LETTER TO JMG

Molecular analysis of the mitochondrial 12S rRNA and tRNA$^{Ser(UCN)}$ genes in paediatric subjects with non-syndromic hearing loss

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Hearing loss is a very common congenital disorder affecting one in 1000 newborns. More than 50% of deafness cases in the paediatric population have a genetic cause with autosomal dominant, autosomal recessive, X-linked, or mitochondrial patterns of inheritance.[1] Mutations in mitochondrial DNA (mtDNA), particularly in the 12S rRNA and tRNA$^{Ser(UCN)}$ genes, have been found to be one of the most important causes of sensorineural hearing loss (SNHL).[2 3] The homoplasmic A1555G mutation in the highly conserved decoding site of the mitochondrial 12S rRNA has been found to be associated with both aminoglycoside-induced and non-syndromic SNHL in many families of different ethnic origins.[4–8] Recently, the homoplasmic C1494T mutation in the same gene has also been found to be associated with aminoglycoside-induced and non-syndromic SNHL in a large Chinese family.[9] In addition, a C-insertion or deletion at position 961 of the 12S rRNA gene has been shown to be associated only with aminoglycoside-induced deafness.[10 11] Furthermore, the mitochondrial tRNA$^{Ser(UCN)}$ appears to be another hot spot for mutations associated with hearing impairment, as five deafness-associated mutations have been identified in the mitochondrial tRNA$^{Ser(UCN)}$ gene: A7445G,[12 13] 7472insC,[14] T7510C,[15] T7511C,[16] and T7512C.[17] However, non-syndromic deafness-associated mtDNA mutations, such as the A1555G[4–8] or A7445G[12–13] mutation, are often not sufficient to produce the clinical phenotype since some individuals carrying these mutations have normal hearing. Thus, other factors including other mtDNA mutations/polymorphisms and/or nuclear backgrounds or environmental factors modulate the phenotypic variability and penetrance of deafness associated with these mtDNA mutations.

However, little is known about the incidence of these deafness-associated mtDNA mutations. Furthermore, it is anticipated that additional deafness-associated mutations will be found in the 12S rRNA and tRNA$^{Ser(UCN)}$ genes. A retrospective database review of all children with SNHL and subsequent molecular analysis has been initiated in the clinical population at the Center for Hearing and Deafness Research (CHDR) at the Cincinnati Children's Hospital Medical Center (CCHMC). In the present study, a mutational analysis of the 12S rRNA and tRNA$^{Ser(UCN)}$ genes was performed in 164 SNHL subjects from this clinic and 226 unaffected individuals from a comparable ethnic background. This analysis led to the identification of 17 nucleotide changes including the novel G to T transversion at position 961 (T961G) in the 12S rRNA gene in five affected subjects. To elucidate the molecular basis for the T961G mutation, we completed the mitochondrial genome sequence from these five affected subjects. To examine the role of the *GJB2* gene in the phenotypic expression of the known or putative deafness-associated mtDNA mutations, we also performed mutational screening of the *GJB2* gene in subjects carrying the mtDNA mutations.

Key points

- We report here the systematic mutational screening of the mitochondrial 12S rRNA and tRNA$^{Ser(UCN)}$ genes in 164 paediatric subjects with sporadic non-syndromic deafness. We showed that the frequency of the A1555G mutation is 0.6% in this Caucasian paediatric population with non-syndromic deafness. The novel homoplasmic T961G mutation in the 12S rRNA gene has been identified in five subjects with non-syndromic deafness but not in 226 controls.
- Sequence analysis of the mitochondrial genome in these five subjects revealed that these subjects exhibited distinct sets of mtDNA polymorphism in addition to the identical T961G mutation. This implies that the T961G mutation may be associated with non-syndromic deafness as insertion or deletion at this position has been found to be associated with aminoglycoside-induced deafness in several genetically unrelated families.
- Furthermore, three variants (A827G, T1180G, and C1226G) in the 12S rRNA gene, which are localised at highly conserved sites, have been found in the affected individuals but are absent in 226 controls, indicating that they may play a role in the pathogenesis of deafness. Surprisingly, we failed to detect the known deafness-associated mutations in the mitochondrial tRNA$^{Ser(UCN)}$ gene.
- These data strongly suggest that the mitochondrial 12S rRNA gene is the hot spot for deafness-associated mutations.

