*Journal of Pediatric Gastroenterology and Nutrition*

49:126–129 *#* 2009 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

Case Report

De Novo Mutations in *POLG* Presenting with Acute Liver Failure or Encephalopathy

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Historically, mitochondrial respiratory chain disorders have been recognized as a multisystemic disease with predominantly neuromuscular dysfunction [(1).](#_bookmark4) To date, most patients with mutations in *POLG* have presented with predominantly neurological features of headaches or focal seizures [(2,3)](#_bookmark4). In 1 study, end-state hepatic disease developed in only 2 of 26 cases [(2).](#_bookmark4) In another study, the only patients who experienced hepatic disease had been exposed to sodium valproate [(3,4).](#_bookmark5) Viral infec- tion may be temporally associated with the development of acute liver failure in pediatric patients [(5).](#_bookmark6) However, the majority of children who contract viral infections do not experience liver failure. This suggests that there must be host factors that differentiate those children who do experience liver failure.

CASE 1

An infant girl was born at term with a normal birth weight to nonconsanguineous white parents. At 2 months of age she had feeding difficulties and hypotonia. Consequently, she underwent an extensive medical evaluation that included determination of plasma electro- lytes, lactate/pyruvate, plasma amino acids, urine organic acids, chromosome karyotyping, fluorescence in situ hybridization for DiGeorge deletion, Angelman/Prader- Willi methylation studies, and magnetic resonance imaging (MRI) of the brain. The results of these tests

Received February 16, 2008; accepted March 28, 2008.

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Drs Lutz and Dimmock contributed equally to this manuscript. Supported in part by a National Institutes of Health fellowship award

K12 RR17665 (D.D.).

The authors report no conflicts of interest.

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were normal. Although the result of her initial newborn hearing screening was normal, subsequent testing showed her auditory brainstem response to be abnormal.

At 4.5 months of age the patient contracted an influ- enza A infection. Concurrently she experienced hepato- megaly associated with severe hypoglycemia and elevations of plasma tyrosine (203 mmol/L, normal range [NR] 35–126), glutamine (994 mmol/L, NR 353– 883), and alanine (1048 mmol/L, NR 152– 459). Her serum lactate was increased to 9.8 mmol/L (NR <2.2). The markedly increased lactate-to-pyruvate ratio (43.9, NR

<15) suggested a defect in the electron transport chain or tricarboxylic acid cycle [(6).](#_bookmark8)

Abnormalities in liver synthetic function were demon- strated by an elevated prothrombin time of 23 seconds (NR 12.4–15.1) and a partial thromboplastin time of 58 seconds (NR 25–39). Her conjugated bilirubin increased to 1.89 mg/dL (NR 0.0–0.3), suggesting a cholestatic process. Conversely, there were only modest elevations in liver hepatocellular enzymes (aspartate aminotransferase [AST] 260 U/L, NR 16–46; alanine transaminase [ALT] 313 U/L, NR 29–46) without significant elevations in creatinine kinase. Ammonia was normal until the final stages of her illness, when it rose to 76 mmol/L (normal

<50).

Her neurological status did not show further decline. She did not experience seizures or nystagmus. However, a lumbar puncture 2 days after her second admission for worsening liver failure showed elevated cerebrospinal fluid (CSF) protein (550 mg/dL, NR 15– 40) with a nearly acellular tap (13 white blood cells, 0 red blood cells). MRI of the brain showed nonspecific extraaxial fluid collection over the frontal cortex and also thickening and enhancement of the nerve roots of the cauda equina. These changes, sometimes seen in demyelinating poly- neuropathies, suggested a possible neurodegenerative disorder.

