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Extremely low penetrance of deafness associated with the mitochondrial 12S rRNA mutation in 16 Chinese families: Implication for early detection and prevention of deafness

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Abstract

Mutations in mitochondrial DNA (mtDNA) have been found to be associated with sensorineural hearing loss. We report here the clinical, genetic, and molecular characterization of 16 Chinese pedigrees (a total of 246 matrilineal relatives) with aminoglycoside-induced impairment. Clinical evaluation revealed the variable phenotype of hearing impairment including audiometric configuration in these subjects, although these subjects share some common features: being bilateral and sensorineural hearing impairment. Strikingly, these Chinese pedigrees exhibited extremely low penetrance of hearing loss, ranging from 4% to 18%, with an average of 8%. In particular, nineteen of 246 matrilineal relatives in these pedigrees had aminoglycoside-induced hearing loss. Mutational analysis of the mtDNA in these pedigrees showed the presence of homoplasmic 12S rRNA A1555G mutation, which has been associated with hearing impairment in many families worldwide. The extremely low penetrance of hearing loss in these Chinese families carrying the A1555G mutation strongly supports the notion that the A1555G mutation itself is not sufficient to produce the clinical phenotype. Children carrying the A1555G mutation are susceptible to the exposure of aminoglycosides, thereby inducing or worsening hearing impairment, as in the case of these Chinese families. Using those genetic and molecular approaches, we are able to diagnose whether children carry the ototoxic mtDNA mutation. Therefore, these data have been providing valuable information and technology to predict which individuals are at risk for ototoxicity, to improve the safety of aminoglycoside therapy, and eventually to decrease the incidence of deafness.

Keywords: Deafness; Mitochondrial 12S rRNA mutation; Aminoglycoside; Penetrance; Ototoxicity; Maternally transmitted; Prevention; Detection; Chinese

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The use of aminoglycoside antibiotics, such as gentamicin, streptomycin, kanamycin, and tobramycin, can lead to irreversible ototoxicity [1,2]. In particular, susceptibility to aminoglycoside-induced hearing loss is maternally inherited in humans in a significant proportion of cases, particularly in the Chinese populations [3–5]. Hu et al. [3] described 36 Chinese families with maternally transmitted predisposition to aminoglycoside ototoxicity, while Higashi reported that 26 of 28 families with streptomycin-induced deafness had maternally inherited transmission [4]. These drugs are known to exert their antibacterial effects by directly binding to 16S ribosomal RNA (rRNA) in the 30S subunit of the bacterial ribosome, causing mistranslation or premature termination of protein synthesis [6,7]. Mitochondrial ribosomes share more similarities to bacterial ribosomes than do cytosolic ribosomes [8,9]. Therefore, it is proposed that the ototoxic site of aminoglycosides is the mitochondrial ribosome. Thus, mutation(s) in mitochondrial DNA (mtDNA), particularly in 12S rRNA gene, could be the molecular basis for this susceptibility [8,9].

Sequence analyses of the mitochondrial genome in patients with aminoglycoside ototoxicity have led to the identification of several ototoxic mtDNA mutations in the 12S rRNA gene [8,9]. Of those, the C1494T mutation in the highly conserved A-site of 12S rRNA has been associated with both aminoglycoside-induced and nonsyndromic hearing loss in a large Chinese family [10,11]. By contrast, the homoplasmic A1555G mutation in the highly conserved A-site of the 12S rRNA has been associated with both aminoglycoside-induced and nonsyndromic hearing loss in many families worldwide [12–21]. In particular, matrilineal relatives within intra-families or with families carrying the A1555G mutation exhibited the variable expressivity and penetrance of hearing impairment [12,13,15]. Incomplete and variable penetrance of hearing loss as well as a mild biochemical defect associated with this mutation indicated that the A1555G mutation itself is not sufficient to produce the clinical phenotype [12,13,15,19,22,23]. Therefore, aminoglycosides, nuclear modifier genes, and/or other mtDNA mutations/polymorphisms modulate the phenotypic variability and penetrance of deafness associated with the A1555G mutation [22–24].

To understand the pathogenesis of maternally inherited aminoglycoside-induced and nonsyndromic hearing loss, a systematic and extended mutational analysis of mitochondrial 12S rRNA has been initiated at Department of Otolaryngology of the Chinese PLA General Hospital [19–21]. In the present study, we report the clinical, molecular, and genetic characterization of 16 Chinese pedigrees with aminoglycoside-induced hearing loss. Clinical and genetic evaluation revealed extremely low penetrance of hearing loss in these Chinese families. Mutational analysis of 12S rRNA has led to the identification of the A1555G mutation in those families. Those results support the idea that the A1555G mutation is not sufficient to produce a clinical phenotype and aminoglycosides are the major modifier factor for the development of deafness in these Chinese families. Thus, these observations provide a direct evidence for the early diction and prevention of deafness at the high risk populations carrying this mtDNA mutations.

Subjects and methods

Subjects and audiological examinations. As the part of the genetic screening program for hearing impairment, 16 Chinese families were ascertained through the Otology Clinic at Chinese PLA General Hospital. A comprehensive history and physical examination performed to identify any syndromic findings, the history of the use of aminoglycosides, and genetic factors related to hearing impairment in members of these pedigrees. 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 Ethic Committee of Chinese PLA General Hospital.

Mutational analysis of mitochondrial genome. Genomic DNA was isolated from whole blood of participants using Puregene DNA Isolation Kits (Gentra Systems). Subject's DNA fragments spanning the entire mitochondrial 12S rRNA gene were amplified by PCR using oligodeoxynucleotides corresponding to positions 618–635 and 1988–2007 [25]. For the detection of the A1555G mutation, the amplified segments were first digested with a restriction enzyme *Bsm*AI [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 laser densitometry after ethidium bromide staining to determine if the A1555G mutation is in homoplasmy in these subjects. To confirm the presence of the A1555G mutation in each proband, the amplified segments were purified and subsequently 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) [26].

Results and discussion

To further elucidate the molecular basis of aminoglycoside ototoxicity, we have performed a mutational analysis of the mitochondrial 12S rRNA gene in a cohort of Chinese subjects, who were diagnosed as aminoglycoside ototoxicity by the Otology Clinic at the Chinese PLA General Hospital. First, DNA fragments spanning mitochondrial 12S rRNA were PCR amplified from each affected subject. Each fragment was digested by restriction enzyme *Bsm*AI and subsequent electrophoresis analysis. Of those subjects, as shown in Fig. 1, 16 subjects with aminoglycoside ototoxicity carry the homoplasmic A1555G mutation in the 12S rRNA gene. To confirm the presence of the A1555G mutation in those subjects, these PCR-amplified segments were then purified and subsequently analyzed by DNA sequencing. Indeed, the sequence analysis confirmed the presence of the A1555G mutation in these subjects.

In fact, all those 16 subjects, as shown in Table 1, had been administered aminoglycosides (3–5 mg/kg/dose every