METHODS

Subjects

A total of 164 Caucasian subjects, who were younger than 19 years and had been diagnosed with non-syndromic sensorineural hearing impairment at the Cincinnati Children's Hospital Medical Center, participated in this investigation. A comprehensive history and physical examination were performed to identify any syndromic findings or genetic

Abbreviations: ABR, auditory brainstem response; CCHMC, Cincinnati Children's Hospital Medical Center; CHDR, Center for Hearing and Deafness Research; mtDNA, mitochondrial DNA; PTA, pure-tone audiometry; SNHL, sensorineural hearing loss; TEOAEs, transiently evoked otoacoustic emissions

Table 1 Variants in the mitochondrial 12S rRNA gene in 164 hearing-impaired pediatric subjects

Position	Replacement	No. of subjects	Conservation* (H/B/M/X)	Frequency†	Previously reported‡
709	G to A	13	G/A/A/-	36/226	Yes
751	A to G	1	A/G/G/-	1/226	No
769	G to A	9	G/A /T/-	4/226	Yes
813	A to G	1	A/A/C/T	1/226	No
825	T to A	1	T/A/A/A	2/226	Yes
827§	**A to G**	1	**A/A/A/A**	0/226	Yes
921	T to C	1	T/C/C/T	2/226	Yes
930	G to A	5	G/G/C/T	9/226	Yes
951	G to A	1	G/G/T/A	1/226	Yes
961	**T to G**	5	**T/T/A/A**	0/226	No
1018	G to A	10	G/A/A/G	2/226	Yes
1180	**T to G**	1	**T/T/T/T**	0/226	No
1189	T to C	3	T/T/T/C	15/226	Yes
1226	**C to G**	1	**C/C/C/C**	0/226	No
1442	G to A	2	G/A/A/C	4/226	No
1555	**A to G**	1	**A/A/A/A**	0/226	Yes
1598	G to A	1	G/A/T/T	3/226	Yes

*Conservation of nucleotide for the 12S rRNA in human (H), bovine (B), mouse (M) and *Xenopus laevis* (X); †Numbers of controls with variant/total controls; ‡See http://www.mitomap.org; §Known and putative pathogenic mutations are indicated in boldface.

factors related to hearing loss. An age-appropriate audiological examination was performed including pure-tone audiometry (PTA) and/or auditory brainstem response (ABR), immittance testing, and transiently evoked otoacoustic emissions (TEOAEs). The PTA was calculated from the sum of the audiometric thresholds at 500, 1000, and 2000 Hz. The severity of hearing impairment was classified into five grades: mild (20–39 dB), moderate (40–54 dB), moderate severe (55–69 dB), severe (70–89 dB), and profound (90 dB or greater). Informed consent was obtained from all subjects prior to their participation in the study, in accordance with the Cincinnati Children's Hospital Medical Center Institutional Review Board. The 226 control DNA used for screening for the presence of mtDNA mutations were obtained from a panel of unaffected individuals from comparable ethnic backgrounds.

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 subjects' DNA fragments spanning the entire mitochondrial 12S rRNA gene or $tRNA^{Ser(UCN)}$ gene were amplified by PCR using oligodeoxynucleotides corresponding to the mitochondrial genome at positions 618–635 and 1988–2007,[9] and 7151–7170 and 8504–8623,[12 18 19] respectively. Each fragment was purified and subsequently analysed by direct sequencing in an ABI 3700 automated DNA sequencer using the BigDye Terminator Cycle Sequencing Reaction Kit. mtDNA sequence alignments were carried out using the SeqWeb program GAP (GCG).

The entire mitochondrial genomes of five affected subjects carrying the G961T mutation were PCR amplified in 24 overlapping fragments using the light- and heavy-strand oligonucleotide primers, as described elsewhere.[20] 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 number: NC_001807).[21]

Quantification of the mtDNA mutations

For detection of the A1555G mutation, the amplified segments were digested with the restriction enzyme *Bsm*AI.[4 8 22] For analysis of the T961G mutation, DNA fragments were amplified by PCR using oligodeoxynucleotides corresponding to the mtDNA at positions 592–613 and 1155–1173, and the resultant PCR fragments were digested with the restriction enzyme *Aci*I as this T961G mutation creates a site for *Aci*I. Equal amounts of various digested samples were then analysed by electrophoresis through 1.5% agarose gel. The proportions of digested and undigested PCR product were determined by laser densitometry after ethidium bromide staining to determine if the A1555G or T961G mutation is present in homoplasmy in these subjects.

