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Mitochondrial tRNA$^{Ser(UCN)}$ gene is the hot spot for mutations associated with aminoglycoside-induced and non-syndromic hearing loss

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Abstract

Mutations in mitochondrial DNA is one of the important causes of hearing loss. Here, we performed a mutational screening of tRNA$^{Ser(UCN)}$ gene in 1542 Chinese subjects with hearing loss. One subject and five subjects carried tRNA$^{Ser(UCN)}$ A7445C and G7444A mutations, respectively, while two subjects harbored both G7444A and 12S rRNA A1555G mutations. Clinical evaluation revealed the variable phenotype of bilateral hearing impairment including severity and audiometric configuration in these subjects. Six pedigrees carrying only G7444A or A7445C mutation exhibited extremely low penetrance of hearing loss, while two families carrying both G7444A and A1555G mutations displayed high penetrance of hearing loss. Of 94 matrilineal relatives in these families, eight subjects suffered from aminoglycoside-induced hearing loss, while seven hearing-impaired subjects did not have a history of exposure to aminoglycosides. Those suggest that G7444A and A7445C mutations themselves are insufficient to produce a clinical phenotype and aminoglycosides are the major modifier factors for the development of deafness in these Chinese families. The combination of A1555G and G7444A mutations increased deafness expression. These observations provide an additional evidence for the early diction and prevention of deafness at the high risk populations carrying these mitochondrial DNA mutations.

Keywords: Hearing loss; tRNA$^{Ser(UCN)}$; Mitochondrial DNA; Penetrance; Mutation; Chinese; Aminoglycoside ototoxicity

Mutations in mitochondrial DNA (mtDNA), especially in the 12S rRNA and tRNA$^{Ser(UCN)}$ genes, are one of the important causes of both aminoglycoside-induced and non-syndromic hearing loss [1,2]. Of these deafness-associated mutations, the A1555G and C1494T mutations in the highly conserved A-site of the 12S rRNA has been associated with both aminoglycoside-induced and non-syndromic hearing loss in many families worldwide [3–10]. Furthermore, six non-syndromic deafness-associated mutations: A7445G [11–13], A7445C [13], G7444A [10,13],

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7472insC [8,14], T7510C [15,16], and T7511C [17,18], have been identified in the mitochondrial $tRNA^{Ser(UCN)}$ gene. These mutations often occur nearly or completely homoplasmically and conferred mild mitochondrial dysfunction [19–21]. In addition, matrilineal relatives of intra-families or inter-families 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 [3,6,11,12,17]. 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 manifestations of those deafness-associated mtDNA mutations [19–23].

To further investigate the molecular mechanism of hearing loss, we have initiated a systematic and extended mutational screening of mtDNA in several cohorts of hearing-impaired subjects [3,9,10,24–31]. In the previous investigation, we showed the highly variable penetrance and expressivity of hearing loss in 36 Han Chinese families carrying the A1555G mutation [9,10,24–30]. Sequence analysis of complete mitochondrial genomes as well as clinical and genetic valuations in these Chinese pedigrees suggested that five mitochondrial tRNA variants: $tRNA^{Glu}$ A14693G, $tRNA^{Thr}$ T15908C, $tRNA^{Arg}$ T10454C, $tRNA^{Ser(UCN)}$ G7444A, and $tRNA^{Cys}$ G5821A, may influence the phenotypic manifestation of the A1555G mutation [10,26,30]. In the present study, we performed a mutational screening of $tRNA^{Ser(UCN)}$ gene in 1542 hearing-impaired Han Chinese subjects ascertained through the Otology Clinic at the first Affiliated Hospital of Wenzhou Medical College, China. Mutational analysis showed that five hearing-impaired subjects carried the G7444A mutation, two hearing-impaired subjects harbored both G7444A and A1555G mutations and one hearing-impaired subject carried the A7445C mutation. Clinical and genetic evaluation revealed extremely low penetrance of hearing loss in six Chinese families carrying only G7444A mutation or A7445C mutation, while two other Chinese families carrying both G7444A and A1555G mutations exhibited high penetrance of hearing loss. Of these, eight matrilineal relatives of these families suffered from aminoglycoside-induced hearing loss. Those results support the idea that deafness-associated mtDNA mutations themselves are insufficient to produce a clinical phenotype and aminoglycosides are the major modifier factor for the development of deafness in these Chinese families.

