Clinical and Molecular Features of Encephalomyopathy Due to the A3302G Mutation in the Mitochondrial tRNA<sub>Leu(UUR)</sub> Gene

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Background: The mitochondrial DNA mutation A3302G in the tRNA<sub>Leu(UUR)</sub> gene causes respiratory chain complex I deficiency. The main clinical feature appears to be a progressive mitochondrial myopathy with proximal muscle weakness.

Objective: To report on clinical and molecular features in 4 novel patients with the A3302G mutation.

Design: Case reports.

Patients: Four patients (3 of whom are from the same family) with a myopathy caused by the A3302G mitochondrial DNA mutation.

Main Outcome Measure: Identification of the A3302G mutation by DNA sequencing.

Pathogenic mutations in mitochondrial DNA affect at least 1 in 15 000 adults.1 Twenty pathogenic point mutations in the mitochondrial tRNA<sub>Leu(UUR)</sub> gene have been reported.2 The clinical manifestations in patients with mutations in this gene have varied significantly but have included 1 or more of the following symptoms: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; myopathy; diabetes mellitus; deafness; chronic progressive external ophthalmoplegia; cardiomyopathy; progressive encephalopathy; and Leber hereditary optic neuropathy.

The A3302G mutation has previously been reported in 4 patients.3-5 The mutation changes the highly conserved and penultimate nucleotide in the aminoacyl acceptor–stem structure of tRNA<sub>Leu(UUR)</sub>.5 The biochemical consequences of the A3302G mutation are a severe respiratory chain complex I deficiency and lowered complex IV activity. Clinically, the patients had progressive proximal myopathy, characterized by proximal muscle weakness. One patient died of cardiorespiratory failure.5 Herein, we report the clinical and biochemical findings in 4 additional patients with the A3302G mitochondrial DNA mutation. Three of the patients are from the same family and 1 is from an unrelated family. All 4 patients had a progressive mitochondrial myopathy with proximal muscle weakness.

Methods

The main features of patients with the A3302G mutation are shown in the Table.

Patient 1A

A 32-year-old man was initially seen with exercise intolerance. Previously a semiprofessional athlete, the exercise intolerance became apparent after resuming physical activity following a break of several years because of injury. Respiratory chain complex activities were measured in skeletal muscle homog-
Complex I activity was 7%, 5%, and 2% of control means relative to protein, citrate synthase, and complex II, respectively. Complex IV activity was 31%, 21%, and 11% of control means relative to protein, citrate synthase, and complex II, respectively. Complex II activity was elevated at 294% relative to protein and 189% relative to citrate synthase.

Patient 2A
A 31-year-old woman had scoliosis at 12 years of age, which was rectified surgically. She had long-standing fatigue and effort intolerance with palpitations. On examination, patient 2A was of slender build, with very little peripheral fat. Muscles were slim in her upper limbs. Power was normal in most groups except for the shoulder girdle and hip girdle where some muscles were 4+/5, and trunk flexion was 3+ of 5.

Patient 2B
This 26-year-old woman is the sister of patient 2A. She was seen with a 6-month history of muscle stiffness and soreness, particularly after even minimal exercise. There was a history of depression, and the patient was taking fluoxetine hydrochloride.
MOLECULAR STUDIES

Patient 1A

DNA was extracted from the skeletal muscle biopsy specimen. The nuclear-encoded exons of the Twinkle, PolG, and ANT1 genes were sequenced, but no pathogenic mutations were found. Sequencing identified the A3302G mutation in the tRNA_{Leu(UUR)} gene. The heteroplasmic level of the A3302G mutation was more than 95% in the skeletal muscle sample.

Patient 2A

The A3302G mutation was detected when sequencing the mitochondrial tRNA_{Leu(UUR)} gene. Quantitation was performed as described using a fluorescent-labeled primer. The mutant load in lymphocyte DNA was 18% and in buccal swab DNA, 37%.

Patient 2B

The mitochondrial tRNA_{Leu(UUR)} gene was sequenced in DNA extracted from blood. Based on peak heights on the chromatogram, we concluded that the level of the A3302G mutation was more than 80%.

Patient 2C

Mutant load was determined as described for patient 2A and found to be 17% in lymphocyte DNA.

COMMENT

We describe 4 patients—3 of whom are from the same family—with a myopathy caused by the A3302G mitochondrial DNA mutation. Inheritance of the myopathy in the family is consistent with a maternally inherited mitochondrial DNA mutation. Because patient 1A had progressive external ophthalmoplegia, we initially investigated the nuclear genes Twinkle, ANT1, and PolG. No mutations were detected in these genes.

The A3302G mutation was present at a high level (>95%) in a skeletal muscle biopsy specimen from patient 1A. The presence of ragged red fibers in patients 1A and 2B and the results of the biochemistry are consistent with findings in other patients with this mutation. The mutation was also detected in blood from patients 2A, 2B, and 2C, but at a lower level than in the muscle biopsy specimen from patient 1A. The A3302G mutation is located 2 nucleotides away from the 5’ terminal tRNA_{Leu(UUR)} nucleotide at position 3304. It has been suggested that the mutation change leads to abnormal processing of the 16S rRNA-tRNA_{Leu(UUR)}-ND1 precursor RNA. This is likely to lead to quantitative and/or qualitative changes in the ND1 messenger RNA, the result of which would explain the relatively severe complex I deficiency. The A3302G mutation could affect the addition of the CCA triplet to the 3’ terminus by the transfer RNA nucleotidyltransferase, although this mechanism was not supported by in vitro studies. It is also possible the mutation negatively affects the reaction carried out by aminoacyl–transfer RNA hydrolase and/or interferes with the correct aminoacylation of the transfer RNA. The observed clinical features might be the result of a combination of several of these mechanisms.

All 4 patients described herein have an adult-onset mitochondrial myopathy. Interestingly, in 2 unrelated patients, upper limb reflexes were absent, with preservation of at least some lower limb reflexes. Other features, such as ptosis, progressive external ophthalmoplegia, recurrent headache, and hearing loss, were also present. One patient, but not the 2 others from the same family, had mild dysmorphism. Depression, which has been reported in mitochondrial encephalomyopathies, was present in 2 patients from the same family. Ragged red fibers were observed in the available muscle biopsy specimens. While the dominant clinical features of the A3302G mutation in our patients were exercise intolerance and proximal muscle weakness, other features of mitochondrial encephalomyopathies such as hearing loss, recurrent headaches, ptosis, and progressive external ophthalmoplegia and depression were present. Two unrelated patients had reflex loss, predominantly in the arms. Thus, the phenotype associated with the A3302G mutation is more than a pure myopathy, and it is better regarded as an encephalomyopathy.

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REFERENCES