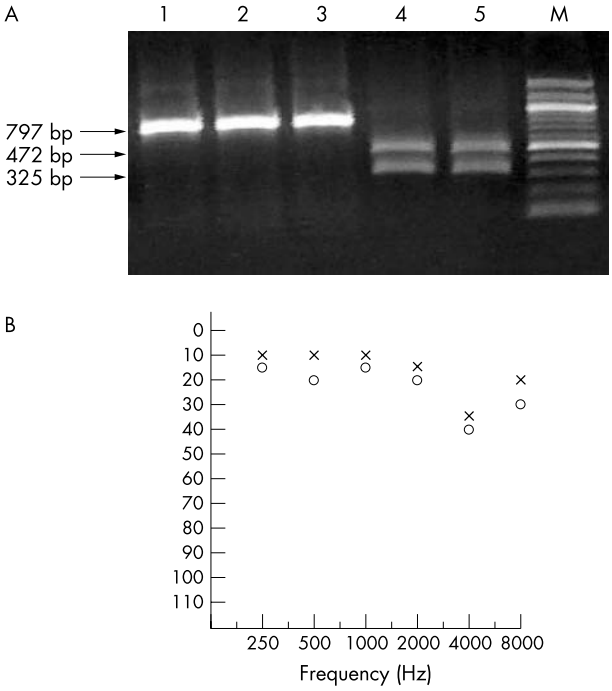

Figure 1 Molecular and audiometric analysis of the affected subject #21 carrying the A1555G mutation. (A) Qualification of the A1555G mutation. PCR products around the A1555G region were digested with *Bsm*AI and analysed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. Lane 1, undigested PCR product; lane 2, #21; lane 3, F11B[22]; lane 4, 143B; and lane 5, control #1. (B) Air conduction audiogram of the affected subject with the A1555G mutation. X, left ear; O, right ear.

Mutational analysis of *GJB2* gene

The DNA fragments spanning the entire coding region of the *GJB2* gene were amplified by PCR using the following oligodeoxynucleotides: forward 5′TATGACACTCCCCAGCAC AG3′ and reverse 5′GGGCAATGCTTAAACTGGC3′. PCR amplification and subsequent sequencing analysis were performed as detailed elsewhere.[23] The results were compared with the wild type *GJB2* sequence (GenBank accession number: M86849) to identify the mutations.

RESULTS AND DISCUSSION

In this study, we have performed a systematic and extended mutational screening of the mitochondrial 12S rRNA and $tRNA^{Ser(UCN)}$ genes in the clinical population of CHDR at the Cincinnati Hospital Medical Center. DNA fragments spanning these two genes were PCR amplified and each fragment was purified and subsequently analysed by DNA sequencing. We failed to detect the presence of the A7445G, T7510C, 7472insC, T7511C, or T7512C mutations in the $tRNA^{Ser(UCN)}$ gene in 164 affected and 226 control subjects. However, the C7476T variant in the $tRNA^{Ser(UCN)}$ gene, which was previously described in a control population,[24] was found in two affected and two control subjects. These data suggest that the deafness-associated mutations in the $tRNA^{Ser(UCN)}$ gene are not common in this Caucasian population.

Comparison of the resultant sequence with the Cambridge consensus sequence[21] identified 17 nucleotide changes in the 12S rRNA gene as shown in table 1. All the nucleotide changes were verified by sequence analysis of both strands and appeared to be homoplasmic. The deafness-associated C1494T mutation in this gene was absent in this hearing impaired population. However, sequence analysis identified one subject carrying the A1555G mutation. In this case, restriction enzyme digestion and subsequent electrophoresis analysis indicated that the A1555G mutation was present in homoplasmy (fig 1A). As shown in fig 1B, audiological evaluation revealed that this subject with the A1555G mutation had mild hearing loss. Further clinical data revealed that this subject had no history of exposure to aminoglycosides. This result indicates a frequency of about 0.6% for the A1555G mutation in this Caucasian sporadic paediatric deafness population. These data are comparable with previous reports that the frequency of this mutation ranged from 0.6 to 1.8% in other ethnic groups.[25–28]

Interestingly, the novel T to G transversion at position 961 (T961G), as shown in fig 2A, was identified in five affected individuals. To determine if the T961G mutation was present with homoplasmy in these individuals, DNA fragments were PCR amplified, digested with *Aci*I, and analysed by agarose gel electrophoresis. As can be seen in fig 2B, there was no detectable wild type DNA in the five mutant individuals, indicating that the T961G mutation appears to be homoplasmic. Furthermore, this T961G mutation was absent in 226 Caucasian and 364 Chinese control subjects.[9] Audiometric studies, as shown in fig 3, revealed variable severity of hearing loss in the individuals, ranging from mild to profound. Audiometric configuration also showed these subjects with flat, U-shaped, and up-sloping patterns, respectively. None of the five patients had mixed or conductive hearing loss. Clinical data revealed that these subjects had no history of exposure to aminoglycosides. Further clinical and genetic evaluations of some matrilineal