Subjects and methods

Subjects and audiological examinations. As the part of genetic screening program for the hearing impairment, eight Chinese families, as shown in Fig. 1, was ascertained through the Otology Clinic at the first Affiliated Hospital of Wenzhou Medical College, China. A comprehensive history and physical examination were performed to identify any syndromic findings, the history of the use of aminoglycosides, 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 (DPOAE). 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.

Identification and quantification of mtDNA mutations at positions 1555, 7444, and 7445. 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 $tRNA^{Ser(UCN)}$ and 12S rRNA genes were amplified by PCR using oligodeoxynucleotides corresponding to the mtDNA at positions 7148–7167 and 8076–8095 and 618–635 and 1988–2007 [32], respectively. For the analysis of mutations at positions 7444 and 7445, the PCR fragments were digested with a restriction enzyme XbaI as the A7445G, A7445C, and G7444A mutations abolish a site for XbaI [10,13]. For the detection of the A1555G mutation, the amplified segments were digested with a restriction enzyme BsmAI [9]. 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 program after ethidium bromide staining to determine if these mtDNA mutations are in the homoplasmy in these subjects. Subsequently, 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 resultant sequence data were compared with the updated consensus Cambridge sequence (GenBank Accession No. NC_001807) [33].

Results and discussion

To further elucidate the molecular basis of hearing loss, we have performed a mutational analysis of the $tRNA^{Ser(UCN)}$ gene in 1542 Han Chinese subjects, who were diagnosed as non-syndromic hearing loss or aminoglycoside ototoxicity by the Otology Clinic of Wenzhou Medical College, China. First, DNA fragments spanning the $tRNA^{Ser(UCN)}$ gene were PCR amplified from each affected subject. Each fragment was digested by restriction enzyme XbaI and subsequent electrophoresis analysis. Of those, PCR fragments derived from eight subjects could not be digested with XbaI, as shown in Fig. 2A, suggesting the presence of the homoplasmic mutations at positions 7444 or 7445. To examine the presence of the A1555G mutation in these eight subjects, DNA fragments spanning 12S rRNA genes were PCR amplified, digested with BsmAI and subsequent electrophoresis analysis. In fact, two of these eight subjects harbored the A1555G mutation. To confirm the presence of mutations at positions 1555, 7444, and 7445 in those subjects, these PCR-amplified segments were then purified and subsequently analyzed by DNA sequencing. The comparison of the resultant sequences with the Cambridge consensus sequence [33] revealed the absence of other deafness-associated mtDNA A7445G, 7472insC, T7510C, T7511C, and C1494T mutations [1,2]. As shown in Fig. 2B, one subject (WZ210-IV2) carried the A74445C mutation, five subjects harbored

