Author Affiliations: Department of Neurology, Columbia University Medical Center, New York, New York (Drs Kurt, Naini, and DiMauro); Center for Metabolic Disease (Dr Jaeken), and Division of Pediatric Neurology (Dr Lagae), University Hospital Gasthuisberg, Leuven, Belgium; Division of Clinical Genetics and Metabolism, The Children’s Hospital, Denver, Colorado (Dr Van Hove); VIB-Department of Molecular Genetics, University of Antwerp, Antwerpen, Belgium (Ms Lofgren); Greenwood Genetic Center, Greenwood, South Carolina (Dr Everman); Division of Genetics and Metabolism, Miami Children’s Hospital, Miami, Florida (Drs Jayakar and Wierenga); Section of Genetics, Department of Pediatrics, Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma (Dr Wierenga); Department of Neurology and Neuromuscular Reference Center, University Hospital of Antwerp, Antwerpen, Belgium (Dr Van Goethem); Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina (Dr Copeland).
Correspondence: Salvatore DiMauro, MD, Columbia University College of Physicians and Surgeons, 630 W 168th St, 4th Floor, Box 31, New York, NY 10032 (sd12@columbia.edu).


Financial Disclosure: None reported.

Funding/Support: This study was supported by grant HD 32062 from the National Institutes of Health and the Marriott Mitochondrial Disorder Clinical Research Fund.

Additional Contributions: The authors wish to thank Augusto Morales, MD, Greenville Hospital System, Greenville, South Carolina, and Kurena Tanji, MD, Columbia University Medical Center, New York, New York, for their investigations of patient 1; and Drs Rudy Van Coster, MD, University of Ghent, Ghent, and Sara Seneca, MD, Free University of Brussels, Brussels, Belgium, for their investigations of patients 3 and 4.

REFERENCES


Topic Collections. Archives offers collections of articles in specific topic areas to make it easier for physicians to find the most recent publications in a field. These are available by subspecialty, study type, disease, or problem. In addition, you can sign up to receive a Collection E-Mail Alert when new articles on specific topics are published. Go to http://archneur.ama-assn.org/collections to see these collections of articles.