Table 2 mtDNA variants in the five affected subjects with the T961G mutation

Gene and position	Nucleotide change	Conservation in H/B/M/X*	CRS†	#91	#221	#222	#246	#256	Previously reported‡
Loop									Yes
143	G→A		G		A	A			Yes
150	C→T		C					T	Yes
152	T→C		T						Yes
195	T→C		T		C	C	C	C	Yes
198	C→T		C	T					Yes
263	A→G		A	G	G	G	G	G	Yes
310	T→TC		T	TC	TC	TC	TC	TC	Yes
16278	C→T		C		T	T			Yes
16293	A→G		A	G	G	G	G	G	Yes
16311	T→C		T	C	C	C	C	C	Yes
12S rRNA									
750	A→G		A	G	G	G	G	G	Yes
961	T→G		T	G	G	G	G	G	No
1438	A→G		A	G	G	G	G	G	Yes
ND2									
4769	A→G		A	G	G	G			Yes
4820	G→A		G	A					No
CO1									
5902	T→C		T	C				C	No
CO2									
7906	C→T		C		T	T			Yes
A8									
8447	A→C (Met to Leu)	M/V/V/V	A	C	C	C	C	C	No
A6									
8860	A→G (Thr to Ala)	T/A/A/T	A	C	G	G	G	G	Yes
8898	C→T		C		T	T			Yes
CO3									
9911	C→T		C	T					No
ND5									
13759	G→A (Ala to Thr)	A/T/T/I	G		A	A	A	A	Yes
ND6									
14587	A→G		A					G	No
Cyto b									
15217	G→C		G					C	No
15326	A→G (Thr to Ala)	T/M/I/I	A	G	G	G	G	G	Yes

*Conservation of amino acid for polypeptide in human (H), bovine (B), mouse (M), and *Xenopus lavis* (X); †Cambridge reference sequence; ‡See the online mitochondrial genome database MITOMAP.

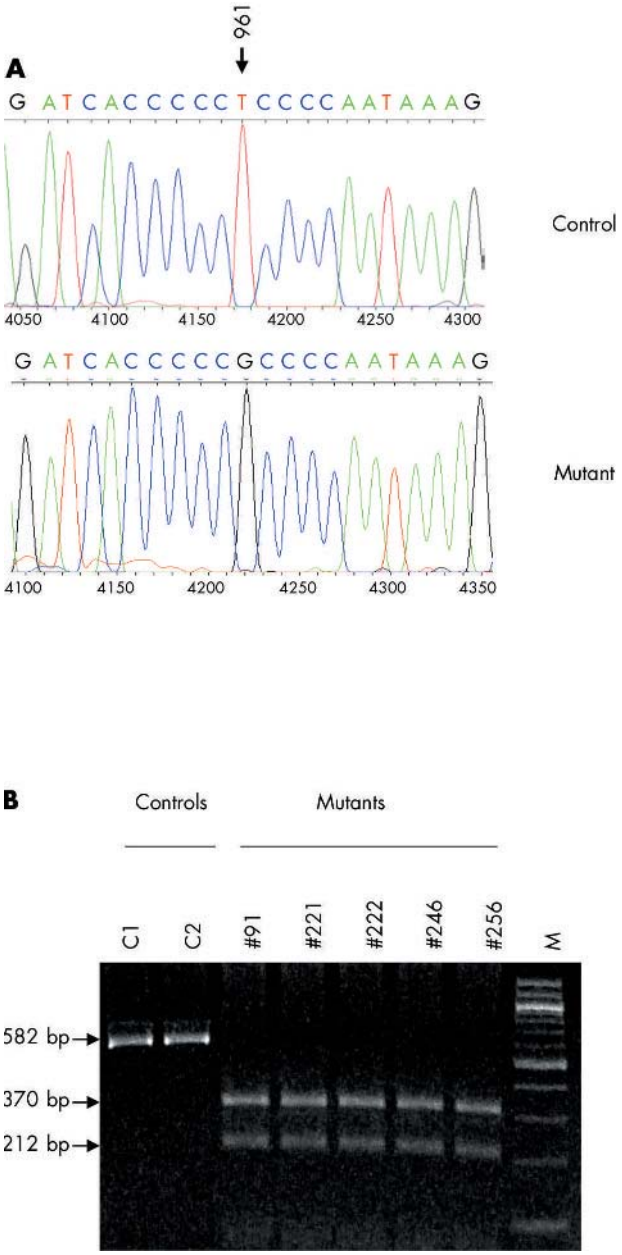

Figure 2 Identification and quantification the T961G mutation. (A). Sequence chromatograms from an affected individual #91 and an unaffected control showing the G to T transversion at position 961 (arrows). (B) Quantification of the T961G mutation. PCR products around the T961G region were digested with *Aci*I and analysed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. Five patients and two control individuals are indicated.