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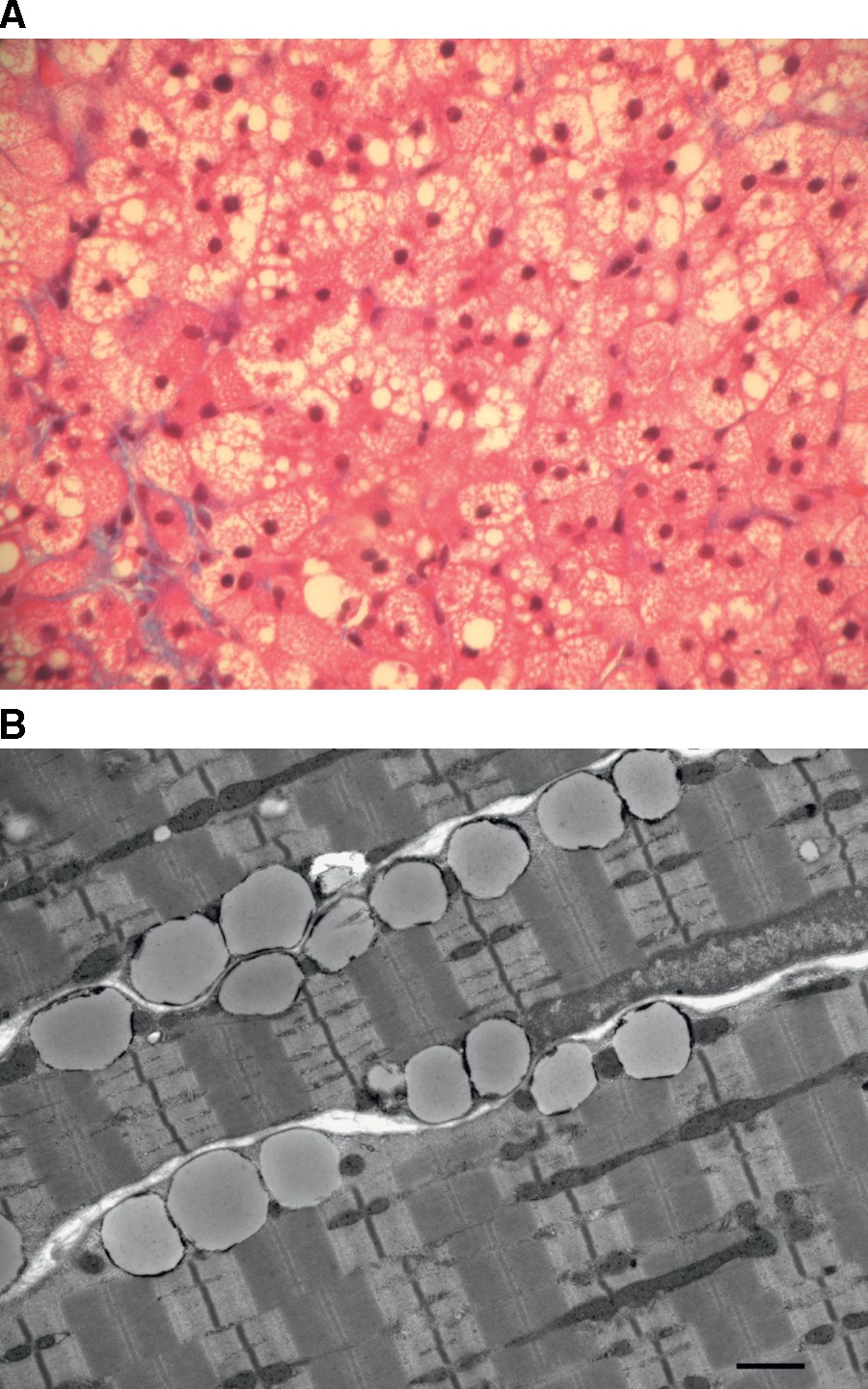


FIG. 1. (A) Liver shows marked accumulation of lipid (microstea- tosis; trichrome stain, original magnification x40). (B) Longitudinal section of skeletal muscle shows marked accumulation of sub- sarcolemmal neutral lipid; mitochondria are unremarkable (elec- tron microscopy, bar equals 1 mm).

The results of other studies, including lysosomal enzymes, plasma very-long-chain fatty acids, and screen- ing for common mutations of mitochondrial deoxyribo- nucleic acid (mtDNA) were normal. She showed signs of ongoing respiratory difficulty, pancreatitis, and renal tubulopathy. Despite aggressive medical therapy, the patient died at the age of 5.5 months.

