

## ONLINE FIRST

# MPV17 Mutations Causing Adult-Onset Multisystemic Disorder With Multiple Mitochondrial DNA Deletions

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**Objective:** To identify the cause of an adult-onset multisystemic disease with multiple deletions of mitochondrial DNA (mtDNA).

**Design:** Case report.

**Setting:** University hospitals.

**Patient:** A 65-year-old man with axonal sensorimotor peripheral neuropathy, ptosis, ophthalmoparesis, diabetes mellitus, exercise intolerance, steatohepatopathy, depression, parkinsonism, and gastrointestinal dysmotility.

**Results:** Skeletal muscle biopsy revealed ragged-red and cytochrome-c oxidase-deficient fibers, and Southern blot analysis showed multiple mtDNA deletions. No dele-

tions were detected in fibroblasts, and the results of quantitative polymerase chain reaction showed that the amount of mtDNA was normal in both muscle and fibroblasts. Exome sequencing using a mitochondrial library revealed compound heterozygous *MPV17* mutations (p.LysMet88-89MetLeu and p.Leu143\*), a novel cause of mtDNA multiple deletions.

**Conclusions:** In addition to causing juvenile-onset disorders with mtDNA depletion, *MPV17* mutations can cause adult-onset multisystemic disease with multiple mtDNA deletions.

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**M**ITOCHONDRIAL DNA (mtDNA) integrity requires nuclear DNA-encoded proteins to maintain deoxy-nucleotide and ribonucleotide balance, to replicate and repair the mitochondrial genome, and to generate the mtDNA-protein nucleoid complex. Disorders in the cross talk between the 2 genomes can compromise the integrity and quantity of mtDNA, leading to pathogenic multiple deletions, point mutations, and depletion of mtDNA.<sup>1</sup>

The clinical presentations of defects of intergenomic communication are heterogeneous. Depletion of mtDNA is associated with infantile-onset myopathy (OMIM #609560) or multisystemic diseases (OMIM #251880 and #203700), whereas multiple deletions of mtDNA have been observed in adult-onset autosomal dominant (ad) or autosomal recessive (ar) progressive external ophthalmoplegia (PEO) (OMIM #157640 and #258450).<sup>1,2</sup> Although mtDNA depletion syndromes were initially considered clinically and etiologically distinct from adPEO and arPEO with

multiple mtDNA deletions, the coexistence of multiple deletions, depletion, and point mutations of mtDNA in mitochondrial neurogastrointestinal encephalomyopathy (OMIM #603041) revealed that qualitative and quantitative defects of mtDNA were part of a spectrum ranging from depletion to multiple deletions.<sup>2,3</sup> This concept was confirmed when mutations in genes initially considered typical causes of adPEO and arPEO with multiple mtDNA deletions, such as *POLG*, encoding mtDNA polymerase gamma, and *C10orf2*, encoding a mtDNA helicase, were later found to also cause depletion of mtDNA.<sup>4,5</sup> Conversely, mutations in *RRM2B* and *TK2*, originally associated with mtDNA depletion and infantile myopathy, were found to cause late-onset arPEO with multiple mtDNA deletions.<sup>6,7</sup>

Mutations in *MPV17* have been identified in patients with severe mtDNA depletion manifesting as early childhood-onset failure to thrive, hypoglycemia, encephalopathy, and hepatopathy progressing to liver failure.<sup>8-11</sup> In addition, the p.R50Q *MPV17* mutation was identified in homozygosity in Navajo Indian patients

with infantile-, childhood-, or juvenile-onset neuro-hepatopathy (Navajo neurohepatopathy, OMIM #256810) and mtDNA depletion.<sup>8</sup> We now report that *MPV17* compound heterozygous mutations can cause multiple mtDNA deletions manifesting as adult-onset multisystemic disease.