relatives have been performed. Indeed, subjects #221 and #222 with moderate non-syndromic hearing impairment are twin sisters. As shown in fig 4, the familial history of subject #246 suggests maternally transmitted inheritance. In this family, seven of 24 matrilineal relatives suffered from hearing impairment. The incomplete penetrance of the T961G mutation, similar to that of other non-syndromic deafness-associated mtDNA mutations such as the A1555G,[4–8] suggests the involvement of other factors such as mitochondrial haplotype or nuclear modifier genes in the phenotypic expression of the T961G mutation. To elucidate the molecular basis and the role of the mitochondrial haplotype in the phenotypic expression of the T961G mutation, we also performed PCR amplification of fragments spanning the entire mitochondrial genome and subsequent DNA sequence analysis in these five subjects. As shown in table 2, subjects #221 and #222 shared an identical mitochondrial genome including the T961G mutation, while other subjects exhibited distinct sets of mtDNA polymorphism, in addition to the identical T961G mutation. The fact that an insertion or deletion at position 961 occurs in genetically unrelated families, including Chinese,[9] Japanese,[29] and Italian families,[10] affected by aminoglycoside-induced deafness, implies that mutations at position 961 are important for the pathogenesis of hearing loss. In fact, the 961insC in the 12S rRNA gene has been implicated as having a role in the phenotypic expression of the A1555G mutation in a large Chinese pedigree.[8] The 961 mutation localises at the C-cluster of the region between loops 21 and 22 of 12S rRNA.[30] This region is not evolutionarily conserved and its function is not well defined. It is possible that alteration of the tertiary or quaternary structure of this rRNA by the 961 mutations may result in a mitochondrial translational defect. To further understand the pathogenetic mechanism of the T961G mutation, it will be necessary to functionally characterise cell lines derived from these affected subjects.

As regards other nucleotide changes, 11 variants in the 12S rRNA gene were previously identified in the control population.[24] Furthermore, the A751G, A813G, T1180G, C1226G and G1442A variants in the 12S rRNA gene are probably novel polymorphisms in the Caucasian population. These variants in the 12S rRNA gene were examined to determine allelic frequency by sequencing the PCR fragments spanning the 12S rRNA gene derived from 226 Caucasian controls. Indeed, three variants (A827G, T1180G, and C1226G) in the 12S rRNA were not present in the 226 controls. These variants in the 12S rRNA genes were further evaluated by phylogenetic analysis of these mtDNA variants and mtDNAs from other organisms. Interestingly, the A827G, T1180G, and C1226G variants in the 12S rRNA gene are localised at sites which are highly conserved in human,[21] mouse,[31] bovine,[32] and *Xenopus laevis*,[33] whereas other variants in the same gene are not evolutionarily conserved. Strikingly, the T1180G variant disrupts a very conservative G-U base pairing at loop 30 of 12S rRNA, while the C1226G variant breaks an evolutionarily

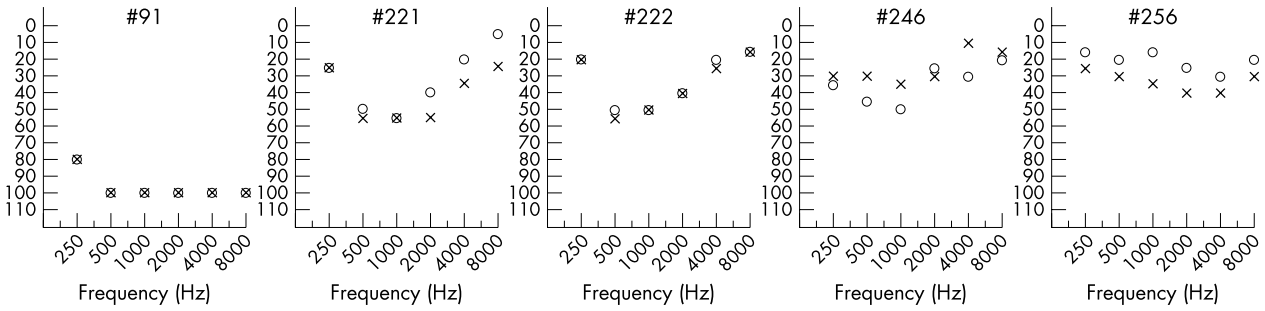

Figure 3 Air conduction audiograms of five affected subject with the T961G mutation. X, left ear; O, right ear.

