Mitochondrial COI/tRNA\textsuperscript{Ser(UCN)} G7444A mutation may be associated with hearing impairment in a Han Chinese family

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Abstract: Mutations in mitochondrial genome have been found to be associated with hearing loss. Of these, the mitochondrial 12S rRNA and tRNA\textsuperscript{Ser(UCN)} are the hot spots for pathogenic mutations associated with deafness. To understand the putative role of mitochondrial DNA (mtDNA) mutations in hearing loss, we recently initiated a mutational screening for the mtDNA mutations in Hangzhou area from Zhejiang Province. In this study, we described a maternally inherited Han Chinese family with high penetrance of hearing loss, notably, the penetrances of hearing loss in this family were 80% and 40%, when the aminoglycoside was included or excluded. Three matrilineal relatives in this pedigree exhibited different levels of hearing loss with different age at onset. In addition, sequence analysis of the complete mitochondrial genome showed the presence of the well-known C1494T mutation in 12S rRNA gene and the G7444A mutation in the COI/tRNA\textsuperscript{Ser(UCN)}. The C1494T mutation had been reported to be a pathogenic mutation associated with aminoglycoside-induced and non-syndromic hearing loss. While the G7444A mutation was considered as a secondary mutation associated with deafness. However, the lack of functional variants in \textit{GJB2} and \textit{TRMU} genes suggested that nuclear modified genes may not play important roles in deafness expression. Thus, the combination of G7444A and C1494T mutations in mitochondrial genome may account for the high penetrance of hearing loss in this Chinese family.

Keywords: Deafness, mtDNA mutations, C1494T, G7444A, Chinese family

Introduction

Hearing loss is one of the most common human health problems, affecting one in 700-1000 newborns [1]. Deafness can be caused by gene alternations and environmental factors including the ototoxic drugs such as aminoglycoside antibiotics. Of the hereditary factors, mutations in mtDNA, especially in 12S rRNA and tRNA\textsuperscript{Ser(UCN)} genes, are the important causes of sensorineural hearing loss [2]. Among these mutations, the homoplasmic A1555G and C1494T mutations in the highly conserved A-site of 12S rRNA has been associated with both aminoglycoside-induced and nonsyndromic hearing loss (AINHL) in many families worldwide [3-6]. Moreover, the A7445G, 7472 insC, T7510C and T7511C mutations have been identified in tRNA\textsuperscript{Ser(UCN)} gene [7]. However, matrilineal relatives within and among families carrying these mutations exhibited a wide range of penetrance, severity and age at onset in hearing loss [8, 9], moreover, functional analysis of the cell lines derived from the matrilineal relatives carrying these primary mutations demonstrated that the A1555G or C1494T mutation led to mild mitochondrial dysfunction and sensitivity to aminoglycosides [10, 11], these findings strongly indicated that A1555G or C1494T mutation was insufficient to produce enough clinical phenotype, thus, other factors, such as aminoglycosides, nuclear genes or mitochondrial haplotype may contribute to the clinical expression of deafness-associated mtDNA mutations.

With the aim of elucidating the molecular basis of hearing loss, an extensive mutational screen-
MtDNA G7444A mutation and deafness

ing for mitochondrial 12S rRNA and tRNA\textsubscript{Ser(UCN)} genes were performed in Hangzhou area of Zhejiang Province. In this report, we described a Han Chinese family with maternally inherited AINHL. Sequence analysis of the mitochondrial genome showed the presence of C1494T and G7444A mutations.