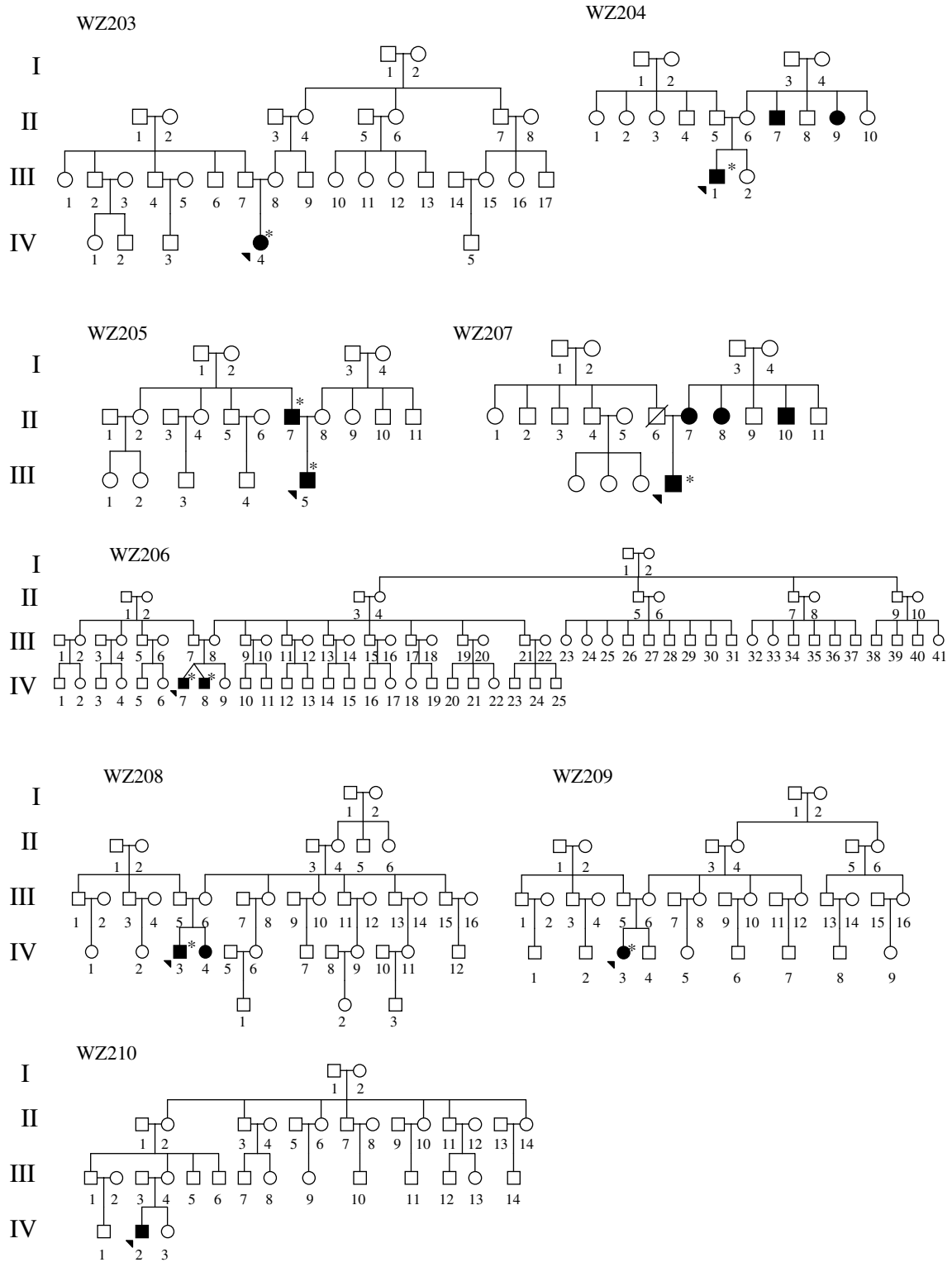

Fig. 1. Eight Chinese pedigrees with aminoglycoside-induced and non-syndromic hearing impairment. Hearing impaired individuals are indicated by filled symbols. Arrow denotes proband. Asterisks denote individuals who had a history of exposure to aminoglycosides.

the G7444A mutation, and two subjects carried both G7444A and A1555G mutations.

