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Coexistence of mitochondrial 12S rRNA C1494T and CO1/tRNA$^{Ser(UCN)}$ G7444A mutations in two Han Chinese pedigrees with aminoglycoside-induced and non-syndromic hearing loss

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Abstract

Mutations in mitochondrial DNA are one of the important causes of hearing loss. We report here the clinical, genetic, and molecular characterization of two Han Chinese pedigrees with maternally transmitted aminoglycoside-induced and nonsyndromic bilateral hearing loss. Clinical evaluation revealed the wide range of severity, age-at-onset, and audiometric configuration of hearing impairment in matrilineal relatives in these families. The penetrances of hearing loss in these pedigrees were 20% and 18%, when aminoglycoside-induced deafness was included. When the effect of aminoglycosides was excluded, the penetrances of hearing loss in these seven pedigrees were 10% and 15%. Sequence analysis of the complete mitochondrial genomes in these pedigrees showed the presence of the deafness-associated 12S rRNA C1494T and CO1/tRNA$^{Ser(UCN)}$ G7444A mutations. Their distinct sets of mtDNA polymorphism belonged to Eastern Asian haplogroup C4a1, while other previously identified six Chinese mitochondrial genomes harboring the C1494T mutation belong to haplogroups D5a2, D, R, and F1, respectively. This suggested that the C1494T or G7444A mutation occurred sporadically and multiplied through evolution of the mitochondrial DNA (mtDNA). The absence of functionally significant mutations in tRNA and rRNAs or secondary LHON mutations in their mtDNA suggest that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the 12S rRNA C1494T and CO1/tRNA$^{Ser(UCN)}$ G7444A mutations in those Chinese families. However, aminoglycosides and other nuclear modifier genes play a modifying role in the phenotypic manifestation of the C1494T mutation in these Chinese families.

Keywords: Hearing loss; Aminoglycoside; Mitochondrial DNA; Mutation; Chinese; 12S rRNA; tRNA; Haplotype; Modifier; Penetrance

Mitochondrial 12S rRNA and tRNA$^{Ser(UCN)}$ genes are the hot spots for mutations associated with hearing loss [1,2]. The 12S rRNA A1555G and C1494T mutations have been associated with both aminoglycoside-induced and nonsyndromic hearing loss in many families worldwide [3–10]. Six mutations in the tRNA$^{Ser(UCN)}$ gene: A7445G [11–13], A7445C [13], G7444A [10,13], 7472insC [8],

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T7510C [14], and T7511C [15], have been associated with aminoglycoside-induced and nonsyndromic hearing loss. These mutations often occur nearly or completely homoplasmically and conferred mild mitochondrial dysfunction [16–20]. Matrilineal relatives within and among families carrying the mtDNA mutation(s) exhibited a wide range of penetrance and expressivity in hearing loss [3,6,9,11,12,15]. These indicated that the mtDNA mutation(s) itself is insufficient to produce the clinical phenotype. Other modifier factors including aminoglycosides, nuclear modifier genes and mitochondrial haplotypes modulate the phenotypic manifestations of those deafness-associated mtDNA mutations [16–20].

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 [3,9,10,21–31]. In the previous investigation, we showed the variable penetrance and expressivity of hearing loss in 40 Chinese families carrying the A1555G mutation [9,10,21–27] and six Chinese pedigrees carrying the C1494T mutation [3,28–30]. Five mitochondrial variants: tRNAGlu A14693G, $tRNA^{Thr}$ T15908C, $tRNA^{Arg}$ T10454C, $tRNA^{Ser(UCN)}$ G7444A, and $tRNA^{Cys}$ G5821A, were implicated to influence the phenotypic manifestation of the A1555G mutation [10,23,27], while the $tRNA^{Ala}$ T5628C variant may modulate the phenotypic manifestation of the C1494T mutation in a large Chinese family [29]. In the present study, a mutational screening of $tRNA^{Ser(UCN)}$ and 12S rRNA genes in a large cohort of hearing-impaired Chinese subjects led to the identification of G7444A and C1494T mutations in two Han Chinese families with aminoglycoside-induced and nonsyndromic hearing loss. We further performed the clinical, molecular, and genetic characterization of these two Chinese pedigrees. To assess the contribution that mtDNA variants make toward the phenotypic expression of the G7444A and C1494T mutations, we performed a PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of those families.

Subjects and methods

Subjects and audiological examinations. As the part of genetic screening program for the hearing impairment, two Chinese families, as shown in Fig. 1, was ascertained through the Otology Clinic of Chinese PLA General Hospital, Beijing. 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 Chinese PLA General Hospital.

