Mitochondrial COI/tRNA$^{Ser(UCN)}$ G7444A mutation may be associated with aminoglycoside-induced and non-syndromic hearing impairment

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Abstract. Mutations in mitochondrial DNA (mtDNA) have been reported to have important roles in aminoglycoside-induced hearing impairment; however, the underlying molecular mechanisms have remained largely elusive. The current study presented a case of a Chinese patient with maternally inherited aminoglycoside-induced hearing impairment. A profound hearing impairment was identified by clinical evaluation; furthermore, analysis of the mitochondrial genome sequence of the patient revealed the presence of an A1555G mutation in the 12S rRNA as well as a G7444A mutation in the COI/tRNASer(UCN) gene. As the G7444A mutation is highly conserved between various species, it may be a modifying factor with regard to the pathological effects of the A1555G mutation.

Introduction

Hearing loss is one of the most common type of sensory disorder, affecting ~120 million patients worldwide and can be caused by gene mutations and external factors, including aminoglycoside antibiotics (1-3). The well-known A1555G mutation in the human mitochondrial 12S rRNA gene has been associated with aminoglycoside-induced and non-syndromic hearing loss (AINHL) in numerous pedigrees all over the world (4-6). Aminoglycoside inevitably induces hearing impairment in individuals carrying the 12S rRNA A1555G mutation. In the absence of aminoglycosides, matrilineal relatives in families carrying the A1555G mutation exhibited a wide range of clinical phenotypes, age of onset as well as various degrees of penetrance and severity of hearing loss (5). In addition, the mitochondrial tRNASer(UCN) gene was shown to be a hotspot for pathogenetic mutations associated with deafness of the sensorineural type, with mutations including A7445G, 7472insC, T7510C and T7511C (7-9). Furthermore, the G7444A mutation in the cytochrome *c* oxidase sub-unit I (COI)/tRNASer(UCN) gene is highly conserved between various species (10).

With the aim of elucidating the molecular basis of hearing loss, an extensive genomic screening analysis for mutations in the mitochondrial (mt)-tRNASer(UCN) and 12S rRNA genes was performed in the Shaoxing area (China). The present study presented the case of a patient from this study cohort who had a family history of AINHL. Sequence analysis of the patient's mitochondrial genome revealed the presence of COI/tRNASer(UCN) G7444A and 12S rRNA A1555G mutations.

Patients and methods

The patient was a 19 year-old male from Zhejiang Province (China) who was treated for hearing loss at Shaoxing People's Hospital (Shaoxing, China). The medical history of the patient and his family was assessed and a physical examination was performed to identify any syndromic manifestations. The patient had a history of treatment with aminoglycoside (3-5 mg/kg gentamycin every 8 h) after hospitalization due to pneumonia with fever at the age of 15 years and developed a bilateral hearing impairment two months later. In addition, the patient's mother, who was also impaired of hearing, had a history of using aminoglycosides (kanamycin) during pregnancy. Informed consent to participate in the present study was obtained from the patient, and the protocol of the present study was approved by the Ethics Committee of Shaoxing People's Hospital (Shaoxing, China). In addition, 268 healthy individuals residing in the Shaoxing area were recruited as controls, whose the DNA was obtained at the Department of Otolaryngology (Shaoxing People's Hospital,

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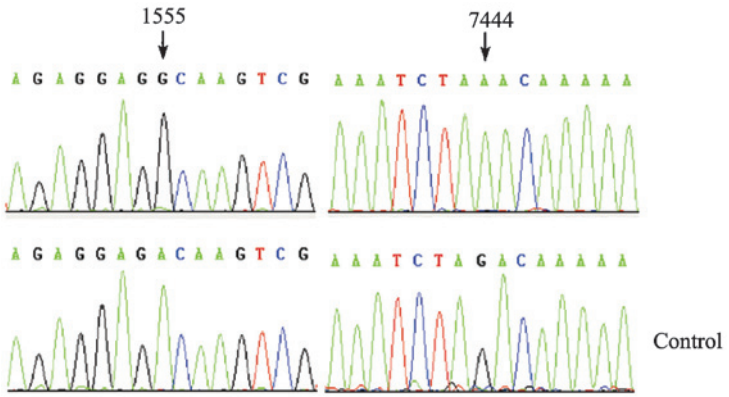

Figure 1. Identification of the 12S rRNA A1555G and COI/tRNASer(UCN) G7444A mutations. Partial sequence chromatograms of the 12S rRNA and COI/tRNA$^{Ser(UCN)}$ genes from the patient. Arrows indicate the 1555 and 7444 positions.

Shaoxing, China) with written informed consent provided by all individuals.

Auditory examinations, including pure-tone audiometry, auditory brainstem response, immittance testing and determination of distortion product otoacoustic emissions were performed. The degree of hearing loss was classified into five levels: Normal, <26 dB; mild, 26-40 dB; moderate, 41-70 dB; severe, 71-90 dB; and profound, >90 dB.

The genomic DNA was isolated from the blood of the patient using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). DNA fragments containing the mt-tRNASer(UCN) and 12S rRNA genes were amplified by polymerase chain reaction as previously described (11). The primers used were as follows: tRNASer(UCN) forward, 5'-ACGAGTACACCGACTACGGC-3' and reverse, 5'-TGG GTGGTTGGTGTAAATGA-3'; 12S rRNA forward, 5'-CGA TCA ACCTCACCACCTCT-3' and reverse, 5'-TGGACA ACCAGCTATCACCA-3'. In addition, the coding regions of connexin 26 (*GJB2*) gene mutations were amplified using the following primers: Forward, 5'-TATGACACTCCCCAGCAC AG-3' and reverse, 5'-GGGCAATGCTTAAACTGGC-3' (9). The PCR primers were supplied by BGI (Shenzhen, China) and the PCR mixture included 200 μM dNTP, 10X buffer, Taq DNA polymerase and 15 mmol/l Mg^{2+} (Takara Biotechnology Co., Ltd., Dalian, China). Each amplified DNA sample was purified and analyzed using the ABI 3700 automated DNA sequencer and the Big Dye Terminator Cycle sequencing reaction kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). For screening of the mutations in the mitochondrial genome, the sequence data were compared with the reversed Cambridge sequence (GenBank accession no. NC_012920) (12) and for screening the mutations in the *GJB2* gene, the results were compared with the wild-type *GJB2* sequence (GenBank accession no. M86849).