Liver biopsy revealed marked panlobular accumu- lation of neutral lipid within hepatocytes, with a pre- dominantly microvesicular pattern ([Fig.](#_bookmark2) 1A). Moderate subacute portal inflammation, mild canalicular and hepa- tocellular cholestasis, and mild pseudoacinar formation were also noted. Glycogen staining was normal. Electron microscopy confirmed the microvesicular steatosis but did not demonstrate any structural abnormalities of mito- chondria. The muscle biopsy revealed prominent accumulation of neutral lipid within myocytes, especially in the subsarcolemmal region, corresponding to a ‘‘lipid

myopathy’’ ([Fig.](#_bookmark2) 1B). The myofibers were abnormally small, measuring from 5 to 10 mm in cross-sectional diameter. There were no ragged-red fibers or cycloox- ygenase-negative fibers. Glycogen staining was normal. Electron microscopy confirmed the excessive accumu- lation of lipid and demonstrated small subsarcolemmal collections of mitochondria in occasional myofibers. Most mitochondria in the specimen contained matrix granules. Some cristae were slightly curved or dilated, but there were no unique structural abnormalities and no paracrystalline inclusions. The mitochondria ranged from 105 to 510 (mean 262) nm in cross-sectional diameter. Abnormally enlarged mitochondria were not present. The lipid droplets ranged from 260 to 1464 nm in diameter. The activities of liver mitochondrial enzymes, including respiratory chain complexes, are summarized in [Table](#_bookmark3) 1.

Three heterozygous *POLG* mutations; c.752C>T (p.T251I), c.1760C>T (p.P587L), and c.3572A>G

(p.K1191R), were identified. The p.T251I and p.P587L missense mutations have been frequently reported in *cis* [(7–9).](#_bookmark9) The novel missense variant, c.3572A>G (p.K1191R), predicts a relatively conservative change of lysine to arginine; however, a p.K1191N mutation at the same amino acid residue has been found in a patient with Alper syndrome [(2).](#_bookmark4) Thus, the p.K1191R alteration is likely to be pathogenic. Testing of the parents’ DNA showed that the mother was heterozygous for the p.T251I and p.P587L missense mutations, confirming her carrier status. Test results from the father were negative for all of the mutations. Paternity was confirmed by compara- tive analysis using 15 unlinked polymorphic markers. Measurement of the mtDNA content in liver using qPCR revealed a severely reduced mtDNA (3% of age-matched mean), consistent with 2 mutated alleles in the *POLG* gene.

CASE 2

A white infant boy was born to healthy unrelated parents. At 18 months of age, he had speech and motor delay. He was able to crawl but not walk. MRI of the spinal cord showed an asymptomatic syrinx between T11 and L1. While at home with his family, he had a new- onset seizure that included eye deviation, jaw clenching, and hypotonia of the trunk and extremities. He experi- enced repetitive generalized tonic-clonic seizures that evolved into refractory status epilepticus. No precipitat- ing event or ingestion was identified, and he was not ill or febrile before the onset of seizures.

Standard treatment with phenobarbital, fosphenytoin, midazolam, lorazepam, and diazepam was unsuccessful. Therefore, the child underwent pentobarbital coma for seizure control. He was not treated with valproic acid. After 30 days of this treatment, his seizures resolved and the pentobarbital was discontinued. He was left with a severe encephalopathy characterized by choreo-athetoid

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TABLE 1. *Mitochondrial respiratory chain activity*

Case 1 Citrate synthase activity

NADH:FeCN

reductase

IþIII Rotenone sensitive

Succinate

dehydrogenase IIþIII

Cytochrome c oxidase (IV)

Liver\* 232 proliferated (370; 100) 509 (206; 56) 30 (16; 4) 65 (369; 100) 14 (119; 32) 7 (27; 7)