Seven of these eight affected subjects, as shown in Table 1, had been administrated aminoglycosides (3–5 mg/kg/dose every 8 h for gentamicin or 15–25 mg/kg/dose every 12 h for streptomycin or 7.5 mg/kg/every 18 h for kamamycin) for various illnesses at the ages from 1 year to 3 years old. They began suffering bilateral hearing impairment within three months after drug administration. As illustrated in Fig. 3, audiological evaluation showed that those

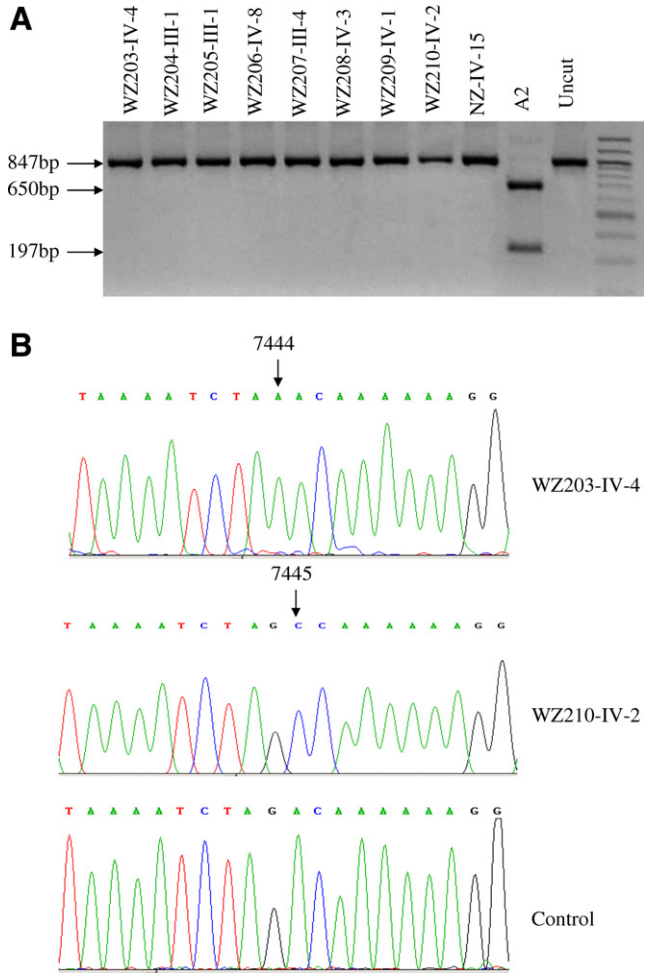

Fig. 2. Identification and qualification of G7444A and A7445C mutations. (A) Quantification of G7444A or A7445C mutation of matrilineal relatives and control subjects derived from these eight Chinese families. PCR products spanning the $tRNA^{Ser(UCN)}$ gene were digested with XbaI and analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. A2 is a Chinese hearing normal control [3] and NZ-IV-15 carried the A7445G mutation [19]. (B) Partial sequence chromatograms of COI/$tRNA^{Ser(UCN)}$ genes from probands of hearing-impaired subjects, and one Chinese control subject. An arrow indicates the location of the base changes at positions 7444 and 7445.

seven subjects had the loss of the high frequencies and their hearing impairment was symmetric. However, subject WZ210-IV2, who was diagnosed as mild hearing impairment at the age of 10 years old, did not have a history of exposure to aminoglycosides. Those subjects, as shown in Table 1, exhibited a variable severity of hearing impairment: four subjects suffered from profound hearing impairment, three individuals had severe hearing impairment and one subject (WZ210-IV2) exhibited mild hearing impairment. Those subjects, as shown in Fig. 3, also displayed variable patterns of audiometric configuration of hearing loss: two subjects had the flat pattern, three subjects showed the sloping pattern, two individuals exhibited the upgrade pattern and one displayed the ravine pattern.

