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Sensorineural hearing loss caused by mutations in two alleles of both GJB2 and SLC26A4 genes

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ABSTRACT

Background: Most studies of the molecular etiology of sensorineural hearing loss have described deafness as a monogenic disease encompassing double-allele mutations for patients with autosomal recessive deafness. Here, we report the first case of autosomal recessive genetic deafness in an enlarged vestibular aqueduct syndrome (EVAS) patient with biallelic mutations in two deafness genes.
Methods: Temporal computed tomography (CT), complete physical and otoscopic examinations, and an audiological study, including tympanometry, pure-tone audiometry or auditory steady-state response (ASSR), were carried out. Exon 2 of *GJB2* and the coding exons of *SLC26A4* were sequenced.
Results: A patient with an enlarged vestibular aqueduct was found to carry c.1229C>T/c.1079C>T compound heterozygous mutations in *SLC26A4*.This individual also carried c.257C>G/c.299-300delAT compound heterozygous mutations in *GJB2*. As a result, the recurrent risk of the patient's siblings increased significantly from 25% for typical autosomal recessive deafness to 43.75%.
Conclusions: The findings of the present study challenge the traditional diagnostic strategy in which testing is generally considered complete upon identification of a double-allele mutation within one gene, with significant implications for genetic counseling and risk prediction. Our results suggest that, with advances in sequencing technology, it will be possible and necessary to test all known deafness genes in the near future, as this will likely allow more accurate genetic counseling of patients.

1. Introduction

Hearing impairment is the most common neurosensory disorder in humans, with an incidence of 1 in 300 to 1 in 1000 children [1–3], and approximately half of all cases are caused by genetic defects. Genetic components underlie both syndromic and non-syndromic forms of deafness with a wide range of heterogeneities. Non-syndromic deafness accounts for 60–70% of cases of inherited hearing impairment, with autosomal recessive being the most common form. In many ethnic populations, the common causes of non-syndromic sensorineural hearing loss are associated with mutations in *GJB2* [4–7], *SLC26A4* [8–10], or mitochondrial *12S rRNA* genes [11,12]. Sensorineural hearing loss is generally considered a monogenic disease in which disorder the inactivation of one of the genes is enough to cause the disease. Accordingly, in most clinics, testing is considered complete upon detection of double-allele mutations (compound heterozygous or homozygous mutations) in one gene, which allows a final diagnosis to be made. Here, we report one enlarged vestibular aqueduct syndrome (EVAS) patient with compound mutations in both *SLC26A4* and *GJB2*. Our results illustrate the complexity of the etiology of hearing impairment, which requires additional steps for the detection of underlying mutations to improve genetic counseling and risk prediction.

2. Materials and methods

A 12-year-old Chinese boy with severe–profound symmetrical sensorineural hearing loss (Fig. 1) was examined at the Department of Otolaryngology, Chinese PLA General Hospital. He suffered prelingual hearing impairment without fluctuant features and absence of goiter. He could communicate with simple words before diagnosis of hearing loss at the age of 2 years. Computed

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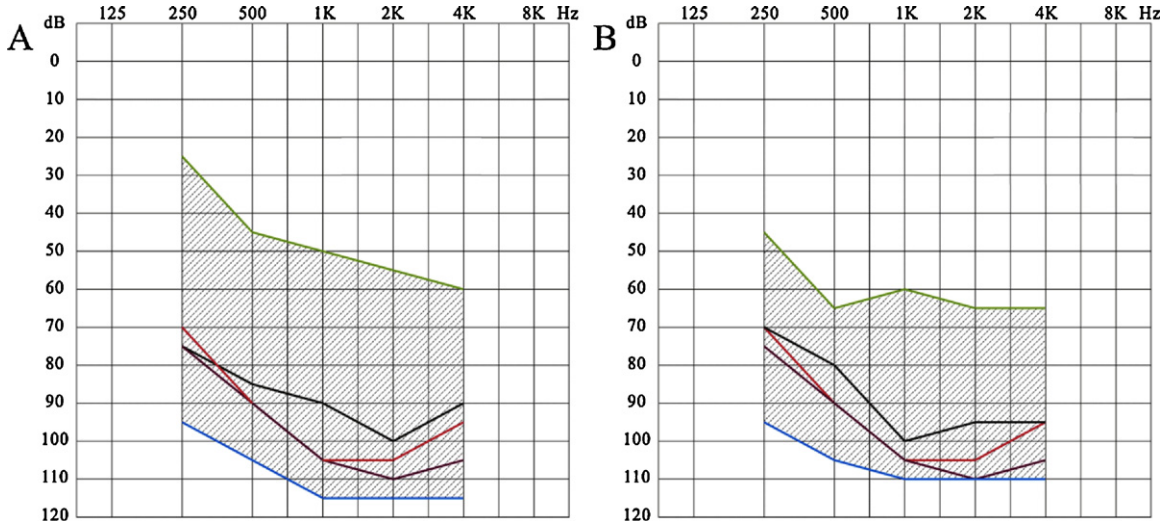