Liver control mean SD\* 62.7 25.8 247 127 188 58.7 17.6 5.3 11.8 3.2 26.4 10.8

Range\* 31.6–117 58.5–438 84.5–281 11.1–26.5 4.8–15.7 7.5–42.8

Muscley 20.67 0.60 1.13 0.09 1.75

Muscle reference rangey 6.86–24.62 0.41–1.21 0.45–1.29 0.42–1.65 1.03–3.38

Mitochondrial respiratory chain enzyme activities in liver and muscle showed significantly reduced complex IþIII and IIþIII and IV in liver before and after correction for citrate synthase and significantly reduced complex IIþIII in muscle. Citrate synthase and succinate dehydrogenase activities, which are not encoded by mtDNA, were found to be increased in liver, consistent with mitochondrial proliferation. IV ¼intravenous; NADH ¼ nicotinamide adenine dinucleotide.

\* Activities are expressed in nmol · min-1 · mg-1 of protein. Numbers in the parentheses represent percentages of the control mean before (first

number) and after (second number) correction to citrate synthase activity. Control mean values were obtained from 10 liver specimens with urea cycle deficiency, which may have below-normal respiratory chain enzyme activities. Thus, the patient values in this table may be overestimated.

y Activities are expressed in mmol · min-1 · g-1 of wet weight tissue. Muscle electron transport chain was performed at Buffalo Children’s Hospital, Biochemical Genetics Laboratory. Reference ranges reflect laboratories’ published reference ranges.

movements, corticovisual impairment, diffuse hypotonia, and severe swallowing dysfunction.

The initial laboratory tests revealed elevated plasma lactate (3.2 mmol/L, NR 0.5– 2.0), AST (85 U/L, NR 16–

46), and ALT (69 U/L, NR 29–46). Ammonia was within normal range. A lumbar puncture was performed. This did not suggest an infectious cause because the CSF was colorless, with a few white cells (18 white blood cells, 4 red blood cells). However, CSF lactate (3.4 mmol/L, NR 0.6– 2.2) and protein (46 mg/dL, NR 15– 40) were both elevated.

Cultures of the blood, urine, and CSF were negative for bacterial or viral processes. Titers for multiple causes of viral encephalitis and herpes simplex virus polymerase chain reaction were negative. Liver transaminases remained mildly elevated throughout his hospitalization. AST peaked at 195 U/L and ALT at 200 U/L; however, liver synthetic dysfunction was not significantly dis- turbed, with a normal coagulation profile (prothrombin time 13.1, international normalized ratio 1.0, partial thromboplastin time 28), bilirubin (maximum total bilir- ubin 1.1 mg/dL, NR 0– 1.5), and maximum direct bilir- ubin (0.6 mg/dL, NR 0.1– 0.5).

Magnetic resonance imaging of the brain showed abnormal restricted diffusion involving the subcortical white matter in the left posterior parietal and occipital lobes, consistent with acute infarction or ischemia. Abnormally increased T2-weighted image and fluid attenuated inversion recovery (FLAIR) signal were seen in the bilateral thalami. He had normal biotinidase, very- long-chain fatty acids, carbohydrate-deficient transferrin, urine organic acids, plasma amino acids, plasma acyl- carnitine profile, palmitoyl-protein thioesterase 1, and tripeptidyl-peptidase 1. Subsequently, he has remained neurologically static, with 1 admission for seizures. He has not had any subsequent elevation in his liver enzymes.

Sequence analysis revealed 2 mutations in *POLG*: p.A467T mutation and a novel splice site mutation,

c.2157þ5\_6GC>AG, in the child. The mother carried the p.A467T mutation. Test results from the father, however, were negative for both mutations, despite 15 unlinked markers being consistent with the stated pater- nity. The patient has an asymptomatic older brother who carries the p.A467T mutation.