A comprehensive history and physical examination as well as audiological examination were performed to identify any syndromic findings, the history of the use of aminoglycosides, genetic factors related to the hearing impairment in all available members of seven Chinese families carrying the G7444A mutation and one Chinese family carrying the A7445C mutation. In fact, comprehensive family medical histories of those probands and other members of these Chinese families showed no other clinical abnormalities, including diabetes, muscular diseases, visual dysfunction, and neurological disorders. Strikingly, the Chinese family carrying A7445C mutation and five Chinese families carrying the G7444A mutation exhibited extremely low penetrance of hearing loss (only one or two hearing-impaired matrilineal relatives in each pedigree). Of these, six hearing-impaired matrilineal relatives of these six Chinese pedigrees had a history of exposure of aminoglycosides, suggesting that the G7444A mutation is associated with aminoglycoside ototoxicity. This result is consistent with our previous data that aminoglycoside-induced and non-syndromic hearing loss is associated with the G7444A mutation in two Chinese families [31]. By contrast, three of eight matrilineal relatives in WZ204 pedigree and four of seven matrilineal relatives in WZ207 pedigree carrying both A1555G and G7444A mutations exhibited hearing loss. In these two pedigrees, two hearing impaired matrilineal relatives had a history of exposure to aminoglycoside, while other members did not receive any treatment of aminoglycosides. These data are in contrast with our previous observation that there is extremely low penetrance of hearing loss in 27 Chinese families carrying the A1555G mutation (27–29). Similar to the fact that other five Chinese pedigrees carrying the A1555G mutation, in conjunction

Table 1
Summary of clinical data for eight Chinese probands with hearing loss

Subject	Gender	Audiometric configuration	Use of aminoglycosides	Age at test (yr)	Age at onset (yr)	PTA (dB) right ear	PTA (dB) left ear	Level of hearing impairment
WZ203-IV-4	F	Flat	Yes	11	3	107	107	Profound
WZ204-III-1	M	Flat	Yes	15	3	88	87	Severe
WZ205-III-1	M	Slope	Yes	17	<1	102	107	Profound
WZ206-IV-8	M	Slope	Yes	10	<1	102	108	Profound
WZ207-III-4	M	Upgrade	Yes	12	<1	82	92	Severe
WZ208-IV-3	M	Slope	Yes	18	1	108	107	Profound
WZ209-IV-1	F	Upgrade	Yes	15	<1	108	90	Severe
WZ210-IV-2	M	Ravine	No	17	10	22	30	Mild