Mutational analysis of 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 $tRNA^{Ser(UCN)}$ 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 the G7444A mutation, the PCR fragments were digested with a restriction enzyme XbaI as the G7444A mutation abolished a site for XbaI [10,13]. 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. The entire mitochondrial genome of two probands and their mothers was PCR amplified in 24 overlapping fragments by use of sets of the light-strand and the heavy-strand oligonucleotide primers, as described elsewhere [32]. 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) [33].

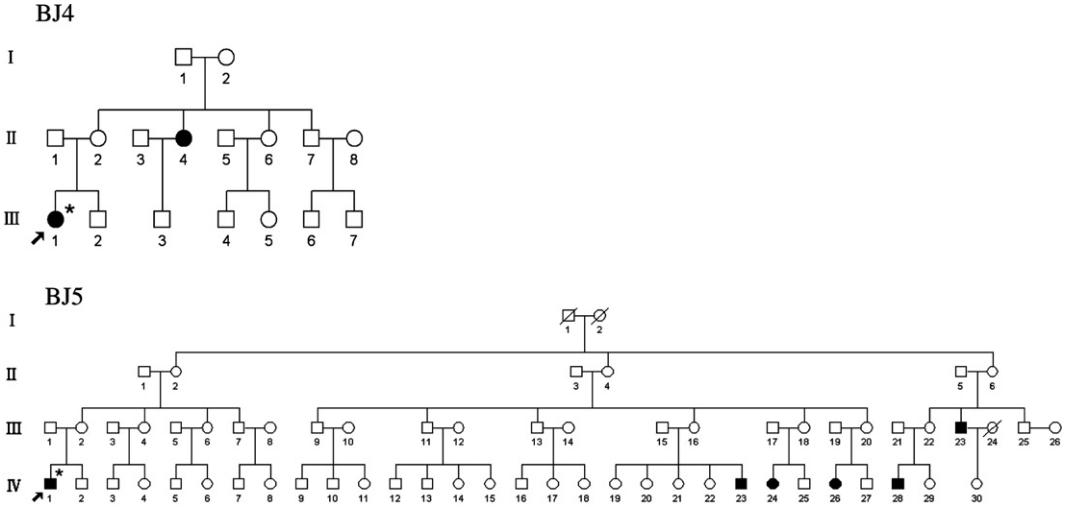

Fig. 1. Two Chinese pedigrees with aminoglycoside-induced and nonsyndromic hearing impairment. Hearing-impaired individuals are indicated by filled symbols. Arrow denotes proband. Asterisks denote individuals who had a history of exposure to aminoglycosides.

Results

Clinical and genetic evaluation of two Chinese pedigrees

Recently, we have initiated a mutational analysis of the tRNA$^{Ser(UCN)}$ and 12S rRNA genes in a large cohort of Chinese subjects, who were diagnosed as nonsyndromic hearing loss or aminoglycoside ototoxicity by the Otology Clinic of Chinese PLA General Hospital. As shown in Fig. 2A, sequence analysis of the PCR-amplified segments spanning these genes revealed that two hearing-impaired Han Chinese subjects carried both G7444A mutation in the CO1/tRNA$^{Ser(UCN)}$ genes and C1494T mutation in 12S rRNA gene. To determine the presence of the homoplasmic mutation, PCR-amplified segments spanning the tRNA$^{Ser(UCN)}$ gene were digested by restriction enzyme XbaI and electrophoresis analysis. Of those, PCR fragments derived from four subjects could not be digested with XbaI, as shown in Fig. 2B, suggesting the presence of the homoplasmic G7444A mutation.

A comprehensive history, physical examination and 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 two Han Chinese pedigrees. 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.

Of these, the proband (III-1) of BJ4 family received gentamycin for high fever at the age of two and half years old. She exhibited bilateral hearing impairment two weeks later after the drug administration. As illustrated in Fig. 3, audiological evaluation showed that she had severe hearing impairment (96 dB at right ear, 76 dB at left ear, with a flat-shaped pattern). Further comprehensive family history and physical examination as well as audiological examination in members of the three-generation family revealed that one (II-4) of other nine matrilineal relatives, without exposure to aminoglycosides, exhibited hearing deficit.

