Complex Neurologic Syndrome Associated With the G1606A Mutation of Mitochondrial DNA

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Objectives: To confirm the pathogenicity of the G-to-A substitution at nucleotide 1606 (G1606A) mutation in the mitochondrial DNA (mtDNA) tRNAVal gene, and to characterize genotype-phenotype correlation.

Patient and Methods: A 37-year-old man since childhood developed a complex clinical picture characterized by hearing loss, migraine, ataxia, seizures, cataracts, retinitis pigmentosa, mental deterioration, and hypothyroidism. Magnetic resonance imaging revealed diffuse calcification of the basal ganglia and cerebral cortical atrophy. Morphologic and biochemical studies of respiratory chain complexes were performed in skeletal muscle. All 22 mitochondrial tRNA genes were screened for mutations by direct sequencing.

Results: Biochemical analysis showed normal activities of respiratory chain enzymes and citrate synthase; morphologic examination showed scattered ragged-red fibers and poor or absent cytochrome c oxidase staining in 10% of the fibers. A heteroplasmic G1606A transition in the mtDNA tRNAVal gene was found. Mutant DNA was 70% of the total in the proband’s muscle. The mutation was absent in blood samples and urinary sediment from his healthy brother and mother.

Conclusion: This second patient with the G1606A mutation confirms both the pathogenicity of the mutation and its association with a characteristic complex neurologic phenotype.

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IN THE PAST 14 years, more than 100 point mutations in transfer RNA (tRNA) genes of human mitochondrial DNA (mtDNA) have been reported, in association with a wide spectrum of clinical features. Although brain and skeletal muscle are most frequently involved, patients may present with a variety of neurologic and nonneurologic features. Some mutations tend to be associated with specific clinical syndromes, but exceptions and “overlap syndromes” abound. This complexity of genotype-phenotype correlation relates mostly to the abundance of mutant mtDNA and its tissue distribution, but also to mtDNA haplotype, mtDNA copy number, and nuclear background.1 In addition, the high frequency of mtDNA polymorphisms makes it difficult to distinguish neutral changes from pathogenic mutations, especially in nonprotein coding genes. By consensus, the pathogenic role of a given mtDNA change cannot be considered conclusively documented until the mutation is identified in different families. We describe a second patient with a complex multisystemic syndrome associated with a G-to-A substitution at nucleotide 1606 (G1606A) mutation in the tRNAVal gene.2 This finding confirms the pathogenic role of the mutation and contributes to define a characteristic genotype-phenotype correlation.

REPORT OF A CASE

A 37-year-old man reported that since the age of 10 years he has had progressive hearing loss and left-sided migrainous headache. At the age of 20 years, he underwent bilateral extracapsular cataract extractions, and, since the age of 32 years, he developed progressive dementia, loss of balance, and retinitis pigmentosa. He also had recurrent episodes of loss of consciousness and developed myoclonic jerks in all limbs, more prominently on the left side.

At the age of 35 years, he was diagnosed as having hypothyroidism and his condition was successfully treated with hormone supplementation. His 76-year-old mother and his only sibling, a brother aged 43 years, are in good health.

Physical examination showed slight dysmetria and mild difficulty with tandem gait. Eye movements were fully preserved and muscle bulk and strength were normal. Examination of cognitive functions, which were clearly impaired, was made difficult by the severe hearing loss and behavioral abnormalities.

Laboratory findings included normal levels for serum creatine kinase, and serum lactate, and pyruvate. The patient refused a spinal tap. The electrocardiogram was normal.
Nerve conduction studies and electromyography demonstrated mild bilateral ulnar neuropathy at the elbow and borderline values of short-duration motor unit potentials in the proximal limb muscles, consistent with early myopathy. Brain magnetic resonance imaging revealed diffuse calcification of the basal ganglia and cerebral cortical atrophy.

**RESULTS**

**HISTOCHEMICAL AND BIOCHEMICAL STUDIES**

Histological and histochemical analysis of a needle biopsy specimen from the left vastus lateralis muscle showed sparse ragged-red fibers. Histochemistry for cytochrome c oxidase showed that 10% of the fibers stained poorly or not at all. No clear excess of sarcoplasmic lipid was noted by oil-red O staining. These findings were consistent with a primary mitochondrial disease. Biochemical analysis showed normal activities of respiratory chain enzymes and citrate synthase.

**GENETIC STUDIES**

Sequencing of the 22 tRNA genes of mtDNA revealed a heteroplasmic G-to-A transition in the tRNAVal gene at nucleotide position 1606 of the reference sequence (Figure 1A). The mutation was heteroplasmic in muscle (70% mutant). It was absent in blood samples and urinary sediment of the proband’s mother and brother (Figure 1B). No other tissue from the proband was available.

**COMMENT**

The G-to-A transition at nucleotide 1606 of the tRNAVal gene was previously described in a patient, who was seen at the age of 20 years with bilateral hearing loss and who at the age of 40 years developed bilateral cataracts, cognitive impairment, ataxia, mild weakness, seizures, and myoclonus. 

