Maternally inherited hearing loss in a large kindred with a novel T7511C mutation in the mitochondrial DNA tRNA<sup>Ser(UCN)</sup> gene

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ABSTRACT

Article abstract Thirty-six of 43 maternally related members of a large African American family experienced hearing loss. A muscle biopsy specimen from the proband showed cytochrome c oxidase (COX)-deficient fibers but no ragged-red fibers; biochemical analysis showed marked reduction of COX activity. A novel T7511C point mutation in the tRNA<sup>Ser(UCN)</sup> gene was present in almost homoplasmic levels (>95%) in the blood of 18 of 20 family members, and was also found in lower abundance in the other two. Single-fiber PCR showed that the mutational load was greater in COX-deficient muscle fibers. The tRNA<sup>Ser(UCN)</sup> gene may be a “hot spot” for mutations associated with maternally transmitted hearing loss.

Sensorineural hearing loss (SNHL) is a common clinical manifestation in patients with mitochondrial disorders. Although SNHL in the context of multisystemic syndromes has been associated with numerous mtDNA point mutations and rearrangements, isolated SNHL has been associated with only three mutations: A1555G, A7445G, and 7472ins. The aim of this study was to identify the mtDNA mutation in a large family with maternally transmitted SNHL.
PATIENTS AND METHODS.

A large African American family had maternally transmitted hearing loss (figure 1). The proband was a 35-year-old woman who sought treatment for bilateral moderately severe hearing loss without systemic symptoms. Details of the clinical findings in this family are published elsewhere. Total DNA from the blood of 29 family members was obtained. An open muscle biopsy specimen obtained from the proband after informed consent was studied by standard histochemical and biochemical techniques.

DNA sequencing.

The entire mitochondrial genome was amplified using 24 sets of primers as described. For each PCR fragment, direct sequencing was performed in an ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA) using the Big Dye Terminator Cycle sequencing reaction kit (Perkin-Elmer Applied Biosystems, Foster City, CA).

PCR/restriction fragment length polymorphism analysis.

mtDNA was amplified by use of PCR with primers corresponding to nt positions 7397–7417 ("forward") and 7633–7613 ("backward"). After 30 cycles, 3 μCi of $\alpha-^{32}P$ was added to each reaction tube for the final PCR cycle ("hot finish PCR"). The mutant mtDNA creates a novel $MboII$ restriction site, which can be detected by restriction fragment length polymorphism (RFLP) analysis (figure 2). The digested PCR product was electrophoresed on a 12% acrylamide gel, then placed in a phosphoimager (Molecular Analyst, Bio–Rad, Hercules, CA) to quantify the percentage of mutation.

Figure 1. Family pedigree and summary of clinical and molecular features. Filled symbols indicate affected individuals. Horizontal bars indicate that audiologic data are available. DNA was obtained from family members marked with an asterisk.

Figure 2. The T7511C mutation in the tRNA$^{Ser(UCN)}$ gene. (A) Automated
sequence analysis showing the T7511C mutation (heavy arrow) and the G7521A neutral polymorphism (light arrow). (B) Wild-type sequence from a control subject. (C) Scheme of the PCR/RFLP analysis and quantitation of the T7511C mutation. The wild-type 226-bp PCR product is cleaved into two fragments, 196 and 30 bp in length, whereas the 226-bp fragment containing the T7511C mutation is cleaved into three fragments, 120, 76 and 30 bp in length. (D) Digestion gel showing the presence of the mutation. M = DNA molecular weight marker, U = undigested PCR fragment. The proband (Patient III–27) shows >95% mutant mtDNA in both muscle and blood, Patient III–22 shows 92% mutant mtDNA, Patient IV–13 (a paternal unaffected relative) shows only wild-type mtDNA, and Patient III–36 shows 90% mutant mtDNA. Patient ID numbers are identical with those used in figure 1.

Single-fiber PCR analysis.

Thirty-micrometer-thick muscle sections from the proband were stained for cytochrome c oxidase (COX) activity. Single fibers were isolated by mouth suction using siliconized microcapillaries under an inverted microscope as described. Nested PCR was performed using 20-mer nucleotide primers corresponding to nt positions 7361–7380 and 7667–7648 before amplifying 10 μL of the PCR product.
using the same primer set for the PCR/RFLP analysis as described above. The mutational load in single muscle fibers with COX deficiency (n = 26) was compared with that in fibers with less severe COX deficiency (n = 24). To confirm small levels of heteroplasmy, the digested PCR product was reamplified by PCR to ensure that the remaining wild-type mtDNA was indeed present.

RESULTS.

Clinical findings.