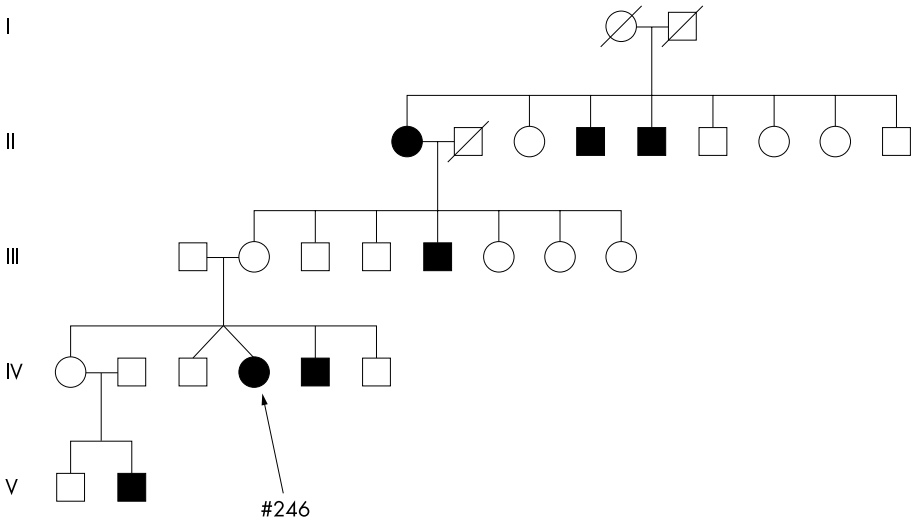

Figure 4 The Caucasian pedigree with non-syndromic hearing impairment. Hearing impaired individuals are indicated by filled symbols. Arrow denotes subject #246.

conservative C-G base pairing at loop 33 of this rRNA.[30] Alteration of the tertiary or quaternary structure of this rRNA by these variants may lead to mitochondrial dysfunction, thereby playing a role in the pathogenesis of non-syndromic deafness.

Our previous data showed that expression of the clinical phenotype of deafness-associated homoplasmic mutation(s) in the 12S rRNA gene, such as the A1555G [4 8 22] and C1494T mutations,[9] requires the contribution of modulating factors including aminoglycosides or nuclear modifier genes. The Connexin 26 (*GJB2)* gene is a potential candidate modifier gene as mutations in this gene are the most common cause of hereditary hearing loss.[34] In particular, it has been implied that *GJB2* mutations modulate the severity of hearing loss associated with the mitochondrial 12S rRNA A1555G mutation.[35] To examine the role of the *GJB2* gene in phenotypic expression of the known or putative deafness-associated mtDNA mutations, we performed mutational screening of the *GJB2* gene in subjects carrying the A1555G, T961G, A827G, T1180G, and C1226G mutations in the 12S rRNA gene. Sequence analysis revealed the absence of mutation in the *GJB2* gene in subjects carrying the A1555G, A827G, T1180G, and C1226G mutations in the 12S rRNA gene. Of five subjects carrying the T961G mutation, the homozygous or heterozygous 35delG mutation in the *GJB2* gene was found in patients #91 with congenital profound hearing impairment and #246 with moderate/late onset hearing impairment, respectively, whereas the other three subjects with mild-moderate/early onset hearing impairment lack the mutation(s) in the same gene (table 3). The profound hearing impairment in subject #91 is likely due to the 35delG mutation in the *GJB2* gene. In fact, the homozygous 35delG mutation accounts for about 30% of genetic causes of non-syndromic SNHL in Caucasians.[34 36] Indeed, the heterozygous 35delG is the prevalent founder allele with normal hearing carrier rates of about 3–4% in individuals of southern European descent.[36] Here, the fact that there are no differences in the severity and age of onset in hearing impairment between a subject with the homoplasmic T961G mutation with the heterozygous 35delG mutation of the *GJB2* gene and another three T961G carriers without mutations in *GJB2* indicates that the heterozygous 35delG mutation of the *GJB2* gene may not be a modifier of the phenotypic effects of the T961G mutation in these subjects.

In summary, the results reported here demonstrate that the frequency of the A1555G mutation is 0.6% in the Caucasian paediatric population with non-syndromic deafness. The novel T961G mutation in the 12S rRNA gene appears to be associated with non-syndromic deafness. Three novel variants, A827G, T1180G, and C1226G, in the 12S rRNA gene, localised at highly conserved sites, may play a role in the pathogenesis of non-syndromic deafness. In this clinical population, the deafness-associated mutations in the mitochondrial $tRNA^{Ser(UCN)}$ gene appear to be less frequent than in the 12S rRNA gene.

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Table 3 Summary of clinical and molecular data for five affected subjects with the T961G mutation in the mitochondrial 12S rRNA gene

Patient	Sex	Audiometric configuation	Age of onset (years)	PTA (dB) right	PTA (dB) left	Level of hearing impairment	35 delG in *GJB2*
91	F	Flat	0	>100	>100	Profound	+/+
221	F	U-shaped	6	48.3	55	Moderate	-
222	F	U-shaped	6	46.7	48.3	Moderate	-
246	F	Up-sloping	~15	41.7	31.7	Moderate	+/-
256	M	Flat	6	18.3	26.7	Mild	-

F, female; M, male.

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Conflict of interest: none declared.

