A New Mitochondrial Point Mutation in the Transfer RNA<sup>Leu</sup> Gene in a Patient With a Clinical Phenotype Resembling Kearns-Sayre Syndrome

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Objective: To report on the molecular identification of a novel heteroplasmic G-to-A transition at mitochondrial DNA position 3249 in transfer RNA<sup>Leu</sup> gene in a patient with a clinical phenotype resembling Kearns-Sayre syndrome.

Patient and Methods: A 34-year-old patient had been suffering for more than 10 years from progressive visual failure, neurosensory hearing loss, exercise intolerance, muscle weakness, paresthesia in the lower limbs, and difficulties swallowing. Clinical examination revealed generalized muscle wasting, ptosis, external ophthalmoplegia, and ataxia. Ophthalmologic examination showed dystrophic features in the cornea and retina. In skeletal muscle, morphologic and biochemical studies of the respiratory chain complexes were performed. Polymerase chain reaction, single-strand conformation polymorphism, and direct sequencing were used to screen for mutations in the 22 mitochondrial transfer RNA genes.

Results: In skeletal muscle, a significantly decreased catalytic activity of complex I was detected by spectrophotometric analysis and numerous cytochrome c oxidase–negative ragged-red fibers were seen on morphologic examination. A G-to-A substitution 3249 (G3249A) mutation was found in the transfer RNA<sup>Leu</sup> gene of the patient and mutant mitochondrial DNA represented 85% of the total in skeletal muscle but only 45% in leukocytes. The mutation was shown to be present in a small fraction in leukocytes from the unaffected mother and to be absent in leukocytes from the healthy sister.

Conclusions: A causal relationship between a heteroplasmic G3249A transfer RNA<sup>Leu</sup> mutation in a patient suffering from progressive external ophthalmoplegia, retinal dystrophy, ataxia, neurosensory hearing loss, and muscle wasting is postulated. To our knowledge, the G3249A mutation has never previously been described and was not detected in control subjects.

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Mitochondrial DNA (mtDNA) is a multi-copy, double-stranded, circular, extranuclear genome encoding 13 proteins, 2 ribosomal RNA, and 22 transfer RNA (tRNA) species, all of which are essential for oxidative phosphorylation. During the past few years, several mtDNA mutations have been identified and shown to be associated with a wide range of human neurologic diseases. Mitochondrial diseases are most often multisystem disorders with a clinical variability often related to the phenomenon of heteroplasmy, defined as a variable mixture of mutant and wild-type mtDNA molecules in the same cell. Phenotypic expression of mitochondrial cytopathies, therefore, is most likely to occur in tissues with high-energy requirements. The central nervous system, as well as skeletal muscle, heart muscle, the kidney, and liver are at highest risk. Mitochondrial DNA lesions range from large-scale rearrangements such as deletions and duplications to single-nucleotide substitutions. To date, more than 70 pathogenic point mutations have been identified in mtDNA, mostly located in tRNA genes.

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In this article, we describe a patient with clinical signs suggestive of a mitochondrial disease resembling Kearns-Sayre syndrome, in whom a novel mutation in a tRNA gene was detected using single-strand conformation polymorphism, a technique commonly used when screening for point mutations in genes, and mtDNA sequence analysis.
The propositus was born as the first child to healthy non-consanguineous parents. The family history was unremarkable, except for diabetes mellitus in the paternal grandmother. He has 2 healthy sons, aged 7 and 4 years. The proband’s mother and sister are in good health. Symptoms were first noticed in the propositus when he was near the age of 22 years. He began to suffer from hearing loss and visual problems. He had the feeling of an uncomfortable sensation in the left eye worse than the right eye) due to enophthalmitis pigmentosa (NARP)/Leigh (T8993C/G) syndrome and neuropathy, ataxia, and retinal dystrophy (MERRF) (A8344G), nor neuropathy, ataxia, and retinal dystrophy. Audiometry demonstrated significant bilateral neurosensory hearing loss. A loss of 50 to 70 dB for low- to high-frequency sounds was detected on the right side, and 40 to 80 dB on the left side. Electrocardiogram, 24-hour electrocardiogram registration, and ultrasonography of the heart were normal. Electromyogram of the deltoid muscles showed bilateral polyphasic potentials with low amplitudes suggestive of myopathy. Amplitudes of motor action potentials were normal, as were motor nerve conduction velocities. Results of an aerobic exercise test revealed low tolerance to physical effort and a low anaerobic threshold. Blood lactate level was 3.2 mmol/L at 40 W increasing to 5.3 mmol/L at 80 W and 8.5 mmol/L at 100 W. Magnetic resonance imaging of the brain showed atrophy of the cerebral cortex and cerebellum, and, on T2-weighted slices, bilaterally a hypointense aspect of the globus pallidus, substantia nigra, and red nucleus (Figure 1).

