A novel mitochondrial tRNAPhe mutation causes MERRF syndrome

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Abstract—A woman with typical features of myoclonic epilepsy with ragged red fibers (MERRF) had a novel heteroplasmic mutation (G611A) in the mitochondrial DNA tRNA phenylalanine gene. The mutation was heteroplasmic (91%) in muscle but undetectable in accessible tissues from the patient and her maternal relatives. Single-fiber PCR analysis showed that the proportion of mutant genomes was higher in cytochrome c oxidase (COX)-negative ragged red fibers (RRFs) than in COX-positive non-RRFs. This report shows that typical MERRF syndrome is not always associated with tRNA lysine mutations.

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More than 120 point mutations in human mitochondrial DNA (mtDNA) genes have been associated with a wide spectrum of disorders that can affect virtually every tissue in the body, although brain and skeletal muscle are most frequently involved.[1]

Thirteen years ago, one of the most common encephalomyopathies, myoclonic epilepsy with ragged red fibers (MERRF), was attributed to a mutation (A8344G) in tRNA lysine (tRNA^{Lys}) gene of mtDNA.[2] Two rarer mutations in the same gene have also been associated with MERRF.[3]

We report a novel mutation in a different gene, tRNA phenylalanine (tRNA^{Phe}), in a patient with typical clinical, histologic, and biochemical features of MERRF.

Case reports. An Italian woman aged 42 years developed normally until age 11 years, when she started having panic attacks. In her 20s, she developed episodes of migraine and progressive limb myoclonus, manifesting as sudden finger flexion, arm abduction, and leg jerks. In her 30s, she also had exercise intolerance, loss of balance, and memory problems. Since age 37 years, she has had progressive sensorineural hearing loss. There was no family history of neuromuscular disorders. She has a healthy daughter aged 15 years.

On examination, she was short, with pes cavus, and bilateral hearing loss. Her upgaze was partially restricted, but lateral eye movements and downgaze were preserved, and she had no ptosis. Funduscopy was normal. Motor examination showed slight spasticity of the legs and mild weakness of neck flexors and quadriceps against resistance. She had frequent myoclonic jerks. There were no signs or symptoms of peripheral neuropathy. Plantar reflex was extensor bilaterally, and there was bilateral clonus of the patella and ankle. She had cerebellar signs, including dysmetria and tremor on finger-to-nose and heel-to-shin tests, dysarthria, and mild hand tremor. She had poor balance with tandem gait and a positive Romberg sign.

Blood and urine routine assays were normal. Resting blood lactate was increased (3.12 mmol/L; normal, 0.45 to 2.45). EKG and echocardiography were normal. EEG showed episodic generalized spikes and spike and wave complexes with background slowing.

Nerve conduction velocities were normal, and EMG showed mild myopathic features. Cognitive functions were mildly impaired based on the Mini-Mental State Examination score (23/30). Brain MRI with T1-weighted sequences showed moderate cerebral and more severe cerebellar atrophy. MR spectroscopy showed a decreased choline peak but no lactate peak.

Methods. *Histochemical and biochemical studies.* Histochemical and biochemical studies of frozen muscle sections were carried out as described previously.[4,5]

Genetic studies. Southern blot analysis and quantitative determination of mtDNA were performed as described previously.[6] A series of PCRs amplified fragments of muscle DNA encompassing all the tRNA genes, which were sequenced directly in an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) following standard procedures.

The presence of the mutation was confirmed by PCR-restriction fragment length polymorphism (RFLP) analysis. The DNA was amplified using the following primers: forward mismatched, 5'-CCTCCTCAAAGCAATACATT-3' and reverse mismatched, 5'-TAAGCGTTTTGAGCTGCATT-3'. PCR conditions were 94 °C for 3 minutes, followed by 35 cycles of 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1 minute, and a final extension step at 72 °C for 7 minutes. Aliquots of PCR products were digested with *Mse*I restriction endonuclease and electrophoresed on a 12% nondenaturing acrylamide gel, which was analyzed in a phosphorimager (Bio-Rad, Hercules, CA) to quantitate the percentage of the mutation.

