Novel Mitochondrial Transfer RNA\textsuperscript{Phe} Gene Mutation Associated With Late-Onset Neuromuscular Disease

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Background: An extensive range of molecular defects have been identified in the human mitochondrial genome (mitochondrial DNA); many are associated with well-characterized, progressive neurological syndromes, but a minority of patients have uncharacteristic phenotypes in which symptoms may be relatively mild.

Objective: To describe a novel transfer RNA\textsuperscript{Phe} mutation of mitochondrial DNA in a late-onset case with a mild phenotype of mitochondrial disease.

Design: Case report.

Patient: A 66-year-old woman presented with a 4-year history of walking difficulties due to exercise intolerance and paresthesia in the feet. Clinical examination results were normal. Her deceased mother had similar walking difficulties, but her sister and 2 children were unaffected.

Results: The demonstration of a marked histochemical defect in cytochrome c oxidase activity on muscle biopsy prompted molecular investigation of mitochondrial DNA, revealing a novel maternally inherited mutation in the variable loop of the mitochondrial transfer RNA\textsuperscript{Phe} gene. This 622G\textsuperscript{H}A transition was heteroplasmic and segregated with cytochrome c oxidase deficiency in single fibers.

Conclusion: This case serves to illustrate that primary defects of the mitochondrial genome should be considered even in older patients with late-onset, mild neuromuscular symptoms.

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Mitochondrial DNA (mtDNA) mutations are important causes of human genetic diseases and are associated with an impressive spectrum of different clinical presentations. Many patients present with severe neurological syndromes such as Kearns-Sayre syndrome or MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes) but there can be widespread clinical and genetic heterogeneity involving a number of organ systems.\textsuperscript{1,2} There are also patients who present with mild phenotypes such as CPEO (chronic progressive external ophthalmoplegia) or patients where exercise intolerance is the predominant feature and onset has occurred in childhood. In this latter group of patients, mtDNA mutations have been detected not only in genes encoding protein subunits of complex I, III, and IV,\textsuperscript{3} but also in mitochondrial transfer RNA (tRNA)\textsuperscript{4,5} genes, including tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Gly}\textsuperscript{4,5}

Here, we report on a patient with a mild myopathy and peripheral neuropathy associated with a novel mutation at nucleotide position 622 (622G\textsuperscript{H}A) in the mitochondrial-encoded tRNA\textsuperscript{Phe} gene.

REPORT OF A CASE

A 66-year-old woman presented with a 4-year history of exercise intolerance and walking difficulties. Previously a keen hiker, fatigue rather than muscle pain had severely reduced her ability to walk long distances, a change from more than 20 km to less than 5 km. She complained of unsteady gait, weakness on climbing stairs, muscle cramps, and paresthesia in both feet ascending symmetrically up to the knees. More recently, she had noted a mild hearing impairment. There was no history of myoglobinuria, seizures, diabetes, or psychiatric problems. Her mother had also had walking difficulties in later life, but no other relatives appear to have been affected. She has 2 healthy children and a healthy sister.

General examination revealed systemic hypertension but was otherwise unremarkable. Neurologically, there was no evidence of muscle atrophy or weakness,
and sensory examination results, tone, deep tendon, and Babinski reflexes were all normal.

Needle electromyogram of the tibialis anterior muscle revealed only very mild neurogenic changes, and nerve conduction studies of the peroneal nerve were consistent with this finding, demonstrating a mild reduction in amplitude with no change in velocity. Sural nerve examination results were normal. An audiogram showed a mild sensorineural hearing impairment with a loss of up to 60 dB in higher frequencies. Resting lactate levels were normal but increased rapidly after gentle exercise to 11 mmol/L (normal, <2.2 mmol/L), suggesting a mitochondrial abnormality. Creatine kinase, vitamin B12, and folate acid levels; full blood cell count results; and erythrocyte sedimentation rate were normal, as were test results for thyroid, renal, and liver function. Examination results of cerebrospinal fluid were unremarkable.

### METHODS

Standard histological and histochemical analyses were performed on frozen sections (10 µm) of biceps brachii muscle. Additionally, electron microscopy was performed. The activities of the respiratory chain complexes and the matrix marker citrate synthase were determined as previously described.6

Total DNA was extracted from several tissues by standard procedures. Rearrangements of mtDNA were investigated by Southern-blot analysis and long-range polymerase chain reaction (PCR) in muscle DNA as described.7 Sequencing of the entire mitochondrial genome was performed using muscle DNA as described elsewhere.8

To determine the level of heteroplasmy, a 235-base pair PCR product spanning the mutation site was amplified using a forward primer (nucleotides 581-602) and a reverse mismatch primer (nucleotides 797-773) 5′-CTAGGCTAAGCGTTTTAATCTGCA-3′ with the mismatch nucleotides shown in bold. The 622G>A mutation introduces a new DraI restriction site into the PCR product. Because of the mismatches in the reverse primer, a further DraI restriction site is created, which cuts wild-type mtDNA. Prior to the last cycle of PCR, 5 µCi (185 Bq) [α-32P]dCTP (3000 Ci/mmol) was added. Labeled products were precipitated, digested with 10 U DraI, separated through a 10% nondeaturing polyacrylamide gel, and the radioactivity in each fragment quantified using ImageQuant software (Amersham Biosciences/GE Healthcare, Little Chalfont, United Kingdom).