## REPORT OF A CASE

A 65-year-old man born of nonconsanguineous parents of European origin developed distal limb weakness and numbness at age 34 years. Nerve conduction studies and electromyographic results showed signs of an axonal sensorimotor peripheral neuropathy, initially diagnosed as Charcot-Marie-Tooth disease. In his 40s, he developed progressive proximal limb weakness, exercise intolerance, diabetes mellitus, ptosis, ophthalmoparesis, hearing loss, severe constipation due to gastrointestinal dysmotility, and depression. Ultrasonography of the abdomen revealed a fatty liver. At age 65 years, he was noted to have parkinsonism with bradykinesia, bilateral resting hand tremor, and mild rigidity.

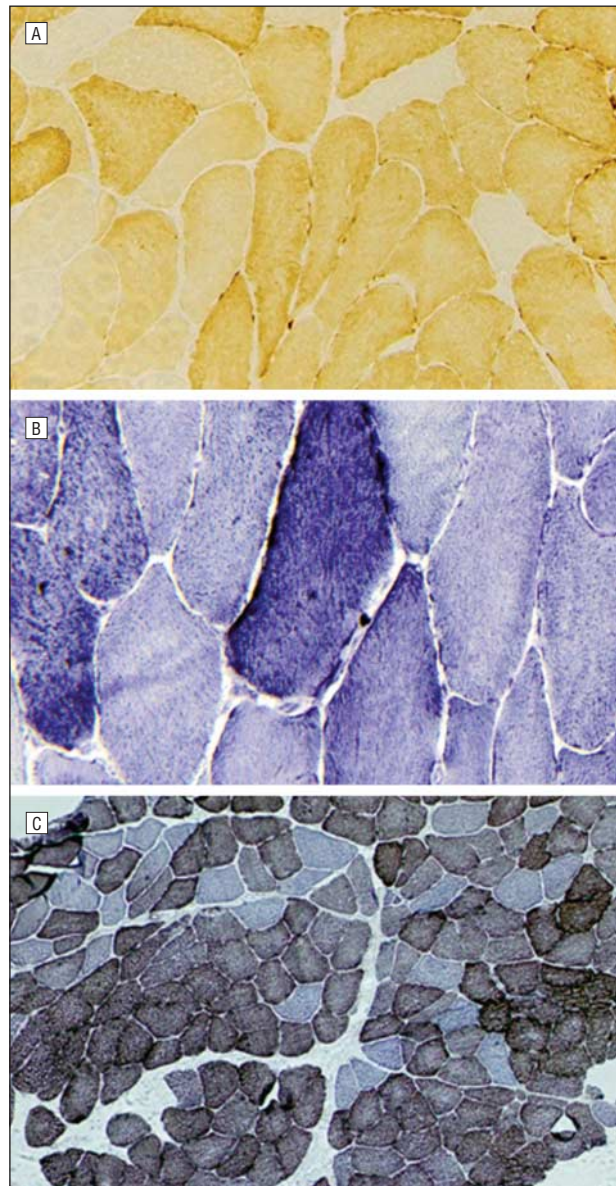
His sister died from unexplained liver failure at 39 years of age. His mother reportedly had ptosis and weakness.

Laboratory studies revealed mildly elevated levels of serum creatine kinase (702 U/L; normal range, 30-220 U/L [to convert to microkatal per liter, multiply by 0.0167]) and venous lactate (2.3 mmol/L; normal range, 0.2-2.2 mmol/L [to convert to milligrams per deciliter, divide by 0.111]). Standard histological studies of a muscle biopsy performed at age 61 years showed chronic myopathy, mild neurogenic abnormalities, 10% of fibers with decreased or no cytochrome-*c* oxidase histochemical activity, and 3% of fibers with excessive subsarcolemmal succinate dehydrogenase staining. With cytochrome-*c* oxidase/succinate dehydrogenase combination staining, approximately half of the cytochrome-*c* oxidase-deficient fibers appeared blue, and scattered cytochrome-*c* oxidase-positive fibers showed increased subsarcolemmal succinate dehydrogenase staining (**Figure 1**). Muscle biochemistry results showed normal activities of all mitochondrial respiratory chain complexes and citrate synthase.