Single-fiber PCR assay was performed in the proband as described using 30-μm-thick muscle sections.[7]

Results. *Histochemical and biochemical studies.* Histochemical examination of the skeletal muscle biopsy showed abundant (23%) ragged red fibers (RRFs), all of which were intensely succinate dehydrogenase (SDH) positive (figure 1A), together with strongly SDH-reactive blood vessels (SSVs; see figure 1A). There were also numerous cytochrome *c* oxidase (COX)-negative fibers (27%) and COX-negative blood vessels (figure 1B). Although RRFs and SSVs are also seen in biopsies from patients with mito-

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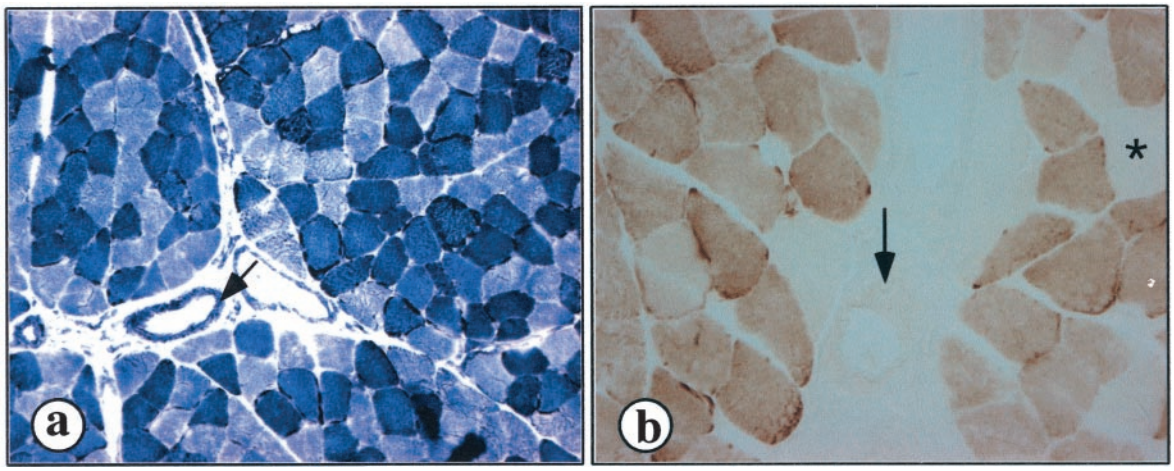

Figure 1. Muscle histochemistry. (A) Succinate dehydrogenase (SDH) stain shows mitochondrial proliferation ("ragged-blue" fibers) and strongly SDH-reactive vessels (SSVs; arrow). (B) Cytochrome c *oxidase (COX) stain shows reduced enzyme activity in individual fibers (asterisk) and a COX-negative SSV (arrow); ×20.*

chondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS), these are typically COX positive in patients with MELAS, whereas they are COX negative in those with MERRF, including our patient.[3] Activities of mitochondrial respiratory chain complexes I, III, and IV were markedly reduced in the patient's muscle extract (25 to 40% of mean control values; data not shown).

Genetic studies. Southern blot analysis excluded the presence of large-scale rearrangements. Sequence analysis of the PCR products showed a heteroplasmic guanine (G)-to-adenine (A) mutation at nucleotide 661 (G661A) in the $tRNA^{Phe}$ gene (figure 2A).[8] The mutation, which was confirmed by RFLP analysis (figure 2B), was abundant in muscle (91%) but undetectable in the patient's blood, urinary sediment, cultured skin fibroblast, and buccal smear. The mutation was also undetectable in blood, buccal smear, and urinary sediment from the patient's mother, brother, and daughter and in blood cells from 100 normal individuals. Single-fiber PCR analysis showed that the proportion of mutant mtDNAs was 93.3 ± 4.0% in 10 COX-negative RRFs and 17.4 ± 7.3% in 10 COX-positive non-RRFs.

