A New Mitochondrial Transfer RNA<sub>Pro</sub> Gene Mutation Associated With Myoclonic Epilepsy With Ragged-Red Fibers and Other Neurological Features

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Background: Pathogenic mutations of the human mitochondrial genome are associated with well-characterized, progressive neurological syndromes, with mutations in the transfer RNA genes being particularly prominent.

Objective: To describe a novel mitochondrial transfer RNA<sub>Pro</sub> gene mutation in a woman with a myoclonic epilepsy with ragged-red fibers–like disease.

Design, Setting, and Patient: Case report of a 49-year-old woman presenting with a myoclonic epilepsy with ragged-red fibers–like disease comprising myoclonic jerks, cerebellar ataxia, and proximal muscle weakness.

Results: Histochemical analysis of a muscle biopsy revealed numerous cytochrome-c oxidase-deficient, ragged-red fibers, while biochemical studies indicated decreased activity of respiratory chain complex I. Molecular investigation of mitochondrial DNA revealed a new heteroplasmic mutation in the T<sub>H</sub>9274C stem of the mitochondrial transfer RNA<sub>Pro</sub> gene that segregated with cytochrome-c oxidase deficiency in single muscle fibers.

Conclusions: Our case serves to illustrate the ever-evolving phenotypic spectrum of mitochondrial DNA disease and the importance of performing comprehensive mitochondrial genetic studies in the absence of common mitochondrial DNA mutations.

Arch Neurol. 2009;66(3):399-402

MITOCHONDRIAL DNA (mtDNA) mutations are associated with an impressive spectrum of clinical presentations ranging from severe encephalopathy in the neonatal period to late-onset progressive external ophthalmoplegia and exercise intolerance in adults. The syndrome of myoclonic epilepsy with ragged-red fibers (MERRF) is one of the most common clinical phenotypes associated with mtDNA mutation, with most patients harboring a specific, maternally inherited point mutation (m.8344A>G) in the transfer RNA<sub>Lys</sub> (tRNA<sub>Lys</sub>) gene. Other MERRF mutations have been identified in both this gene (eg, m.8356T>H11022C<sup>3</sup>) and m.8363G>A<sup>4</sup>) and other mitochondrial tRNA genes,<sup>2</sup> but unlike mutations causing mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes that exhibit considerable genetic heterogeneity, these appear to be restricted to tRNA genes.

Here we describe a patient with clinical and diagnostic features of MERRF and additional neurological complications due to a novel mutation (m.15967G>A) in the mitochondrial-encoded tRNA<sub>Pro</sub> gene that may have arisen sporadically in the patient.

METHODS

PATIENT

A 49-year-old woman presented with a 2-year history of progressive loss of balance and weakness of her arms. At age 27 years, she developed myoclonic jerks and generalized seizures that showed excellent response to sodium valproate. At age 37 years, she developed acute bilateral hearing loss. At age 47 years, she underwent bilateral cataract surgery. Her mother has type 2 diabetes mellitus and her maternal grandmother had type 1 diabetes mellitus. The youngest of her 3 sisters has learning disability and migraine; the other 2 sisters are healthy. She has 3 children, 2 of whom are healthy and 1 of whom has severe dyslexia. All of the 3 children later declined genetic testing.
On examination, she was found to have a Mini-Mental State Examination score of 24 out of 30. She had bilateral deafness, and some pigmentary change was noted in her retina. Her speech was dysarthric. She had proximal muscle weakness and her reflexes were either absent or attenuated. Cerebellar ataxia was demonstrated in all of her limbs and her gait was ataxic. All of her modalities of sensation were normal.

Visual evoked responses were normal. Electroencephalography showed a burst of high-amplitude, 4- to 5-Hz, multiple spike and wave activity with left-sided emphasis. Nerve conduction study results were normal. Mild neurogenic change was noted on electromyography. Her audiogram revealed bilateral sensorineural hearing loss. Brain magnetic resonance imaging showed extensive, high signal change in the cerebral white matter, basal ganglia, and thalami as well as cerebral and cerebellar atrophy (Figure 1). Creatine kinase level, renal and hepatic profiles, thyroid function, vitamin B12 and folate levels, autoantibody screen results, serum immunoglobulin level, serum lactate level, full blood cell count, and syphilis serology results were all either normal or negative. Examination results of the cerebrospinal fluid were unremarkable.

A screen of the m.3243A>G and m.8344A>G mtDNA mutations in blood revealed no abnormalities, prompting muscle biopsy for further investigations.

ANALYSIS

Standard histological and histochemical analyses of quadriceps muscle biopsy were performed on fresh frozen sections (10 µm), while the activities of the respiratory chain complexes and the matrix marker citrate synthase were determined as previously described. Total DNA was extracted from several tissues, including blood and muscle, by standard procedures. Mitochondrial DNA rearrangements were investigated in muscle DNA prior to direct sequencing of the entire mitochondrial genome.