At age 33 years, he was examined by one of us (R.V.C.). He was cooperative and answered questions adequately. His face was long and sharp with bilateral ptosis, mild external ophthalmoplegia, and lack of facial expression. He was very thin owing to generalized muscular atrophy. His weight was 36 kg, his height 164 cm, and his head circumference 51 cm. Muscle strength was reduced especially in the legs. His gait was severely ataxic. Dystonia was detected bilaterally. Tendon reflexes and plantar responses were normal. Routine blood study results, including complete blood cell count, transaminase levels, creatinine level, and creatinine kinase level, and lipoprotein profile, were normal. Serum lactate and pyruvate concentrations measured on several occasions during the course of 1 day were only minimally increased. Maximum lactate concentration was 2.2 mmol/L (reference range, <2.0 mM) and the pyruvate level was 0.17 mmol/L (reference range, <0.14 mmol/L). Results of an amino acid profile in serum and urine, the acylcarnitine levels, and very long-chain fatty acids and phytanic acid concentrations were normal. Urinary organic acid profile results were normal.

A skeletal muscle biopsy was performed. Morphologic examination showed the presence of numerous ragged-red fibers on modified Gomori trichrome staining. Increased staining for nicotinamide adenine dinucleotide–tetrazolium reductase stain (Figure 2) and decreased staining for cytochrome c oxidase contrasted with the significantly decreased staining for cytochrome c oxidase in the ragged-red fibers. Electronmicroscopic examination revealed increased numbers of enlarged mitochondria with abnormal cristae and type I paracrystalline inclusions.

In skeletal muscle, the activity of complex I was significantly decreased. The activities of complexes II, III, and IV were normal (Table 1). Neither gross rearrangements nor any of the common point mutations associated with classic mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (A3243G), myoclonic epilepsy and ragged-red fibers (MERRF) (A8344G), nor neuropathy, ataxia, and retinitis pigmentosa (NARP)/Leigh (T8993C/G) syn-
dromes were found in our patient. A screening of all mitochondrial tRNA genes was performed. Single-strand conformation polymorphism analysis revealed a novel tRNA mutation: a G-to-A transition at position 3249 (Figure 3). The mutation was heteroplasmic. It was located in the tRNA gene for leucine and was not found in more than 100 normal control samples.

The mutation creates an NciI restriction site and PCR-restriction fragment length polymorphism analysis with NciI was used to quantitate the proportion of mutant mtDNA in the patient and his maternal relatives. Restriction fragment length polymorphism analysis of PCR fragments amplified from mtDNA extracted from muscle and leukocytes showed that the transition at 3249 was heteroplasmic, with 45% of the mutant molecules in leukocytes and 85% in skeletal muscle. The patient had inherited the mutation from his mother. She carried less than 5% mutant mtDNA molecules in the blood. In the patient’s sister the mutation was not detectable.

**COMMENT**

We report the identification of a novel mtDNA mutation located in the tRNA<sup>Leu</sup> gene. The patient suffered from neurosensory hearing loss; progressive external ophthalmoplegia; retinopathy, ataxia, and generalized muscle atrophy; and difficulties swallowing. The finding of progressive external ophthalmoplegia in a patient as part of
a multisystemic syndrome suggested an underlying mitochondrial defect. The initial diagnosis based on these clinical findings was Kearns-Sayre syndrome, but neither large-scale mtDNA deletions nor rearrangements were detected by molecular analysis. Previous reports have shown that patients with point mutations in mitochondrial tRNA genes, including the MELAS (A3243G) mutation in tRNA\[^{Leu}\] can also present with progressive external ophthalmoplegia (Table 2). Signs of retinopathy are an almost constant finding in Kearns-Sayre syndrome and NARP (neuropathy, ataxia, and retinitis pigmentosa) but can be seen in patients with MELAS and MERRF, although less frequently. Corneal transparency in our patient was decreased owing to stromal edema and possibly endothelial cell dysfunction. Corneal cells are normally metabolically active. Stromal lactate accumulation, localized acidosis, and increased osmotic solute load can account for corneal edema. To our knowledge, corneal dystrophy has never been reported in patients with mitochondrial defects until now. Cerebellar atrophy was the most obvious magnetic resonance imaging abnormality in the proband, which is not an unusual finding in patients with Kearns-Sayre syndrome and in patients with advanced stages of MELAS. Infarctlike lesions suggestive of MELAS were not detected in the proband.