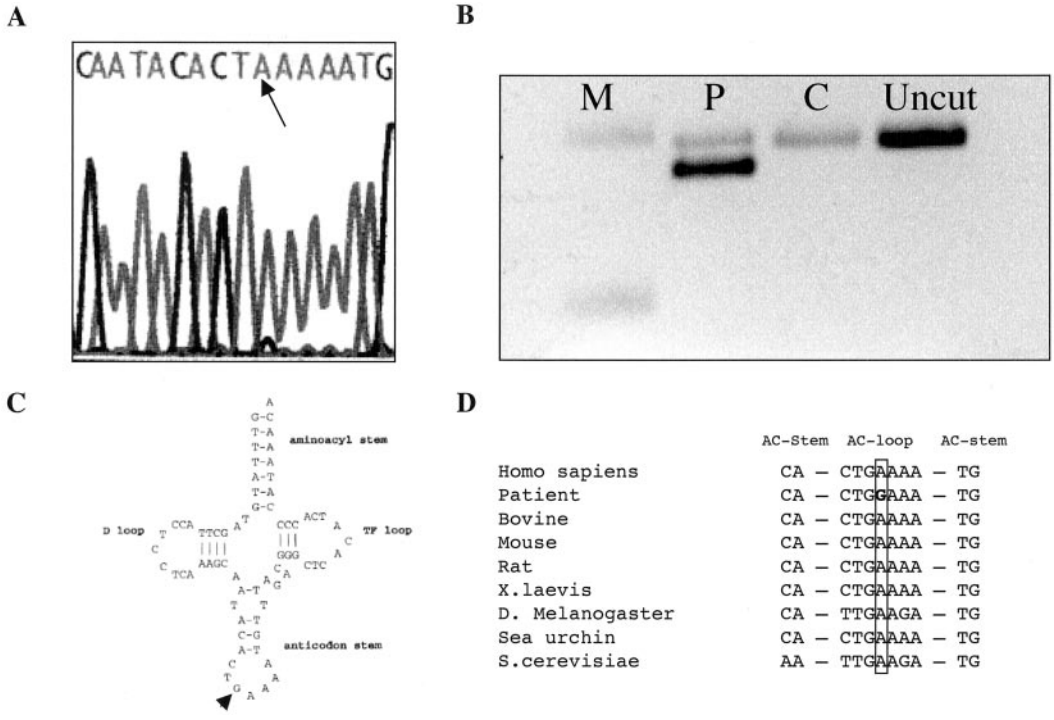

Figure 2. (A) Electropherogram of the mitochondrial DNA region encompassing the G611A mutation (arrow). (B) PCR-restriction fragment length polymorphism analysis of the G611A mutation. M = molecular marker; P = patient's muscle; and C = control muscle. (C) Proposed secondary structure of the human mitochondrial tRNA phenylalanine ($tRNA^{Phe}$) gene (the arrow indicates the G→A change). (D) Comparison of mitochondrial $tRNA^{Phe}$ among several species. The 611-nucleotide is boxed.

Discussion. We describe a novel G>A mutation in the mtDNA $tRNA^{Phe}$ gene of a woman with all canonical features of MERRF, myoclonic epilepsy, hearing loss, exercise intolerance, lactic acidosis, and RRFs. She also had other symptoms and signs frequently associated with MERRF, including ataxia, short stature, pes cavus, pyramidal signs, and mild ophthalmoparesis.[3]

Virtually all patients with typical MERRF described to date have had mutations in the $tRNA^{Lys}$ gene: the A8344G mutation is found in 80 to 90% of patients with MERRF, and two other mutations, T8356C and G8363A, account for most of the remaining cases.[3]

All reported MERRF-like syndromes caused by mutations in different tRNA genes overlap with other mitochondrial encephalomyopathies, such as Kearns–Sayre syndrome[9] or MELAS.[8]

Ours appears to be the first case of typical MERRF not associated with mutation in the $tRNA^{Lys}$ gene.

We consider the G611A mutation pathogenic for several reasons. First, it is consistent with the clinical presentation, the histochemical finding of COX-negative RRFs, and the biochemical observation of multiple defects involving respiratory chain complexes containing mtDNA-encoded subunits. Second, the mutation disrupts a highly conserved base pair in the anticodon stem of $tRNA^{Phe}$ (figure 2, C and D), which is likely to alter the double helix of the stem and affect the stability of the tRNA secondary structure. Third, the mutation is heteroplasmic, and heteroplasmy is a common marker of pathogenic mtDNA mutations. Fourth, the correlation between abundance of mutant mtDNA, mitochondrial proliferation (RRF), and COX negativity shown by single-fiber PCR suggests that the mutation is responsible for the abnormal phenotype observed in muscle. Finally, the mutation was absent in muscle from 100 control subjects.

Although the mitochondrial $tRNA^{Lys}$ should still be considered the "MERRF locus," other tRNA genes ought to be screened in the rare patients with clinical features of MERRF but without mutations in the $tRNA^{Lys}$ gene.

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