In the family BJ5, the proband IV-1 was a 14 year boy. He was treated with aminoglycosides for illness at the age of 3 years old. He began suffering bilateral hearing impairment within 1 month after the first drug administration. As shown in Fig. 3, he had severe hearing loss (79 dB at right ear, 96 dB at left ear, with a slope-shaped pattern). Familiar history and clinical evaluation revealed that five of other 33 matrilineal relatives without exposure to aminoglycosides suffer from bilateral and sensorineural hearing impairment as the sole clinical symptom.

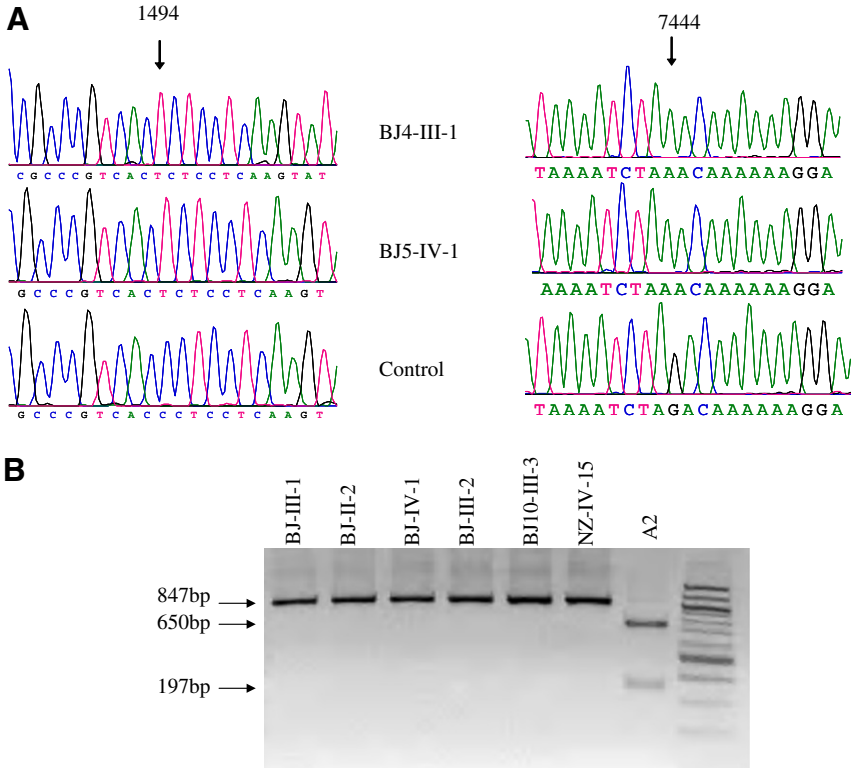

Fig. 2. Identification and qualification of G7444A and C1494T mutations. (A) Partial sequences chromatograms of COI/tRNA$^{Ser(UCN)}$ and 12S rRNA genes from two probands and one Chinese control. (B) Quantification of G7444A mutation of matrilineal relatives derived from these two Chinese families and control subjects. 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], BJ10-III-3 harbored the G7444A mutation [10] and NZ-IV-15 carried the A7445G mutation [16].

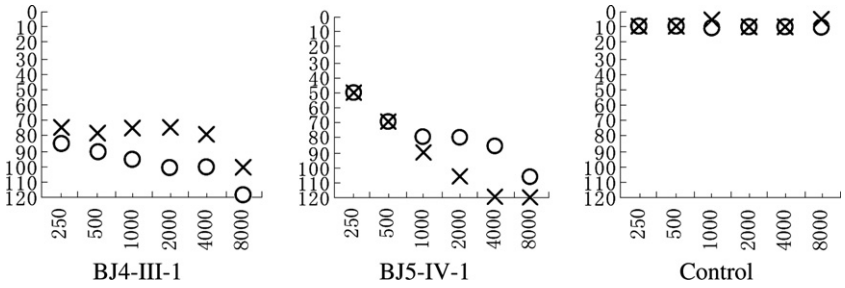

Fig. 3. Air conduction audiogram of probands of two Chinese families and one control. Symbols: X–left, O–right ear.