Materials and methods

Subjects

As a part of genetic screening program for the hearing loss, a three-generation Han Chinese family, as shown in Figure 1, was ascertained in the Department of Otolaryngology, Hangzhou First People’s Hospital. Informed consent, blood samples were obtained from all participants prior to their participation in the study, in accordance with the Ethics Committee of Hangzhou First People’s Hospital. In addition, a comprehensive history and physical examination were performed to identify any syndromic findings, the history of the use of aminoglycosides, as well as the genetic factors related to the hearing impairment in members of this pedigree. An age-appropriate audiological examination was performed, and this examination included pure tone audiometry (PTA) and auditory brainstem response (ABR), immittance testing and distortion product otoacoustic emissions. The PTA was calculated from the sum of the audiometric thresholds at 500, 1000, 2000, 4000 and 8000 Hz. The severity of hearing impairment was classified into five grades: normal < 26 dB, mild 26~40 dB, moderate 41~70 dB, severe 71~90 dB and profound > 90 dB. Moreover, 300 healthy DNAs were obtained from a panel of unaffected Han Chinese subjects with the age and gender matched were used as controls.

Analysis of the mutations in mitochondrial genome

Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Gentra Systems). First, three matrilineal relatives (I-2, II-1, III-2) and control subject’s DNA fragments spanning the mitochondrial 12S rRNA and tRNA\textsubscript{Ser(UCN)} genes were amplified by PCR using oligodeoxynucleotides as previously described [12]. Subsequently, the entire mitochondrial genomes of the deafness patients (I-2, II-1, III-2) and controls were PCR-amplified in 24-overlapping fragments by using the set of light-strand and the heavy-strand primers [12]. After PCR amplification, each fragment was purified and analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. The sequence data were compared with the reversed Cambridge sequence to detect the mutations (GenBank accession no. NC_012920) [13].

Phylogenetic analysis

A total of 17 vertebrates’ mitochondrial genome sequences were used in the interspecific analysis. These include Bos Taurus, Cebus albifrons, Gorilla gorilla, Homo sapiens, Hylobates lar, Lemur catta, Macaca mulatta, Macaca sylva-nus, Mus musculus, Nycticebus coucang, Pan paniscus, Pan troglodytes, Pongo pygmaeus, Pongo abelii, Papio hamadryas, Tarsius bancanus, and Xenopus laevis (GenBank). The conservation index (CI) was calculated by comparing the human nucleotide variants with 16 other vertebrates. Notably, the CI ≥ 75% was regarded as having functional potential.

Mutational screening for GJB2 gene

The DNA fragments spanning the entire coding region of GJB2 gene were amplified by PCR using the following primers: forward: 5'-TATG-
MtDNA G7444A mutation and deafness

ACACTCCCCAGCACAG-3', and reverse: 5'-GG-GCAATGCTTAACTGACG-3'. PCR amplification and sequencing analysis were performed as described elsewhere [14]. The results were compared with the wild-type GJB2 sequence to identify the mutations (GenBank Accession No. M86849).

Mutational analysis of TRMU gene

Previously study showed that the TRMU exon 1 A10S mutation may modulate the phenotypic manifestation of deafness-associated mitochondrial 12S rRNA mutations [15]. To see whether TRMU played an active role in deafness expression, we conducted a mutational screening for the TRMU exon 1 in matrilineal relatives in this pedigree and the healthy controls. The primers for detecting the A10S mutation were as follows: forward: 5'-ACAGCGCAAAGAAGAGCAGT-3', and reverse: 5'-ACACGCGCCACGACGGACG-3'. The PCR segments were analyzed and compared with the TRMU genomic sequence (Accession No. AF_448221).

Statistical analysis

Statistical analyses were performed using the SPSS statistical package, version 16.0, and statistical significance was established at P < 0.05. We performed Fisher’s exact test to evaluate the difference in G7444A mutation between deafness patients and controls.

Results

Clinical features of the Han Chinese family with AINHL

All patients from the Han Chinese family lived in the Hangzhou city of Zhejiang Province. The proband (III-2) was an infant born in Hangzhou First People’s Hospital. As shown in Table 1 and Figure 2, the proband exhibited bilateral hearing impairment (90 dB right ear and 95 dB left ear). A comprehensive history and physical examination were performed to identify any syndromic findings, the history of the use of aminoglycosides. Moreover, we noticed that the proband’s mother (II-5), a young woman at the age of 26 years; had been administered gentamicin (5 mg/kg/dose, 10 days) for fever when she was 18-years-old. She developed the profound hearing loss 2 months after the drug administration. It is interesting to note that two matrilineal relatives (I-2, II-5), who had a history of exposure to gentamicin and streptomycin, exhibited a severity of hearing impairment in this maternal kindred, suggesting that the aminoglycosides may play important role for this disorder.

Screening for the mutations in mitochondrial genome

The maternal transmission of hearing loss in this family suggested the mitochondrial involvement and led us to analysis the mitochondrial genome of matrilineal relatives (I-2, II-2, III-1) and the healthy subjects. We first examined the known mtDNA mutations associated with deafness by PCR-amplification. As shown in Figure 3, PCR-Sanger sequencing identified 2 known mutations: the C1494T in 12S rRNA gene and the G7444A in COI/tRNASer(UCN). However, we did not detect either the presence of A1555G mutation in 12S rRNA gene or A7445G, T7510C, T7511C mutations in tRNASer(UCN) gene in those matrilineal relatives.

To elucidate the molecular basis for maternally transmitted deafness, 24 overlapping DNA fragments spanning the entire mitochondrial genome were PCR-amplified and sequenced. The comparison of the resultant sequence with the Cambridge consensus sequence identified a set of polymorphisms, as shown in Table 2. Among these, there were 5 mutations in D-loop, 2 known mutations in 12S rRNA and 2 mutations in 16S rRNA genes, while other mutations were mainly localized at protein-coding genes. Moreover, we noticed that there were 4 amino acid substitutions caused by corresponding mtDNA mutations occurred in different poly-
peptides. These missense mutations included the ND1 C3497T (A64V), ND A8860G (T112A), ND3 A10398G (T114A) and Cytb A15326G (T194A). These mutations in rRNAs and poly-peptides were further evaluated by phylogenetic analysis from other organisms including mouse [16], bovine [17] and Xenopus laevis [18]. However, none of mutations in the poly-peptides were highly evolutionarily conserved and implicated to have functional consequence.

Mutational analysis of GJB2 and TRMU genes

To examine the role of GJB2 and TRMU genes in phenotypic expression of the C1494T mutation, we performed the mutational screening of GJB2 and TRMU exon 1 in matrilineal relatives who carried the C1494T mutation. However, none of variants in GJB2 and TRMU genes were found, suggesting that the GJB2 and TRMU genes may not play important roles in this Chinese family.

Mitochondrial G7444A mutation may be acted as a risk factor for AINHL

We proposed that the mitochondrial G7444A mutation to be a risk factor for AINHL based on the following reasons: first, this mutation was presented only in the deafness patients of this family but was absent in 300 controls (P < 0.05); second, this mutation may alter the secondary structure of tRNA Ser(UCN) as well as CO1 gene, therefore, the G7444A mutation may lead to the impairment in mitochondrial protein translation; third, this mutation localized at the position which was very conserved from different species (CI=100%).

Discussion

In this study, we have performed clinical, genetic and molecular characterization of a three
Table 2. MtDNA sequence variants in this family with hearing impairment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Replacement</th>
<th>Conservation</th>
<th>Previously reported</th>
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<td>D-loop</td>
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<td>A to G</td>
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<td>73</td>
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<td></td>
<td>152</td>
<td>T to C</td>
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<td></td>
<td>263</td>
<td>A to G</td>
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<td></td>
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<td></td>
<td>16223</td>
<td>C to T</td>
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<td></td>
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<td></td>
<td>16519</td>
<td>T to C</td>
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<td></td>
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<tr>
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</tr>
<tr>
<td></td>
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<td>G/G/A/A</td>
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<tr>
<td></td>
<td></td>
<td>A to G</td>
<td>A/G/A/A</td>
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<td>15326</td>
<td>A to G (Thr to Ala)</td>
<td>T/M/I/I</td>
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</table>

a. Conservation of amino acid for polypeptides or nucleotide for RNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X). b. See the online mitochondrial genome database [http://www.mitomap.org](http://www.mitomap.org).

Figure 4. Location of deafness-associated mutations in tRNAser(UCN) and adjacent COI. Arrow indicate the A7445G and G7444A mutations in the precursor of this tRNA and adjacent sequence of COI from wild-type (WT) and mutant (MT).

In addition, the mitochondrial haplotype has been shown to influence the penetrance of hearing loss associated with mtDNA primary mutations. In particular, mtDNA mutations at positions 4216 and 13708 acted as second

Sequence analysis of the mitochondrial genome showed the presence of C1494T mutation in 12S rRNA gene, in fact, this mutation was first identified in a large Chinese family with AINHL [6]. Functional characterizations of cell lines derived from the C1494T mutation led to only mild mitochondrial dysfunction and sensitive to aminoglycosides [11]. In addition, three affected matrilineal relatives exhibited the various severities, age at onset of hearing loss, suggesting that the C1494T mutation itself was insufficient to produce the clinical phenotypes; other modified factors such as environmental factors, aminoglycosides, mitochondrial haplotype and nuclear genes were involved in deafness expression.

As shown in Figure 1, this family exhibited a high penetrance of hearing loss, in particular, the penetrance of hearing loss in this family was 80% and 40%, when aminoglycoside was included and excluded.
Lebers’ hereditary optic neuropathy (LHON) mutations were implicated to increase the penetrance of the deafness-associated A7445G mutation [19]. Moreover, the T5628C mutation in tRNA\textsubscript{Ala} was thought to have a modifying role in the phenotypic manifestation of the C1494T mutation in a Han Chinese family [20]. In this study, the sequence analysis of the entire mitochondrial genome identified a set of polymorphisms, apart from C1494T and G7444A mutations, other mutations in mitochondrial genome showed no evolutionary conservation. As shown in Figures 3 and 4, the G7444A mutation resulted in a read-through of the stop codon AGA of the COI message, thereby adding three amino acids (Lys-Gln-Lys) to the C-terminal of the polypeptide. Thus, the mutated polypeptide may retain a partial function. Alternatively, the G7444A mutation was adjacent to the site of 3’ end endonucleolytic processing of the L-strand RNA precursor, spanning tRNA\textsubscript{Ser(UCC)} and ND6 mRNA [19]. Previous study showed that the A7445G mutation in the precursor of tRNA\textsubscript{Ser(UCC)} led to a failure in the processing of the L-strand RNA precursor, thereby causing a marked decrease of the steady-state levels of tRNA\textsubscript{Ser(UCC)} and ND6 mRNA [19]. Thus, the G7444A mutation, similar to the A7445G mutation, may also cause a defect in the processing of the L-strand RNA precursor, thus causing mitochondrial dysfunction. Although aminoglycoside was the predominant factor for hearing impairment, the G7444A mutation may also play an important role in the phenotypic expression of the C1494T mutation in this Chinese family. Moreover, due to the lack of any functional variants in GJB2 and TRMU genes indicated those nuclear genes may not play active roles in deafness expression. Taken together, our data showed that the combination of the C1494T and G7444A mutations in mitochondrial genome, as well as the aminoglycosides may account for the high penetrance and expressivity of hearing loss in this family.

Conclusions

Our study indicated that the combination of C1494T and G7444A mutations in mitochondrial genome, combined with the aminoglycosides may account for the high penetrance and expressivity of AINHL in this family. Moreover, the incomplete penetrance, variable degree of hearing loss in matrilineal relatives suggested that other modified factors, such as epigenetic modification and environmental factors may contribute to the clinical expression of hearing loss in this family.

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Disclosure of conflict of interest

None.

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