### RESULTS

Histological examination of the proband’s muscle biopsy showed several atrophic fibers. Remarkably, histochemistry revealed a marked number (>35%) of cytochrome c oxidase (COX)–deficient fibers (Figure 1A), with many of these (15%) showing evidence of subsarcolemmal mitochondrial proliferation. Electron microscopy revealed enlarged mitochondria with paracrystalline inclusions (Figure 1B). Respiratory chain enzyme analysis of a skeletal muscle homogenate showed apparent normal absolute values of respiratory chain complexes but a marked increase in the activity of both citrate synthase (149%) and succinate dehydrogenase (143%). When respiratory chain complex activities were expressed relative to the activity of citrate synthase, the activities of complex I (50%), complex III (68%), and complex IV (55%) were shown to be decreased as compared with the lower normal value of controls.

Southern-blot analysis excluded the presence of large-scale mtDNA rearrangements in the patient’s muscle DNA. Direct sequencing of the entire mitochondrial genome revealed an unreported 622G>A transition in the mitochondrial tRNA^the gene (Figure 2A). Polymerase chain reaction–restriction fragment length polymorphism analysis showed that the mutation was heteroplasmic and present at the highest level in the patient’s muscle (88% mutant load) with lower levels in hair shafts (70%), urinary epithelia (66%), buccal epithelia (63%), and blood (36%) (Figure 2B). Analysis of several tissues from her asymptomatic daughter revealed low levels of the mutation (10%-20%), confirming maternal transmission.

Single muscle fiber analysis was performed to determine whether the amount of mutated mtDNA correlated with the observed biochemical phenotype in individual cells. We detected substantially higher levels of the 622G>A mutation in COX-deficient fibers (mean±SD, 93 ± 1.0%, n = 11) than in the COX-positive fibers (mean±SD, 82 ± 8.5%, n = 14) (P<.001, 2-tailed t test), confirming segregation of the 622A genotype with respiratory chain dysfunction (Figure 2C).
Exercise intolerance is not only a typical symptom of mitochondrial disease but is observed in many other neurological and age-related disorders. Additionally, our patient showed other symptoms (neuropathy with mild signs of axonal damage and hearing impairment) that are frequently observed in older patients but which may also be a feature of mitochondrial disease.\textsuperscript{1,2} Our patient’s case was remarkable not just because of the late onset of her mitochondrial disease, but also because of the striking disparity between the mild clinical presentation and the marked histochemical changes, which prompted a detailed molecular genetic investigation of the mitochondrial genome to identify the causative mutation.

The pathogenicity of the 622G\textgreater{}A transition is proposed by the following canonical criteria.\textsuperscript{1,9} It is not a recognized neutral polymorphic variant because the mutation was not represented in 2 databases of human mtDNA sequences (Neil Howell, oral communication, October 2005; http://www.genpat.uu.se/mtDB/index.html); it was heteroplasmic and present at higher levels in postmitotic skeletal muscle than mitotic cells (Figure 2B); single muscle fiber analysis clearly demonstrated that the mutation segregates with COX-deficient fibers (Figure 2C); and the mutation affects a nucleotide that is phylogenetically conserved within the tRNAPhe structure (http://mamit-trna.u-strasbg.fr) located in the variable loop (Figure 3).

Although the overwhelming majority of pathogenic mitochondrial tRNA mutations occur in the stem structures, disrupting Watson-Crick base pairing,\textsuperscript{9} there are exceptions; a mutation in the variable loop of the tRNA\textsuperscript{Val} is associated with adult-onset Leigh syndrome,\textsuperscript{10} and both the common 3243A\textgreater{}G MELAS and 8344A\textgreater{}G MERRF (myoclonic epilepsy with ragged-red fibers) point mutations occur within loop structures of their respective tRNA molecules. Finally, the biochemical analysis of complex activities revealed a moderate combined defect of complex I, III, and IV, a finding entirely consistent with a tRNA mutation disrupting mitochondrial protein synthesis.

Five other pathogenic mtDNA mutations have previously been described in the tRNA\textsuperscript{Phe} gene associated with classical mitochondrial syndromes such as MELAS and MERRF but also with phenotypes predominantly affecting muscle. However, in these patients, severe muscle weakness was noted on clinical examination.\textsuperscript{11,12}
Given their maternal transmission, identifying disease-causing point mutations within mtDNA has significant consequences for other family members. Her clinically unaffected daughter, the only maternal relative to agree to be tested, has mutation levels in several tissues below the threshold to cause symptoms. Although we do not know the level of mutation in muscle, there is still a risk that she may be affected in later life given the late onset of symptoms in her mother and that pathogenic, mtDNA tRNA point mutations can accumulate over time, even in postmitotic muscle. 13

In conclusion, our case highlights the importance of including mitochondrial DNA disorders as a possible differential diagnosis even in patients with late-onset, mild neuromuscular symptoms (myopathy and peripheral neuropathy) who do not present with a classical mitochondrial syndrome.

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References