Mutational analysis of mitochondrial genome

To assess the role of mtDNA variants in the phenotypic expression of the C1494T and G7444A mutations, we performed a PCR-amplification of fragments spanning entire mtDNA and subsequent DNA sequence analysis in two probands and their mothers. In addition to the C1494T and G7444A mutations, as shown in Table 1, these subjects exhibited distinct sets of mtDNA polymorphism. Of other nucleotide changes in these mitochondrial genomes, there are 10 known variants in the non-coding region D-loop, three known variants in 12S rRNA gene, three known variants in the 16S rRNA gene, one known variant in the $tRNA^{Pro}$ gene, 16 known and two novel silent variants in the protein encoding genes as well as nine known and one novel missense mutations in the protein encoding genes [34]. These missense mutations are the A8508G (N48S) in the A8 gene, the G8584A (A20T), A8701G (T59A), and A8860G (T112A) in A6 gene, the A10398G (T114A) in the ND3 gene, the G11969A (A406T) in the ND4 gene, the T14318C (N117S) in the ND6 gene, and the C14766T (T7I), T15204C (I155T), and A15326G (T194A) in the cyto *b* gene. These variants in RNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from other organisms including mouse [35], bovine [36], and *Xenopus laevis* [37]. The 2313insAA mutation in the 16S rRNA gene locates at the highly conserved base of this rRNA, but is the haplogoup C4a1-specific variant [38]. However, none of other variants showed evolutionary conservation and the implication of functional significance.

Discussion

In the present study, we have performed the clinical, genetic, and molecular characterization of two Chinese pedigree with aminoglycoside-induced and nonsyndromic hearing impairment. Hearing impairment as a sole clinical phenotype was mostly present in the maternal lineage of those pedigrees. The C1494T and G7444A mutations were identified to be homoplasmy in matrilineal relatives of these Chinese families. The G7444A mutation has been associated with both aminoglycoside-induced and nonsyndromic hearing loss in several genetically unrelated families [10,13,31]. The C1494T mutation was identified in six Chinese families with maternally inherited aminoglycoside-induced and nonsyndromic hearing impairment [3,28–30] and three Spanish families [39]. As shown in Table 2, the penetrances of hearing loss (affected matrilineal relatives/total matrilineal relatives) in BJ4 and BJ5 pedigrees were 20% and 18%, respectively, while the penetrances of hearing loss in other six Chinese pedigrees were 28%, 20%, 15%, 51%, 78%, and 23% [3,28–30], when aminoglycoside-induced deafness was included. When the effect of aminoglycosides was excluded, the penetrances of hearing loss in BJ4 and BJ5 pedigrees were 10% and 15%, respectively, whereas the penetrances of hearing loss in other six Chinese pedigrees were 21%, 13%, and 8%, 31%, 22%, and 20% [3,28–30], respectively. This suggested that the average penetrance of hearing loss in the absence of aminoglycosides is approximately 18% in these Chinese pedigrees carrying the C1494T mutation. However, three Spanish pedigrees exhibited higher penetrance of hearing loss than these Chinese pedigrees [39].

A wide range of penetrance and severity of hearing loss among these pedigrees indicated the involvement of modifier factors including aminoglycosides, nuclear, and mitochondrial modifiers in the phenotypic manifestation of the C1494T mutation. The fact that two matrilineal relatives of these pedigrees suffered from aminoglycoside-induced hearing loss indicated that the aminoglycosides caused the deafness expression of the C1494T mutation. The phenotypic variability of matrilineal relatives within and among families suggests a role of nuclear modifier genes in the phenotypic manifestation of the C1494T mutation as described other pedigrees carrying the A1555G mutation [19,20]. Furthermore, the mtDNA variants have been shown to modulate the phenotypic manifestation of the deafness-associated mtDNA mutations. The haplogroup J specific variants T4216C and G13708A may increase the penetrance of hearing loss associated with A7445G mutation [16], while the haplogroup L1b specific variants ND1 T3308C and $tRNA^{Ala}$ T5655C likely caused higher penetrance of deafness in an African pedigree than Japanese and French families carrying the T7511C mutation [15,17]. mtDNA variants A14693G, T15908C, T10454C, G7444A, and G5821A may contribute to higher penetrance of hearing loss in five Han Chinese pedigrees carrying the A1555G mutation [10,23,27], while the T5628C variant may influence the phenotypic