Fig. 1. Hearing level of the patient at different ages. Red curve: The hearing level of the patient at 10 years old. Purple curve: The hearing level of the patient at 12 years old. The green, blue, and black lines represent the best, worst, and average hearing levels, respectively, at frequencies of 0.25, 0.5, 1.0, 2.0, 4.0 kHz of two groups of patients with similar *GJB2* (Part A) or *SLC26A4* (Part B) mutation combinations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

tomography (CT) indicated that he had a bilateral enlarged vestibular aqueduct (EVA, Fig. 2). Other members of his family have normal hearing. A complete physical and otoscopic examination as well as audiological studies were carried out, including pure tone audiometry and tympanometry. No individuals in the family were found to have any goiter or syndromic signs in other systems.

After obtaining informed consent, this study was performed according to the protocol approved by the Ethics Committee of the Chinese PLA General Hospital. DNA was extracted from peripheral blood leukocytes using a commercially available DNA extraction kit (Watson Biotechnology, Inc., Shanghai, China). DNA sequence analysis of the *GJB2* and *SLC26A4* genes was performed by polymerase chain reaction (PCR) amplification of the coding exons plus approximately 50–100 bp of the flanking intron regions using primers described previously [13,14]. The reaction mixture contained 100 ng of DNA, 1.5 units of DNA Taq polymerase (TaKaRa, Dalian, China), 200 μM dNTPs, 3 pmol of each forward and reverse primer, and 2.5 μL of 10× buffer (containing 2.5 mM $MgCl_2$), and the final reaction mixture was made up to 25 μL with ddH_2O. The exons of the genes were amplified according to the PCR conditions. The PCR amplification products were sequenced and analyzed using an ABI 3130 DNA sequencing machine (ABI, Foster City, CA) and ABI 3130 Analysis Software (v.3.7 NT), according to the manufacturer's instructions.

To compare the hearing level of this patient with other patients with identical mutations, we selected 33 EVAS patients with the p.T410M and c.919-2A>G compound heterozygous mutations (27 cases) or p.A360V and c.919-2A>G compound heterozygous mutations (6 cases), 23 with the p.T86R and c.235delC compound heterozygous mutations (20 cases) or p.T86R and c.299-300delAT compound heterozygous mutations (3 patients) as the control groups.

3. Results

Mutational analysis identified compound heterozygosity for the c.257C>G (p.T86R) and c.299-300delAT mutations in *GJB2*, and the c.1229C>T (p.T410M) and c.1079C>T (p.A360V) mutations in *SLC26A4* in the proband. His father was confirmed to carry the c.299-300delAT mutation in *GJB2* and p.A360V in *SLC26A4*, and his mother carried the p.T86R mutation in *GJB2* and p.T410M in *SLC26A4*. The results suggested that his parents were both coincident *GJB2* and *SLC26A4* mutation carriers, but with normal hearing (PTA of the parents was 11.25 dB HL and 12.5 dB HL respectively) and CT results (Fig. 2).

The patterns and severity of hearing loss in the 33 EVAS patients with the p.T410M or p.A360V mutations and 23 with the p.T86R and c.299-300delAT (or p.T86R and c.235delC) compound heterozygous mutations were comparable to the results in our patient (see examples in Fig. 1). The severity of hearing impairment in the control cohorts with GJB2 and SLC26A4 is very wide ranging, from moderate to profound deafness.

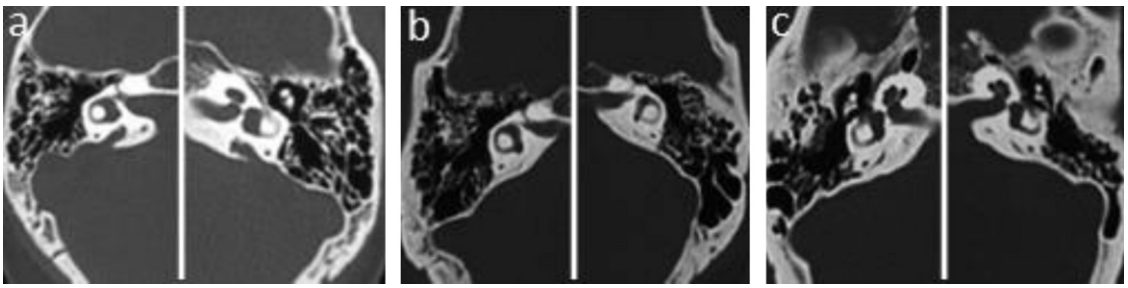

Fig. 2. