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Aminoglycoside-induced and non-syndromic hearing loss is associated with the G7444A mutation in the mitochondrial COI/tRNA$^{Ser(UCN)}$ genes in two Chinese families

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Abstract

We report here the clinical, genetic, and molecular characterization of two Chinese families with aminoglycoside induced and non-syndromic hearing impairment. Clinical and genetic evaluations revealed the variable severity and age-of-onset in hearing impairment in these families. Strikingly, there were extremely low penetrances of hearing impairment in these Chinese families. Sequence analysis of the complete mitochondrial genomes in these pedigrees showed the distinct sets of mtDNA polymorphism, in addition to the identical G7444A mutation associated with hearing loss. Indeed, the G7444A mutation in the CO1 gene and the precursor of tRNA$^{Ser(UCN)}$ gene is present in homoplasmy only in the maternal lineage of those pedigrees but not other members of these families and 164 Chinese controls. Their mitochondrial genomes belong to the Eastern Asian haplogroups C5a and D4a, respectively. In fact, the occurrence of the G7444A mutation in these several genetically unrelated subjects affected by hearing impairment strongly indicates that this mutation is involved in the pathogenesis of hearing impairment. However, there was the absence of other functionally significant mtDNA mutations in two Chinese pedigrees carrying the G7444A mutation. Therefore, nuclear modifier gene(s) or aminoglycoside(s) may play a role in the phenotypic expression of the deafness-associated G7444A mutation in these Chinese pedigrees.

Keywords: Hearing loss; Mitochondrial DNA mutation; tRNA; Aminoglycoside; Non-syndromic; CO1; Processing; ND6; Penetrance; Haplogroup; Chinese

Mutations in mitochondrial DNA (mtDNA), especially in 12S rRNA and tRNA$^{Ser(UCN)}$ genes, have been shown to be one of the important causes of hearing loss [1,2]. Of these, four mutations in mitochondrial 12S rRNA including A1555G [3–7], C1494T [8,9], T1095C [10,11], and T961G or 961insC [12–14] have been associated with aminoglycoside-induced and non-syndromic hearing loss in many families worldwide. In the mitochondrial tRNA$^{Ser(UCN)}$ gene, four non-syndromic deafness-associated mutations, A7445G [15,16], 7472insC [17,18], T7510C [19,20], and T7511C [21,22], have been

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identified in families from various ethnic backgrounds. These mutations often occur nearly or completely homoplasmically. These mtDNA mutations, such as the A1555G mutation, often exhibit incomplete penetrance, evidenced by the fact that some individuals carrying the mutations have normal hearing [4,6,23]. In addition, matrilineal relatives of intra-families or inter-families, despite carrying the same deafness-associated mtDNA mutation(s), exhibited the variable penetrance and expressivity including the severity, age-of-onset, and progression in hearing loss [3,4,8,23]. These indicated that the mtDNA mutation(s) itself is not sufficient to produce the clinical phenotype, and other modifier factors including aminoglycosides, nuclear modifier genes, and mitochondrial haplotypes are required for the phenotypic expression of those deafness-associated mtDNA mutations [24–26].

To further investigate molecular pathogenetic mechanism of maternally inherited hearing loss, a systematic and extended mutational screening of the 12S rRNA and $tRNA^{Ser(UCN)}$ genes has been initiated by an international collaboration project between Wenzhou Medical College and Cincinnati Children's Hospital Medical Center. In previous investigation, we carried out a mutational screening of the mitochondrial 12S rRNA gene in 128 Chinese pediatric subjects with sporadic aminoglycoside-induced and non-syndromic hearing loss [27]. In particular, we showed that the incidence of the A1555G mutation is approximately 13% and approximately 2.9% in this Chinese pediatric population with aminoglycoside-induced and non-syndromic hearing loss, respectively [27]. In the present study, clinical and genetic evaluation revealed extremely low penetrance of hearing loss in two Chinese families with aminoglycoside-induced and non-syndromic hearing loss. Molecular analysis demonstrated the presence of the homoplasmic G7444A mutation in the COI/the precursor of $tRNA^{Ser(UCN)}$ genes in these families. To elucidate the role of mitochondrial haplotype in the phenotypic expression of the G7444A mutation, we performed PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of these families.

Subjects and methods

Subjects and audiological examinations. As a part of genetic screening program for the hearing impairment, two Chinese families, as shown in Fig. 1, were ascertained through the Otology Clinic at the first Affiliated Hospital of Wenzhou Medical College. A comprehensive history and physical examination were performed to identify any syndromic findings, the history of the use of aminoglycosides, and 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/or auditory brainstem response (ABR), immittance testing, and Distortion product otoacoustic emissions (DPOAEs). The PTA was calculated from the sum of the audiometric thresholds at 500, 1000 and 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. Informed consent was obtained from participants prior to their participation in the study, in accordance with the Cincinnati Children's Hospital Medical Center Institutional Review Board and Ethnic Committee of The first Affiliated Hospital of Wenzhou Medical College. The164 control DNA samples used for screening for the presence of mtDNA mutations were obtained from a panel of unaffected and unrelated subjects from Chinese ancestry.

Mutational screening of the mitochondrial genome. Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Gentra Systems). First, affected and control subject's DNA fragments spanning the entire mitochondrial 12S rRNA gene or $tRNA^{Ser(UCN)}$ gene were amplified by PCR using oligodeoxynucleotides corresponding to the mtDNA at positions 618–635 and 1988–2007, and 7148–7167 and 8076–8095 [28], respectively. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit.

The entire mitochondrial genomes of two affected matrilineal relatives IV-1 Of WZ201 pedigree and III-1 of WZ202 pedigree were PCR amplified in 24 overlapping fragments by use of sets of light-strand and the heavy strand oligonucleotide primers, as described elsewhere [28]. Each fragment was purified and subsequently submitted for sequence analysis as described above. The resultant sequence data were compared with the updated consensus Cambridge sequence (GenBank Accession No. NC_001807) [29]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG).

Quantification of the mtDNA G7444A mutation. For the analysis of G7444A mutation, DNA fragments were amplified by PCR using oligodeoxynucleotides corresponding to the mtDNA at positions 7148–7167 and 8076–8095, and the resultant PCR fragments were digested with a restriction enzyme *Xba*I as this G7444A mutation abolishes a site for *Xba*I [30,31]. Equal amounts of various digested samples were then analyzed by electrophoresis through 1.5% agarose gel. The proportions of digested and undigested PCR product were determined by using the IMAGE-QUANT

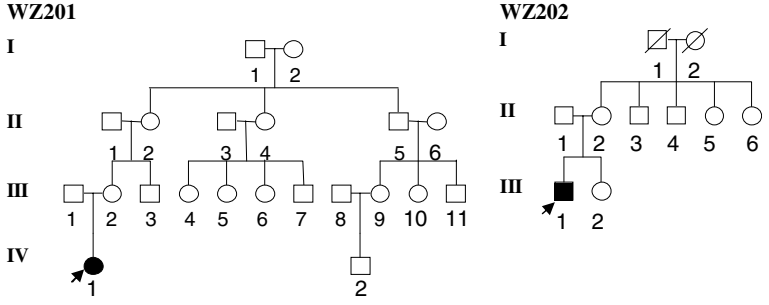

Fig. 1. Two Chinese pedigrees with aminoglycoside-induced and non-syndromic hearing impairment. Hearing impaired individuals are indicated by filled symbols. Arrow denotes proband.