Thirty-six of 43 maternally related family members experienced hearing loss. Pure tone audiometric studies performed on 18 maternally related and 9 paternally related family members confirmed SNHL in 14 maternal relatives but none of the paternal relatives (see figure 1). Hearing loss was symmetric and gradually progressive. Age at onset was variable, ranging from childhood to old age. There was no history of sudden hearing loss or asymmetrical involvement.

Morphologic and biochemical studies.

Muscle histochemical analysis showed COX–deficient muscle fibers but no ragged–red fibers on Gomori trichrome or succinate dehydrogenase staining. COX deficiency involved both Type I and Type II fibers (figure 3). Biochemical analysis of respiratory chain enzymes showed marked reduction in COX activity (1.01 μmoles/min/g tissue; normal 2.80 ± SD 0.52). Other respiratory chain enzyme activities were normal.

Figure 3. Muscle biopsy specimen of (a) control subject and (b) the proband (Patient III–27) showing partial cytochrome c oxidase (COX) deficiency in the patient. COX activity is diffusely reduced in both type I and II fibers.
DNA sequencing.

Direct sequencing of mtDNA identified several changes from the Cambridge sequence; most were previously reported polymorphisms or neutral changes, except mutations at T3308C, T5655C, and T7511C. Because the T3308C and T5655C mutations were homoplasmic and were found in several control subjects (data not shown), we did not deem them pathogenic. The T7511C mutation was heteroplasmic in two family members (see figure 2), and we did not find any of the previously reported pathogenic mutations associated with hearing loss (A1555G, A3243G, T7445C, T7472Cins, and A8344G).

PCR and RFLP analysis.

PCR/RFLP analysis showed that the mutation was present in almost homoplasmic levels (94% to 100%) in blood from 18 maternal relatives and in lower abundance in another 2 (see figure 2). There was no correlation between severity of the hearing loss and mutational load in blood. The T7511C mutation was not detected in 9 paternally related family members or 250 control subjects.

Single-fiber PCR analysis.

Two observations from single–fiber PCR analysis suggested that the T7511C mutation was pathogenic. First, the mutational load was greater in single fibers with more severe COX deficiency (95%, SD 9.5) than in fibers with more intense COX staining (90%, SD 10.2). Second, homoplasmacy for the T7511C mutation was more common in COX-deficient single fibers (50%) than in fibers with higher COX activity (16%).

DISCUSSION.

We report a large kindred with isolated SNHL associated with a novel T7511C point mutation and with morphologic and biochemical evidence of COX deficiency in muscle.

The hearing loss was progressive, often severe, and symmetric, but the age at onset was variable. This family further extends the clinical spectrum of SNHL associated with mtDNA–related disorders, which can also be congenital, precipitated by aminoglycosides, insidious in onset, gradually progressive or with steplike deteriorations, or asymmetric.1,4 This is the fourth pathogenic mutation in the tRNA^Ser(UCN) gene to be associated with SNHL, although mutations in this gene have also been reported in patients with diverse systemic manifestations, including ataxia,7 stroke, and epilepsy.8–10 One other mutation in the tRNA^Ser(UCN) gene has been associated with a myopathic presentation in the absence of hearing loss.9

The pathogenicity of the T7511C mutation is supported by the following: 1) it disrupts a highly conserved site in the acceptor stem of the tRNA^Ser(UCN), 2) it was heteroplasmic in some family members, 3) it was absent in 250 control subjects, and 4) quantitative PCR on single fibers showed that the mutation was present in higher abundance in affected (COX-deficient) fibers. Although there was no correlation between clinical severity and mutational load in blood from our patients, if quantitative analysis could be performed in affected tissues, such as auditory structures, such correlation might become apparent.
Like other reported mtDNA mutations associated with hearing loss, the T7511C transition was present in almost homoplasmic levels in blood, suggesting that this mutation also has a relatively low pathogenic potential. Unlike the A1555G mutation, which is thought to enhance aminoglycoside binding to the 12S ribosomal RNA, mutations in the tRNA$^{\text{Ser(UCN)}}$ are not predicted to affect aminoglycoside binding. It is noteworthy that all reported patients with mutations in this tRNA gene had biochemical or histochemical evidence of COX deficiency in the absence of ragged-red fibers in muscle biopsy specimens, suggesting that altered function of the tRNA$^{\text{Ser(UCN)}}$ gene may impair transcription or translation of COX. Alternatively, there may be a tissue-specific factor in the auditory system that amplifies what in other tissues is only a mild or subclinical abnormality.

Mutations in the tRNA$^{\text{Ser(UCN)}}$ gene are commonly associated with hearing loss, but their pathogenic mechanism remains unclear. From a clinical point of view, patients with undiagnosed maternally transmitted hearing loss in the presence or absence of other neurologic features should be investigated for mtDNA mutations in the tRNA$^{\text{Ser(UCN)}}$ gene.

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