In skeletal muscle from the patient, numerous cytochrome c oxidase–negative ragged-red fibers were observed. In patients with mtDNA defects affecting protein synthesis, including mtDNA deletions, mtDNA depletion, or tRNA mutations, ragged-red fibers are cytochrome c oxidase–negative. In the skeletal muscle from the proband, however, mtDNA deletions and mtDNA depletion were excluded by molecular analysis. Cytochrome c oxidase–negative ragged-red fibers can also be found in mtDNA mutations located in the cytochrome c oxidase I, II, or III genes, but spectrophotometrical analysis in the proband showed normal cytochrome c oxidase activity in isolated mitochondria. Taking these findings into consideration, the most likely hypothesis was a mutation in a tRNA gene in the proband.

The classic MELAS (A3243G), MERRF (A8344G), and NARP/MILS (maternally inherited Leigh syndrome) (T8993C/G) mutations were absent in the proband. In contrast using PCR, single-strand conformation polymorphism, and direct sequence analysis, a G3249A mutation in tRNA\[^{Leu}\] was detected. Sufficient evidence can be found to substantiate the causative role of this mutation. The base change was heteroplasmic, a common feature of pathogenic mtDNA mutations. Moreover, the mutation was represented in higher proportions in skeletal muscle than in leukocytes. The nucleotide involved is evolutionarily well conserved\(^25\) and has not been observed in more than 100 control samples. The mutation was maternally inherited and associated with morphologic and biochemical abnormalities in skeletal muscle.

The G3249A mutation is located adjacent to a cluster of point mutations at nucleotides 3250, 3251, 3252, 3254, and 3256, all positioned in the dihydrouridine loop, or dihydrouridine stem of the tRNA\[^{Leu}\] gene.\(^{14,26-30}\) These mutations, as well as the classic A3243G (MELAS) mutation, are known to disrupt the tRNA function and are associated with a wide range of phenotypical presenta-

<table>
<thead>
<tr>
<th>Complex</th>
<th>Enzyme Activities, nmol/min per Milligram of Protein</th>
<th>Enzyme Activity–Citrate Synthase Activity Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls Mean (P(<em>5)-P(</em>{95}))(^3)</td>
</tr>
<tr>
<td>NADH-Q(_{\circ}) reductase</td>
<td>45</td>
<td>100 (38-265)</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>418</td>
<td>117 (72-326)</td>
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<tr>
<td>Succinate cytochrome c reductase</td>
<td>337</td>
<td>157 (81-334)</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>1829</td>
<td>799 (436-2073)</td>
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<tr>
<td>Citrate synthase</td>
<td>2647</td>
<td>1110 (428-1829)</td>
</tr>
</tbody>
</table>

\(^*\)P\(_5\)-P\(_{95}\) indicates fifth to 99th percentile; control population (n = 30) free of outliers.

\(^1\)NADH-Q\(_{\circ}\)Q indicates nicotinamide adenine dinucleotide–coenzyme Q.
tions such as MELAS, pure myopathy, cardiomyopathy, and encephalopathy. Until now, more than 10 different pathogenic mutations have been identified in the tRNALeu(UUR) gene.

Therefore, this gene represents a mutational hot spot in the mitochondrial genome.

CONCLUSIONS

We conclude that the G3249A mutation in tRNALeu should be tested for in patients with clinical symptoms suggestive of Kearns–Sayre syndrome in whom large-scale mtDNA deletions or rearrangements and classic MELAS and MERRF point mutations have been excluded. If negative, a thorough screening for mtDNA mutations, especially in the tRNA genes, is a worthwhile test. We would like to emphasize that it is impossible to predict that the G3249A mutation always presents with the Kearns–Sayre–like phenotype, since phenotypic variability of this point mutation cannot be assessed by this single patient and his asymptomatic mother.

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