DNA extracted from skeletal muscle and fibroblasts using real-time polymerase chain reaction revealed a normal concentration of mtDNA. Southern blot analysis revealed multiple deletions in muscle but not in fibroblasts (**Figure 2**). Mutations in the *POLG* gene were excluded by sequencing all exons and flanking introns.

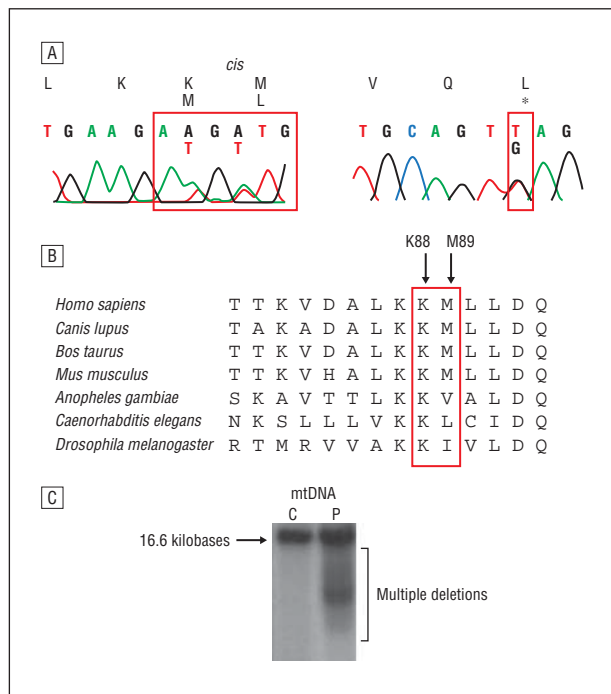
Targeted exome sequencing was performed on whole-genome-amplified DNA obtained from the muscle of the patient. A solution hybrid capture method was used to isolate 4.3 megabases of targeted DNA that included the 16-kilobase mtDNA, as well as all coding and untranslated exons of 1381 nuclear genes, 1013 mitochondrial genes from the MitoCarta database,<sup>12</sup> 21 genes with recent strong evidence of mitochondrial association, and 347 additional genes, which were then sequenced on Illumina GA-II platform.<sup>13</sup>

After filtering out common variants with high allele frequency (exceeding 0.005) within dbSNP132 and the



**Figure 1.** Histochemical staining of the muscle biopsy showing (A) cytochrome-*c* oxidase-negative (original magnification  $\times 200$ ), (B) succinate dehydrogenase hyperreactive (original magnification  $\times 400$ ), and (C) ragged-red (original magnification  $\times 100$ ) fibers.

“1000 Genomes Project,” we detected 2 heterozygous variants, c.263A>T (p.Lys88Met) and c.265A>T (p.Met89Leu), present in exon 3 of the *MPV17* gene. These amino acid residues showed high evolutionary conservation, with identical amino acids observed in 37/37 aligned vertebrate species (p.Lys88) and 35/37 aligned vertebrate species (p.Met89). The PolyPhen algorithm (<http://genetics.bwh.harvard.edu/pph/>) predicted the first variant to be “probably damaging” and the second variant to be “benign.” These variants are in *cis*, based on co-occurrence in sequence reads, and were both present in maternal DNA. Using Sanger sequencing, we screened all 7 exons of *MPV17*, confirming the exome sequencing data and identifying the third nonsynonymous variant in exon 6, c.428T>G (p.L143\*) (Figure 2), that was absent in the mother and is likely inherited from the fa-



**Figure 2.** A, Sequence of *MPV17* showing the c.263A>T, c.265A>T, and c.428T>G pathogenic variants. B, Evolutionary conservation of sites of mutations p.LysMet88-89MetLeu. C, Southern blot analysis showing mitochondrial DNA (mtDNA) multiple deletions in muscle biopsy. C indicates control; P, patient.

ther (DNA not available). This heterozygous variant was supported by the targeted exome sequence data (3/10 aligned reads), although the depth and quality of the reads were insufficient for us to confidently detect a heterozygous variant using automated algorithms to detect a heterozygous variant.