DISCUSSION

In the past few years there has been a substantial increase in the understanding of the molecular basis of infantile liver failure caused by mtDNA depletion. At least 3 nuclear genes are known to be responsible of the hepatic form of mtDNA depletion. These are *POLG*, deoxyguanosine kinase (*DGUOK*), and the recently dis- covered *MPV 17* [(10)](#_bookmark10). In general, CNS symptoms are the predominant clinical features of cases with mutations in *POLG* [(11);](#_bookmark11) infantile liver failure, or Navajo neurohepa- topathy in *MPV 17* deficiency [(10,12](#_bookmark10)), whereas *DGUOK* mutations may present with either liver and neurological symptoms or isolated liver failure without CNS symp- toms [(13,14).](#_bookmark12)

Alper syndrome is a severe autosomal recessive dis- order caused by mutations in the *POLG* gene. The minimum diagnostic criteria for Alper syndrome are refractory seizures, psychomotor regression, and charac- teristic hepatic disease with or without acute liver failure

[(15).](#_bookmark13) The majority of patients present with central ner- vous system disease, most notably headaches or seizures. In 1 series, only 9 of 26 patients experienced liver failure, and in the majority of those it was precipitated by sodium valproate exposure [(3).](#_bookmark5) A similar relation to sodium valproate was seen in another series [(2).](#_bookmark4) In the context of isolated liver failure without neurological signs, the clinical diagnosis of Alper syndrome cannot be established.

Influenza A viral infection has been associated with hepatic decompensation in adults with preexisting liver

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disease [(16](#_bookmark14)). Similarly, acute hepatic disease was observed concurrent with influenza A infection in 4 apparently healthy children, 1 of whom was subsequently suspected to have a mitochondrial cytopathy [(4)](#_bookmark7). Inasmuch as the 13,000 other children from Colorado with influenza infec- tion during the time of that study did not experience liver failure, the postulates of Kock were not fulfilled. It is most likely that this difference reflects a host susceptibility. We believe that case 1 in our report represents another example in which an underlying genetic susceptibility leads to acute liver failure in the face of a hepatotropic viral infection. Additionally, we recently described twin boys, with mutations in *DGUOK*, 1 of whom experienced acute liver failure apparently precipitated by a herpesvirus infection. The condition of this patient acutely deterio- rated, and he died at 9 months of age [(17](#_bookmark15)). His twin brother, aside from modest elevation in his AST and ALT, remains well at 3.5 years with the same mutations. These cases may begin to answer the question why only some children experience acute liver failure in the face of viral infections whereas most do not. Furthermore, they suggest that evaluation for the mtDNA depletion syndromes should be considered in patients with infan- tile acute liver failure even in the presence of an apparent viral trigger.

Case 2 demonstrates that the full triad of Alper syn- drome may not always be seen at presentation. Therefore, *POLG* testing should be considered in patients with intractable seizures even without liver failure.

Both individuals seem to have a mutation in both copies of *POLG* consistent with autosomal recessive disease. The p.K1191R and c.2157þ5\_6GC>AG muta- tions have not been reported previously. The p.K1191R affects a highly conserved amino acid residue, and the c.2157þ5\_6GC>AG splice site mutation produces a truncated polypeptide. Furthermore, they are not present in 250 individuals whose *POLG* gene has been sequenced in our laboratory. These results, in the context of the clinical presentation, strongly support the pathogenic role of these mutations. Comparative analysis on both fathers and their affected children at 15 unlinked polymorphic markers is consistent with the stated paternity. Thus, the data support the presence of a de novo mutation in both cases. It is likely that both novel mutations occur in the paternally derived chromosome. This observation is consistent with those in many other diseases, such as achondroplasia, in which paternal chromosomes are the source of de novo point mutations, as reviewed by Crow

[(18).](#_bookmark16) This is the first report of de novo mutations in the *POLG* gene. To date, more than 100 mutations in *POLG* have been found in patients with a broad clinical spectra of mitochondrial diseases [(19)](#_bookmark17). Contrary to a recent report [(20)](#_bookmark18), the discovery of 2 novel de novo mutations in a total of 28 patients with Alper syndrome identified in our laboratory suggests that the *POLG* gene may be susceptible to de novo or somatic mutations.

Acknowledgment: The authors thank Melissa S. Holzapfel, BS, for the electron microscopy of muscle and preparation of electron micrographs.

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*J Pediatr Gastroenterol Nutr, Vol. 49, No. 1, July 2009*

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