Table 1
mtDNA variants in two Chinese families with hearing loss

Gene	Position	Replacement	Conservation (H/B/M/X)[a]	CRS[b]	BJ4	BJ5	Previously reported[c]
D-loop	73	A to G		A	G	G	Yes
	194	C to T		C	T	T	Yes
	249	Del A		A	A	A	Yes
	310	T to CTC		T	CTC	CTC	Yes
	489	T to C		T	C	C	Yes
	16093	T to C		T	C	C	Yes
	16129	G to A		G	A	A	Yes
	16223	C to T		C	T	T	Yes
	16298	T to C		T	C		Yes
	16519	T to C		T	C		Yes
12S rRNA	750	A to G	A/G/G/-	A	G	G	Yes
	1438	A to G	A/A/A/G	A	G	G	Yes
	1494	C to T	C/C/C/C	C	T	T	Yes
16S rRNA	1715	C to T	C/A/A/A	C	T	T	Yes
	2213	A to AAA	A/A/A/A	A	AAA	AAA	Yes
	2706	A to G	A/G/A/A	A	G	G	Yes
ND1	3552	T to A		T	A	A	Yes
ND2	4715	A to G		A	G	G	Yes
	4769	A to G		A	G	G	Yes
CO1	6026	T to A		G	A	A	Yes
	6488	T to C		T	C	C	No
	7028	C to T		C	T	T	Yes
	7196	C to A		C	A	A	Yes
	7444	G to A (Ter to Lys)		G	A	A	Yes
A8	8508	A to G (Asn to Ser)	N/N/K/N	A	G	G	No
A6	8584	G to A (Ala to Thr)	A/V/V/I	G	A	A	Yes
	8701	A to G (Thr to Ala)	T/S/L/Q	A	G		Yes
	8860	A to G (Thr to Ala)	T/A/A/T	A	G	G	Yes
CO3	9540	T to C		T	C	C	Yes
	9545	A to G		A	G	G	Yes
ND3	10398	A to G (Thr to Ala)	T/T/T/A	A	G	G	Yes
	10400	C to T		C	T	T	Yes
ND4	10873	T to C		T	C	C	Yes
	11969	G to A (Ala to Thr)	A/A/G/A	G	A	A	Yes
	11719	G to A		G	A	A	Yes
ND5	12672	A to G		A	G	G	No
	12705	C to T		C	T	T	Yes
	13263	A to G		A	G	G	Yes
ND6	14318	T to C (Asn to Ser)	N/N/D/S	T	C	C	Yes
CYTB	14766	C to T (Thr to Ile)	T/S/I/S	C	T	T	Yes
	14783	T to C		T	C	C	Yes
	15043	G to A		G	A	A	Yes
	15204	T to C (Ile to Thr)	I/I/I/K	T	C	C	Yes
	15301	G to A		G	A	A	Yes
	15326	A to G (Thr to Ala)	T/M/I/I	A	G	G	Yes
	15487	A to T		A	T	T	Yes
$tRNA^{Pro}$	15968	T to C	T/T/A/G	T	C	C	Yes

[a] Conservation of amino acid for polypepides or nucleotide for rRNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X).
[b] CRS: Cambridge reference sequence [33].
[c] See the online mitochondrial genome database http://www.mitomap.org.

manifestation of the C1494T mutation in a large Chinese family [29]. The distinct sets of sequence variations in their mitochondrial genomes of these Chinese pedigrees, as shown in Table 2, belong to Eastern Asian haplogroups C4a1 [38], while other six Chinese mitochondrial genomes belong to haplogroups F1a1 and D5a2, D, R, and F1 [3,28–30] and mtDNAs from three Spanish families belong to haplogroups H, U5b, and U6a [39], respectively. Furthermore, other three Chinese mitochondrial genomes harboring the G7444A mutation belong to haplogroups C5a and D4a, respectively [10,31]. This suggests that the C1494T or G7444A mutation, similar to the A1555G mutation [23,24], occurred sporadically and multiplied through evolution of the mtDNA. Despite the presence of distinct sets of mtDNA variants, there was the absence of other functionally significant mtDNA mutations in these two Chinese families. These data suggest that these mtDNA haplogroup-specific variants may not play an important role in the deafness expression of the C1494T and G7444A mutations in those Chinese families.

Table 2
Summary of clinical and molecular data for eight Chinese families carrying the C1494T mutation

Pedigree	Number of matrilineal relatives	Penetrance (including the use of drugs)[a] (%)	Penetrance (excluding the use of drugs) (%)	mtDNA haplogroup
BJ4	10	20	10	C4a1
BJ5	34	18	15	C4a1
BJ1[b]	40	51	31	D
BJ2[c]	9	77.8	22.2	R
BJ3[d]	65	23.1	20	F1
WZD101[e]	39	28.2	20.5	F1a1
WZD102	30	20	13.3	F1a1
WZD103	13	15.4	7.7	D5a2a

[a] Affected matrilineal relatives/total affected matrilineal relatives.
[b] Zhao et al. (2004) [3].
[c] Wang et al. (2006) [28].
[d] Han et al. (2007) [29].
[e] Chen et al. (2007) [30].

Acknowledgments

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