All 3 sequence variants are extremely rare. They were not observed in 628 individuals with low-pass whole-genome sequence data available in the 1000 Genomes Project,<sup>14</sup> 371 healthy individuals of European ancestry from the National Institute of Mental Health control collection with available whole-exome data, 5379 individuals with available whole-exome data from the Exome Sequencing Project,<sup>15</sup> or 100 control subjects whose *MPV17* gene was sequenced.

## COMMENT

*MPV17* is a mitochondrial inner-membrane protein with unknown function. Mutations in *MPV17* have been reported in 29 patients with infantile-onset progressive liver dysfunction, hypoglycemia, failure to thrive, or neurological symptoms leading to early death in the absence of a liver transplant.<sup>8-10</sup>

The homozygous p.R50Q mutation, first described in a large consanguineous Southern Italian family,<sup>9</sup> causes a neurohepatopathy with high incidence (1 in 1600 live births) in the Navajos of Arizona and Utah.<sup>16-18</sup> The exclusion of a recent common ancestor between the Italian family and Navajo neurohepatopathy families by single-nucleotide polymorphism analysis suggested to us that the p.R50Q mutation causes divergent phenotypes

in the 2 ethnic groups owing to environmental or genetic modifiers.<sup>18</sup>

We detected 2 deleterious variants in *MPV17* in a patient with adult-onset neurohepatopathy-plus syndrome with multiple deletions of mtDNA in muscle. *MPV17* is an inner mitochondrial membrane protein, which is predicted to contain 4 transmembrane (TM) segments. A putative protein kinase C phosphorylation site is predicted to reside between TM2 and TM3 and is the hot spot for most previously described mutations.<sup>11</sup> One of the patient's mutations affects 2 adjacent codons, resulting in p.LysMet88-89MetLeu substitutions within the protein kinase C phosphorylation site, whereas the second mutation, p.L43Leu\*, is predicted to truncate the protein in TM4. The pathogenicity of these missense mutations is supported by the report of another mutation of the same amino acid (p.Lys88Glu).<sup>11</sup> Maternal DNA was shown to contain the missense mutations but not the p.L43Leu mutation, thus supporting the compound heterozygosity of these variants. The pathogenicity of these mutations in our patient is also supported by studies of the *Sym1* (homologue of *MPV17*) mutant yeast model that showed mtDNA rearrangements, impairment of mitochondrial bioenergetics, and morphologically abnormal mitochondria under stress conditions.<sup>19</sup> Owing to the severity of the observed mutations in the patient and the well-established link between recessive *MPV17* mutations and mtDNA depletion, we posit that these mutations cause the observed phenotype in the patient.

The disease presentation in our patient is both similar to and different from the disease presentations in previously described patients with *MPV17* defects. It is similar to the juvenile form of Navajo neurohepatopathy because the neurological manifestations (namely, axonal sensory-motor neuropathy and PEO) preceded liver dysfunction in our patient. Our patient had other clinical manifestations (including gastrointestinal dysmotility, depression, and parkinsonism) that are also common features of *POLG* or *ANT1* mutations with multiple mtDNA deletions.<sup>20-22</sup> Thus, our report reveals that *MPV17* mutations can cause an adult-onset disorder with clinical features overlapping those of other defects of intergenomic communication, which may lead to diagnostic difficulties. In our patient, the presence of multiple deletions of mtDNA in the skeletal muscle reinforces the notion that the *MPV17* protein is critical for the maintenance of mtDNA integrity.

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