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REFERENCES

1 **Morton CC**. Genetics, genomics and gene discovery in the auditory system. *Hum Mol Genet* 2002;**11**:1229–40.
2 **Fischel-Ghodsian N**. Mitochondrial deafness mutations reviewed. *Hum Mutat* 1999;**13**:261–70.
3 **Guan MX**. Molecular pathogenetic mechanism of maternally inherited deafness. *Ann NY Acad Sci* 2004;**1011**:259–271.
4 **Prezant TR**, Agapian JV, Bohlman MC, Bu X, Öztas S, Qiu WQ, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, Shohat M, Fischel-Ghodsian N. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet* 1993;**4**:289–94.
5 **Estivill X**, Govea N, Barceló A, Perelló E, Badenas C, Romero E, Moral L, Scozzari R, D'Urbano L, Zeviani M, Torroni A. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment with aminoglycosides. *Am J Hum Genet* 1998;**62**:27–35.
6 **Matthijs G**, Claes S, Longo-Bbenza B, Cassiman J-J. Non-syndromic deafness associated with a mutation and a polymorphism in the mitochondrial 12S ribosomal RNA gene in a large Zairean pedigree. *Eur J Hum Genet* 1996;**4**:46–51.
7 **del Castillo FJ**, Rodriguez-Ballesteros M, Martin Y, Arellano B, Gallo-Teran J, Morales-Angulo C, Ramirez-Camacho R, Cruz Tapia M, Solanellas J, Martinez-Conde A, Villamar M, Moreno-Pelayo MA, Moreno F, del Castillo I. Heteroplasmy for the 1555A>G mutation in the mitochondrial 12S rRNA gene in six Spanish families with non-syndromic hearing loss. *J Med Genet* 2003;**40**:632–6.
8 **Li R**, Xing G, Yan M, Cao X, Liu XZ, Bu X, Guan MX. Cosegregation of C-insertion at position 961 with A1555G mutation of mitochondrial 12S rRNA gene in a large Chinese family with maternally inherited hearing loss. *Am J Med Genet* 2004;**124A**:113–7.
9 **Zhao H**, Li R, Wang Q, Yan Q, Deng JH, Han D, Bai Y, Young WY, Guan MX. Maternally inherited aminoglycoside-induced and non-syndromic deafness associated with the novel C1494T mutation in the mitochondrial 12S rRNA gene in a large Chinese family. *Am J Hum Genet* 2004;**74**:139–52.
10 **Bacino C**, Prezant TR, Bu X, Fournier P, Fischel-Ghodsian N. Susceptibility mutations in the mitochondrial small ribosomal RNA gene in aminoglycoside induced deafness. *Pharmacogenetics* 1995;**5**:165–72.
11 **Casano RA**, Johnson DF, Bykhovskaya Y, Torricelli F, Bigozzi M, Fischel-Ghodsian N. Inherited susceptibility to aminoglycoside ototoxicity: genetic heterogeneity and clinical implications. *Am J Otolaryngol* 1999;**20**:151–6.
12 **Fischel-Ghodsian N**, Prezant TR, Fournier P, Stewart IA, Maw M. Mitochondrial mutation associated with non-syndromic deafness. *Am J Otolaryngol* 1995;**16**:403–8.
13 **Reid FM**, Vernham GA, Jacobs HT. A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum Mutat* 1994;**3**:243–7.
14 **Tiranti V**, Chariot P, Carella F, Toscano A, Soliveri P, Girlanda P, Carrara F, Fratta GM, Reid FM, Mariotti C, Zeviani M. Hearing impairment and neurological dysfunction associated with a mutation in the mitochondrial $tRNA^{Ser(UCN)}$ gene. *Hum Mol Genet* 1995;**4**:1421–7.
15 **Hutchin TP**, Parker MJ, Young ID, Davis AC, Pulleyn LJ, Deeble J, Lench NJ, Markham AF, Mueller RF. A novel mutation in the mitochondrial $tRNA^{Ser(UCN)}$ gene in a family with non-syndromic sensorineural hearing impairment. *J Med Genet* 2000;**37**:692–4.
16 **Sue CM**, Tanji K, Hadjigeorgiou G, Andreu AL, Nishino I, Krishna S, Bruno C, Hirano M, Shanske S, Bonilla E, Fischel-Ghodsian N, DiMauro S, Friedman R. Maternally inherited hearing loss in a large kindred with a novel T7511C mutation in the mitochondrial DNA $tRNA^{Ser(UCN)}$ gene. *Neurology* 1999;**52**:1905–8.
17 **Jaksch M**, Klopstock T, Kurlemann G, Dorner M, Hofmann S, Kleinle S, Hegemann S, Weissert M, Muller-Hocker J, Pongratz D, Gerbitz KD. Progressive myoclonus epilepsy and mitochondrial myopathy associated with mutations in the $tRNA^{(Ser(UCN)}$ gene. *Ann Neurol* 1998;**44**:635–40.