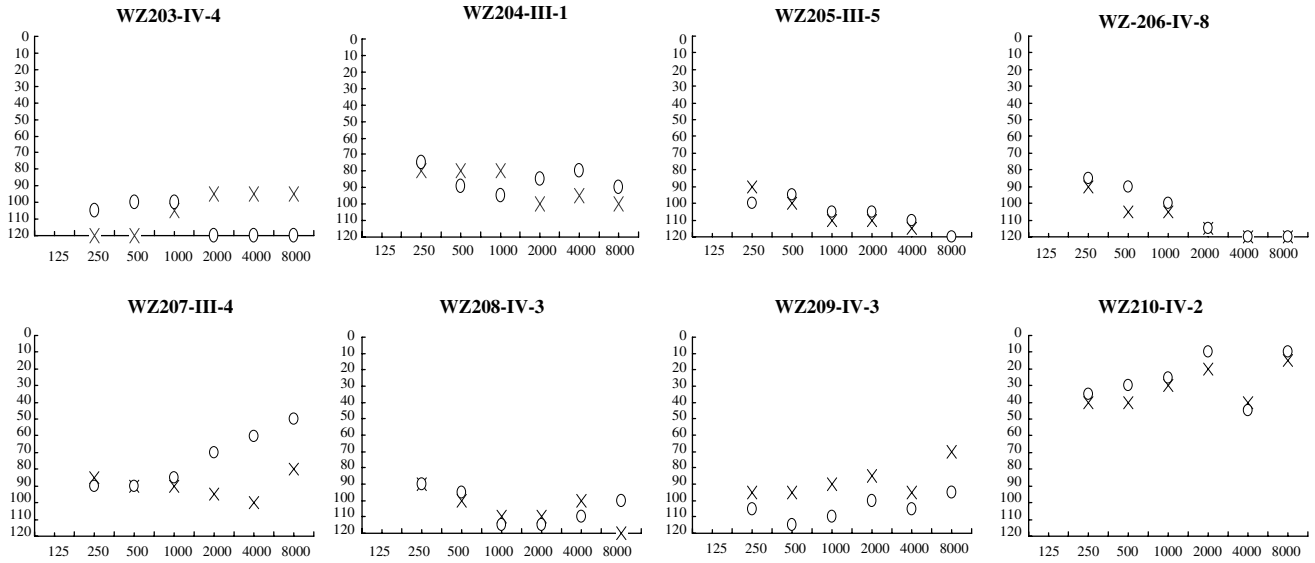

Fig. 3. Air conduction audiogram of probands of eight Chinese families. Symbols: X-left, O-right ear.

with one of these variants: $tRNA^{Glu}$ A14693G, $tRNA^{Thr}$ T15908C, $tRNA^{Arg}$ T10454C, $tRNA^{Ser(UCN)}$ G7444A, and $tRNA^{Cys}$ G5821A, had higher penetrance of hearing loss [10,26,30], the A1555G and G7444A mutations likely contribute to higher penetrance of hearing loss in these two families than other seven Chinese families carrying the G7444A mutation [31] and 27 Chinese families carrying the A1555G mutation (27–29).

The G7444A mutation in the COI/precursor of $tRNA^{Ser(UCN)}$ genes was implicated to be associated with hearing loss [13] or to influence the phenotypic expression of hearing loss associated with the A1555G mutation [10,13] and visual loss associated with the primary LHON-associated mtDNA mutations [34]. The A7445C mutation in the COI gene/the precursor of $tRNA^{Ser(UCN)}$ gene was associated with hearing loss in the Mongolia pedigrees [13]. The occurrence of the G7444A and A74445C mutations in these several genetically unrelated subjects affected by hearing impairment but absence of 164 Chinese controls [31] strongly indicates that these mutations are involved in the pathogenesis of hearing impairment including aminoglycoside ototoxicity. The G7444A and A7445C mutations cause a read-through of the stop condon AGA of the COI message on the H strand of mtDNA, thereby adding three amino acids (Lys-Gln-Lys) [10,31] and (Ser-Gln-Lys) to the C-terminal of the polypeptide, respectively. However, the mutated polypeptide may retain a partial function. Alternatively, the G7444A or A7445C mutation is adjacent to the site of 3′ end endonucleolytic processing of L-strand RNA precursor, spanning $tRNA^{Ser(UCN)}$ and ND6 mRNA [19,20,35]. Our previous data showed that the A7445G mutation led to a failure in the processing of the L-strand RNA precursor, thereby causing a marked decrease of the steady-state levels of $tRNA^{Ser(UCN)}$ and ND6 mRNA [19,36,37]. Thus, the G7444A or A74445C mutation, similar to the A7445G mutation, may also cause the defective processing of the L-strand RNA precursor, thus causing mitochondrial dysfunctions. However, the biochemical defects caused by G7444A mutation or A7445C mutation are likely below the proposed threshold level to support a normal respiratory phenotype. The extremely low penetrance of hearing loss, the possible mild biochemical defects indicated that the G7444A or A7445C mutation itself is not sufficient to produce the clinical phenotype. Thus, the modifier factors including nuclear backgrounds, mitochondrial haplotypes and aminoglycosides are necessary for the phenotypic manifestation of the G7444A mutation. In particular, children carrying these ototoxic mtDNA mutations are likely susceptible to the exposure of aminoglycosides, thereby inducing or worsening hearing impairment, as in the cases of the Chinese families carrying the A1555G or C1494T mutation (3,29). Therefore, these observations provide an additional evidence for the early diction and prevention of deafness at the high risk populations carrying these mitochondrial DNA mutations.

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