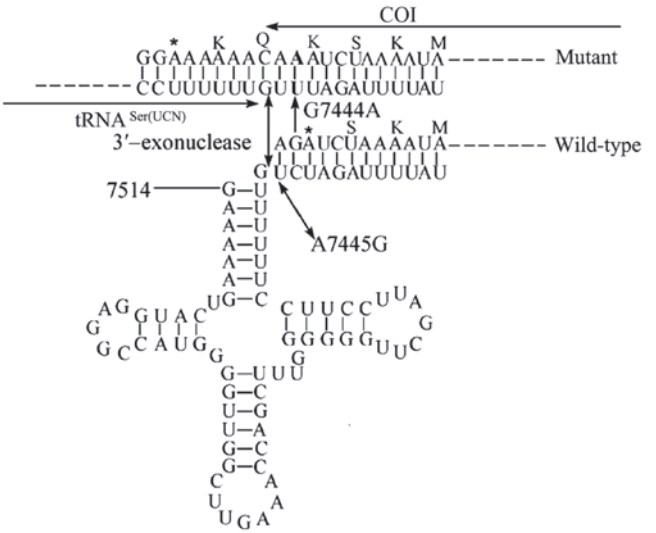

Figure 2. Location of the G7444A mutation in tRNA$^{Ser(UCN)}$ and adjacent COI. The processing site for the 3'-end of tRNA$^{Ser(UCN)}$ precursor, determined by 3'-endonuclease, is indicated by an arrow. COI, cytochrome *c* oxidase sub-unit I.

Results and Discussion

Clinical evaluation showed that the patient had a profound hearing impairment. The patient had been treated with gentamycin at the age of 15 years and developed a bilateral hearing impairment two months after the drug administration. The patient's father's hearing was normal; however, the patient's mother had a hearing impairment and had been treated with kanamycin during pregnancy. Due to these clinical characterizations, the mitochondrial genome of the patient was screened for mutations. The mitochondrial gene sequence data (Fig. 1) were compared with the Mitomap database (http://www.mitomap.org/MITOMAP), which revealed the presence of the homoplasmic 12S rRNA A1555G and tRNASer(UCN) G7444A mutations. To further elucidate the putative role of *GJB2* gene mutations in the phenotypic outcome of the A1555G mutation, the patient was subjected to mutational screening of *GJB2*; however, as no sequence variants in the *GJB2* gene were identified, it is unlikely to be involved. Other nuclear genes may contribute to the phenotypic outcome of the A1555G mutation.

In 1993, Prezant *et al* investigated the underlying molecular mechanisms of AINSHL in three Chinese families and a large Arab-Israeli family with maternally inherited hearing impairment by complete mitochondrial genome analysis to identify an A-to-G replacement at the 1555 position in the 12S rRNA gene (13). In fact, the homoplasmic A1555G mutation was localized at the highly evolutionarily conserved

aminoacyl-tRNA acceptor site (A-site) of the small ribosomal sub-unit (14). This mutation was found in numerous families with maternally inherited, non-syndromic hearing loss and also in patients with hearing loss following use of aminoglycosides (15). Biochemical characterization of cybrid cells containing the A1555G mutation revealed that they exhibited reduced mitochondrial protein synthesis, oxygen consumption and growth rate in galactose medium (16). However, individuals carrying the A1555G mutation presented with a variety of clinical phenotypes, including incomplete penetrance and varying degrees of hearing loss, which indicated that other factors, including environmental factors, nuclear genes and mitochondrial haplogroups, may contribute to the clinical manifestation of hearing impairment associated with the A1555G mutation (17). However, the present study did not detect any common variants in the *GJB2* gene, which suggested that this nuclear gene may not be involved in the manifestation of hearing loss due to the A1555G mutation.

Sequence analysis of the mitochondrial tRNASer(UCN) gene led to the identification of a homoplasmic G7444A mutation (Fig. 1). The G7444A mutation was located in the COI/precursor of tRNASer(UCN) genes; this mutation was previously found to be associated with Leber's Hereditary Optic Neuropathy and is considered to be a secondary aberration leading to increases in the penetrance of the primary mutation, whereas the G7444A mutation alone did not produce the clinical phenotype (18). Furthermore, the homoplasmic A7445G mutation was reported to reduce tRNASer(UCN) levels by ~70% and to cause a 45% reduction in mitochondrial protein synthesis in cybrid cells containing this mutation (19). Structurally, the G7444A mutation is similar to the A7445G mutation and causes a read-through of the stop-codon AGA in the COI sequence, leading to the addition of three amino acids (Lys-Gln-Lys) to the C-terminus of the polypeptide (Fig. 2). This leads to the hypothesis that the G7444A mutation may inhibit mitochondrial protein synthesis, which in turn affects the synthesis of adenosine triphosphate, increases the production of reactive oxygen species and consequently leads to damage of hair cells and cochlear neurons in the ear. Therefore, the combination of the G7444A and A1555G mutations may be responsible for the hearing impairment of the patient of the present study.

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