18 **Guan MX**, Enriquez JA, Fischel-Ghodsian N, Puranam R, Lin CP, Marion MA, Attardi G. The deafness-associated mtDNA 7445 mutation, which affects $tRNA^{Ser(UCN)}$ precursor processing, has long-range effects on NADH dehydrogenase ND6 subunit gene expression. *Mol Cell Biol* 1998;**18**:5868–79.
19 **Li X**, Fischel-Ghodsian N, Schwartz F, Yan Q, Friedman RA, Guan MX. Biochemical characterization of the mitochondrial $tRNA^{Ser(UCN)}$ T7511C mutation associated with nonsyndromic deafness. *Nucleic Acids Res* 2004;**32**:867–77.
20 **Rieder MJ**, Taylor SL, Tobe VO, Nickerson DA. Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. *Nucleic Acids Res* 1998;**26**:967–73.
21 **Anderson S**, Bankier AT, Barrell BG, deBruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Rose BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young I. Sequence and organization of the human mitochondrial genome. *Nature* 1981;**290**:457–65.
22 **Guan MX**, Fischel-Ghodsian N, Attardi G. Biochemical evidence for nuclear gene involvement in phenotype of non-syndromic deafness associated with mitochondrial 12S rRNA mutation. *Hum Mol Genet* 1996;**5**:963–71.
23 **Lim LH**, Bradshaw JK, Guo Y, Pilipenko V, Madden C, Ingala D, Keddache M, Choo DI, Wenstrup R, Greinwald JH Jr. Genotypic and phenotypic correlations of DFNB1-related hearing impairment in the Midwestern United States. *Arch Otolaryngol Head Neck Surg* 2003;**129**:836–40.
24 **Kogelnik AM**, Lott MT, Brown MD, Navathe SB, Wallace DC. MITOMAP: a human mitochondrial genome database – 1998 update. *Nucleic Acids Res* 1998;**26**:112–5.
25 **Usami SI**, Abe S, Akita J, Namba A, Shinkawa H, Ishii M, Iwasaki S, Hoshino T, Ito J, Doi K, Kubo T, Nakagawa T, Komiyama S, Tono T, Komune S. Prevalence of mitochondrial gene mutations among hearing impaired patients. *J Med Genet* 2000;**37**:38–40.
26 **Hutchin TP**, Thompson KR, Parker M, Newton V, Bitner-Glindzicz M, Mueller RF. Prevalence of mitochondrial DNA mutations in childhood/congenital onset non-syndromal sensorineural hearing impairment. *J Med Genet* 2001;**38**:229–31.
27 **Kupka S**, Tóth T, Wróbel M, Zeißler U, Szyfter W, Szyfter K, Niedzielska G, Bal J, Zenner HP, Sziklai I, Blin N, Pfister M. Mutation A1555G in the 12S rRNA gene and its epidemiological importance in German, Hungarian and Polish patients. *Hum Mutat* 2002;**19**:308–9.
28 **Tekin M**, Duman T, Bogoclu G, Incesulu A, Comak E, Fitoz S, Yilmaz E, Ilhan I, Akar N. Frequency of mtDNA A1555G and A7445G mutations among children with prelingual deafness in Turkey. *Eur J Pediatr* 2003;**162**:154–8.
29 **Yoshida M**, Shintani T, Hirao M, Himi T, Yamaguchi A, Kikuchi K. Aminoglycoside-induced hearing loss in a patient with the 961 mutation in mitochondrial DNA. *ORL J Otorhinolaryngol Relat Spec* 2002;**64**:219–22.
30 **Neefs JM**, Van de Peer Y, De Rijik P, Goris A, De Wachter R. Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Res* 1991;**19**(suppl):1987–2018.
31 **Bibb MJ**, Van Etten RA, Wright CT, Walberg MW, Clayton DA. Sequence and gene organization of mouse mitochondrial DNA. *Cell* 1981;**26**:167–80.
32 **Gadaleta G**, Pepe G, De Candia G, Quagliariello C, Sbisa E, Saccone C. The complete nucleotide sequence of the Rattus norvegicus mitochondrial genome: cryptic signals revealed by comparative analysis between vertebrates. *J Mol Evol* 1989;**28**:497–516.
33 **Roe A**, Ma DP, Wilson RK, Wong JF. The complete nucleotide sequence of the Xenopus laevis mitochondrial genome. *J Biol Chem* 1985;**260**:9759–74.
34 **Friedman TB**, Griffith AJ. Human nonsyndromic sensorineural deafness. *Annu Rev Genomics Hum Genet* 2003;**4**:341–402.
35 **Abe S**, Kelley PM, Kimberling WJ, Usami SI. Connexin 26 gene (GJB2) mutation modulates the severity of hearing loss associated with the 1555A>G mitochondrial mutation. *Am J Med Genet* 2001;**103**:334–8.
36 **Estivill X**, Fortina P, Surrey S, Rabionet R, Melchionda S, D'Agruma L, Mansfield E, Rappaport E, Govea N, Mila M, Zelante L, Gasparini P. Connexin-26 mutations in sporadic and inherited sensorineural deafness. *Lancet* 1998;**351**:394–8.