To determine the level of heteroplasmy of a novel m.15967G>A variant, we designed a polymerase chain reaction (PCR)–restriction fragment length polymorphism assay as follows: a 131–base pair (bp) PCR product spanning the mutation site was amplified using a forward primer (nucleotides 15879-15897) and an M-13 tailed (lowercase) reverse mismatch primer (nucleotides H15991-15986), 5’-caggacagcagctatgaccGGGTGC-TAATGGTGGAGTTA-3’, with the single mismatch nucleotide shown in bold. In combination with the m.15967G>A mutation, this serves to introduce a novel Alw261 restriction site, permitting the detection of mutated mtDNA that is cut to 2 smaller bands (94 bp and 37 bp). Prior to the last cycle of PCR, 5 µCi of [α-32p]-labeled deoxycytidine triphosphate (3000 Ci/mmol) was added (to convert microcuries to becquerels, multiply by 37 000; to convert curies per millimole to becquerels per millimole, multiply by 3.7 × 1010). Labeled products were precipitated, digested with 10 U of Alw261, and separated through a 12% non-denaturing polyacrylamide gel, and the radioactivity in each fragment was quantified using ImageQuant software (GE Healthcare, Chalfont St Giles, England).

Enzyme histochemistry of the proband’s muscle biopsy revealed a significant number of fibers (>10%) exhibit-
Pathogenic mtDNA mutations can manifest as distinct clinical syndromes, but sometimes patients may present with additional clinical features that can complicate the diagnosis. Our patient presented with the clinical features of MERRF, including a long-standing history of myoclonic epilepsy. Additionally, she presented with other clinical features (proximal myopathy, sensorineural deafness, cerebellar ataxia, and pigmentary retinopathy) that are typical in patients with mitochondrial genetic disease. Muscle biopsy revealed histochemical and biochemical abnormalities indicative of mitochondrial involvement, and once common mtDNA mutations were excluded, we undertook detailed molecular genetic investigation of the mitochondrial genome, which identified the causative mutation.

The pathogenicity of the m.15967G>A tRNA\textsuperscript{Pro} mutation is suggested based on its fulfillment of established canonical criteria.\textsuperscript{7,8} First, it is not a recognized neutral polymorphic variant as the mutation was not represented in either of the large, publicly available databases of human mtDNA sequences.\textsuperscript{9,10} Second, it was clearly heteroplasmic and present at higher levels in postmitotic skeletal muscle than in mitotic cells such as urinary epithelial cells (Figure 2C). Third, single-muscle-fiber PCR–restriction fragment length polymorphism analysis clearly demonstrated that the mutation segregates with COX-deficient fibers (Figure 2D). The highest level of mutated mtDNA detected in any of the COX-positive fibers was 90%, implying a high biochemical threshold for the m.15967G>A mutation. Fourth, the mutation affects a nucleotide that, although showing poor phylogenetic conservation within the tRNA\textsuperscript{Pro} structure, is predicted to disrupt a highly conserved Watson-Crick base pair (Figure 3).

Surprisingly, although more than 120 different pathogenic mitochondrial tRNA gene mutations have now been described, mutations in the tRNA\textsuperscript{Pro} gene have been described in only 3 previous patients. The m.15990C>T anticodon swap mutation was identified in a young girl with pure myopathy,\textsuperscript{12} and the m.16002A>G mutation was described in an adult patient with muscle weakness.
and exercise intolerance. The other patient presented with mild fatigability, dysphagia, and unsteady gait due to 2 heteroplasmic mtDNA mutations, including m.15995G>A in tRNAPro.14

A further interesting aspect of this tRNAPro mutation is that it was not detected in tissues (urinary epithelial cells and blood) from either the patient’s mother or her 2 clinically unaffected siblings, suggesting that the m.15967G>A mutation arose in the germline as a de novo mutational event. It is conceivable that the mutation has been transmitted and is present at detectable levels in post-mitotic muscle but not in rapidly dividing cells where deleterious mitochondrial tRNA mutations may be actively selected against. Recent data from 2 mouse models have indicated evidence of a strong purifying selection against the transmission of pathogenic mtDNA mutations at the level of the female germline.16,17 Although the overwhelming majority of disease-associated tRNA mutations are maternally inherited, examples of sporadic, nontransmissible tRNA point mutations are documented.18,19 This demonstrates the importance of performing extensive family studies in newly diagnosed patients to assess the risk of transmission from mothers to their offspring and in doing so providing accurate and informative genetic counseling.

Accepted for Publication: September 9, 2008.

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Financial Disclosure: None reported.

Funding/Support: This work was supported by the Wellcome Trust, the Newcastle upon Tyne Hospitals NHS Trust, and the UK National Commissioning Group for Rare Mitochondrial Disorders of Adults and Children (Drs Turnbull and Taylor).

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