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Biochemical and Biophysical Research Communications 325 (2004) 1503–1508
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Clinical evaluation and sequence analysis of the complete mitochondrial genome of three Chinese patients with hearing impairment associated with the 12S rRNA T1095C mutation

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Received 21 October 2004
Available online 11 November 2004

Abstract

Mutations in mitochondrial DNA (mtDNA), particularly those in the 12S rRNA gene, have been shown to be associated with sensorineural hearing loss. Here we report the clinical and sequence analysis of the entire mitochondrial genome in three Chinese subjects with aminoglycoside-induced and non-syndromic hearing impairment. Clinical evaluation showed a variable phenotype of hearing impairment including the age of onset and audiometric configuration in these subjects. Sequence analysis of the complete mitochondrial genomes in three subjects showed the distinct sets of mtDNA polymorphism, in addition to the identical mitochondrial 12S rRNA T1095C mutation. This mutation was previously identified to be associated with hearing impairment in three families from different genetic backgrounds. The T1095C mutation was absent in 364 Chinese control. In fact, the occurrence of the T1095C mutation in these several genetically unrelated subjects affected by hearing impairment strongly indicates that this mutation is involved in the pathogenesis of hearing impairment. Among other nucleotide changes, the A2238G and T2885C mutations in the 16S rRNA, the I175V mutation in the CO2, the F16L mutation in the A6 and the V112M mutation in the ND6 exhibited a high evolutionary conservation. These data suggest that the T1095C mutation may be associated with aminoglycoside-induced and non-syndromic hearing impairments and A2238G and T2885C mutations in the 16S rRNA, the I175V mutation in the CO2, the F16L mutation in the A6 and the V112M mutation in the ND6 may contribute to the phenotypic expression of the T1095C mutation in these subjects.

Keywords: Hearing loss; Mitochondrial DNA; 12S rRNA; Mutation; Aminoglycoside ototoxicity; T1095C; Chinese; Variant

Mitochondrial DNA (mtDNA) mutations have been found to be associated with sensorineural hearing loss [1,2]. In particular, the 12S rRNA gene has been shown to be a hot spot for aminoglycoside-induced and non-syndromic hearing loss. Several deafness-associated mtDNA mutations have been identified in this gene. Of those, the A1555G mutation in the highly conserved decoding site of the 12S rRNA has been associated with both aminoglycoside-induced and non-syndromic hearing loss in many families of different ethnic backgrounds [3–7]. Similarly, the C1494T mutation in the highly conserved decoding site of this rRNA has been associated with both aminoglycoside-induced and non-syndromic hearing loss in a large Chinese family [8]. In addition, a C-insertion or deletion at position 961 of the 12S rRNA gene has been shown to be associated only with

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doi:10.1016/j.bbrc.2004.10.199

aminoglycoside-induced deafness [9,10], while the novel T961G mutation has been implicated to be responsible for the non-syndromic hearing loss in five Caucasian pediatric subjects [11]. Furthermore, the T1095C mutation has also been shown to be associated with hearing impairment [12–14].

With the aim of identifying mtDNA mutations associated with hearing loss, a systematic and extended mutation screening of the mitochondrial 12S rRNA gene has been initiated in the large clinical population of Otology Clinic at the Chinese PLA General Hospital. As a consequence of this study, 34 pedigrees with a maternally inherited pattern of non-syndromic and aminoglycoside-induced hearing loss have been identified, including 15 pedigrees carrying the A1555G mutation and one pedigree carrying the C1494T mutation in the 12S rRNA gene [8]. In the present study, we have performed a clinical characterization and sequence analysis of the complete mitochondrial genome in three Chinese patients with hearing impairment associated with the T1095C mutation in the 12S rRNA gene.

Subjects and methods

Subjects and audiological examinations. As part of genetic screening program for the hearing impairment, three hearing-impaired subjects were ascertained at the Otology Clinic at PLA General Hospital. A comprehensive history and physical examination were performed to identify any syndromic findings or the history of the use of aminoglycosides, genetic factors related to the hearing impairment. 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 the participant 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, the 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 mitochondrial genome at positions 618–635 and 1988–2007 [8,15] and 7148–7167 and 8076–8095 [16,17], 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. mtDNA sequence alignments were carried out using seqweb program GAP (GCG).

The entire mitochondrial genomes of the subject carrying the T1095C mutation were PCR amplified in 24 overlapping fragments by use of sets of the light-strand and the heavy-strand oligonucleotide primers, as described elsewhere [18]. 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) [19].

Results

Clinical presentation

The subject #78 came to the otology clinic at the Chinese PLA General Hospital at the age of 9 years. He received a dose of gentamicin (40 mg) for fever at the age of 5. He began suffering bilateral hearing impairment one month after administration. As illustrated in Fig. 1, the audiological evaluation, including the pure-tone audiometry, immittance, and ABR, showed that he had moderate hearing impairment (62 dB at right ear, 55 dB at left ear) with flat-shaped pattern.

The subject #081 complained of fluctuant hearing loss at the age of 10 years. As shown in Fig. 1, the audiological evaluation revealed that he suffered from moderate hearing impairment (58 dB at right ear, 58 dB at left ear) with U-shaped pattern. He did not have a history of aminoglycoside administration.

The subject #101 is a male of 55 years. Although his hearing was normal according to the assessment criteria, pure-tone audiometry test showed a Hill-shaped pattern, as shown in Fig. 1. In particular, his hearing was reduced to 30 dB at 250 Hz and 55 dB at 8000 Hz. He has not received aminoglycoside administration.

Comprehensive family medical histories of those three subjects showed no other clinical abnormalities, including diabetes, muscular diseases, visual problems, and neurological disorders. Furthermore, karyotype

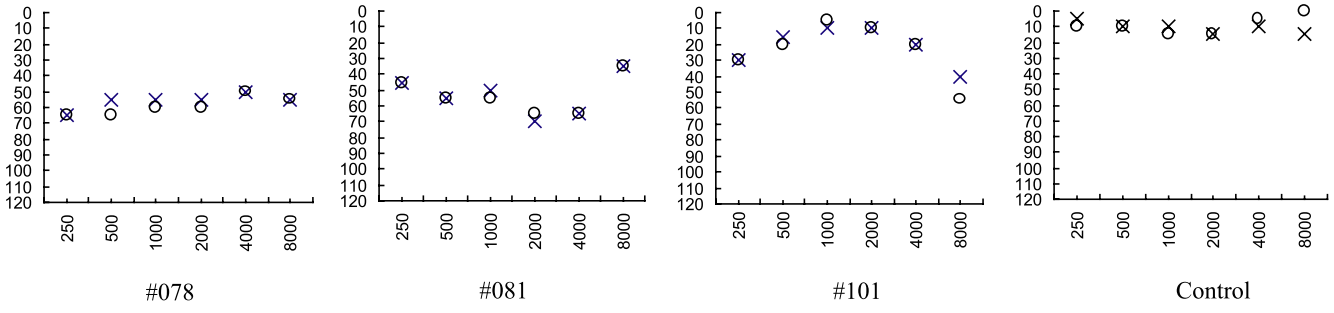

Fig. 1. Air conduction audiogram of three affected subjects with the T0195C mutation. Symbols: (×) left and (○) right ear.