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**METHODS**

Histochemical analysis of a muscle biopsy specimen and measurements of respiratory chain enzyme activities were performed as described. All 22 mitochondrial tRNA genes were amplified using suitable oligonucleotide primers. The resulting polymerase chain reaction (PCR) fragments were subjected to direct sequencing with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit and 310 Automatic Sequencer (Applied Biosystem; Perkin Elmer, Foster City, Calif).

For restriction fragment length polymorphism analysis mtDNA from the proband, his mother, and brother was amplified using the following primers: CTACGCAATTATATTAGGAGGAC (nt1534→1554), and a mismatched backward primer TGGGTGCTTTGTTAAAGCTGC (nt1629→1608, 1609 A→G) designed to create a BsmI site in the mutant allele. Polymerase chain reaction conditions were 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. After 35 cycles, 4 µCi of α32P-dCTP [where 32P indicates deoxyctosine triphosphorus labeled with phosphorus 32] (3000 Ci/mmol) were added and a last PCR cycle was performed. Aliquots (10 µL) of PCR products were digested with the appropriate restriction endonuclease and electrophoresed in 20% nondenaturing acrylamide gel. The proportion of mutant mtDNA was evaluated in a phosphor-imager (Molecular Analyst, BioRad, Hercules, Calif) using Image-Quant software (Molecular Dynamics, Sunnyvale, Calif).

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**Figure 1.** A. Sequence of the complementary strand of the mitochondrial DNA tRNAVal gene showing the C-to-T transition at nucleotide 1606. B. Polymerase chain reaction–restriction fragment length polymorphism analysis of the mutation. Amplified fragments of 96 base pairs (bp) are spliced into 70-bp and 26-bp fragments after digestion with BsmI when harboring the mutation. Lanes: 1, patient muscle; 2, brother’s blood sample; 3, brother’s urinary sediment; 4, mother’s blood sample; 5, mother’s urinary sediment; 6, normal muscle control; 7, uncut fragment; and M, 100-bp marker.
Figure 2. The generic transfer RNA cloverleaf with standard nucleotide numbering. Five sets of pathogenic mutations are boxed. Mutations at the same position (regardless of the specific tRNA in which the mutation is located) are associated with 0 similar phenotypes (modified from Schon et al).

onset and additional symptoms, including migraine, hypothyroidism, and retinitis pigmentosa. While retinitis pigmentosa and migraine are common in mitochondrial encephalomyopathies, hypothyroidism has been reported only in association with large-scale rearrangements of mtDNA and with the A-to-G substitution at nucleotide 3243 (MELAS) mutation. Although both patients had similar mutational loads in muscle, our patient had only electrophysiologic and pathologic evidence of myopathy without overt weakness. Whereas the G1606A mutation was maternally inherited in the patient described by Tiranti et al, it seemed sporadic in our patient, although we cannot exclude that his mother and brother had mutational levels below the threshold of detection in blood and urinary tract cells.

In both patients, the mutation did not impair respiratory chain enzyme activities in skeletal muscle, where mutational levels around 70% are probably just above the pathologic threshold. Assuming a uniform distribution of the tRNA levels around 70% are probably just above the pathologic threshold. Assuming a uniform distribution of the tRNA levels around 70% are probably just above the pathologic threshold. Assuming a uniform distribution of the tRNA levels around 70% are probably just above the pathologic threshold. Assuming a uniform distribution of the tRNA levels around 70% are probably just above the pathologic threshold. Assuming a uniform distribution of the tRNA levels around 70% are probably just above the pathologic threshold.

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In both patients, the mutation did not impair respiratory chain enzyme activities in skeletal muscle, where mutational levels around 70% are probably just above the pathologic threshold. Assuming a uniform distribution of the in all tissues, a functional defect may become evident only in tissues with a very high energy requirement, eg, auditory receptors, retina, and brain. As both patients were alive when studied no data exist on the percentage of heteroplasmy in these tissues.

It has been observed that mutations at the same position in the cloverleaf structure of different tRNA genes are associated to similar clinical phenotypes. For example, mutations at nt−4285 in the tRNA valued at nt−5703 in the tRNA valued, both at the position 27 of the tRNA structure, are associated with progressive external ophthalmoplegia (for other examples see Figure 2).

The G1606A mutation breaks a G-C pair in the acceptor stem of the tRNA valued at the same position on the tRNA structure as reported on T7512C mutation in the tRNA valued gene. In 3 patients with the T7512C mutation, the clinical phenotype was similar to that causing the G1606A mutation and consisted of hearing loss, ataxia, cognitive impairment, seizures, and myoclonus. Although these patients had more severe symptoms, these may be related to higher percentages of mutant mtDNA (>90% in muscle). These findings add further credence to the hypothesis that the genotype-phenotype relationship of mtDNA tRNA mutations may be related not only to alterations of the DNA sequence, but to disruptions of the secondary and tertiary structures. Our study confirms the pathogenic role of the G1606A mutation of the tRNAval gene and the association between this genotype and a complex syndrome characterized by ataxia, hearing loss, seizures, cataracts, and mental deterioration.

CONCLUSIONS

Patients presenting with these clinical features, with or without maternal inheritance, should be investigated for the presence of either the G1606A tRNAval mutation or the T7512C tRNAsuc mutation. Identifying more patients will help to define both the frequency of these mutations and the consistency of their clinical expression.

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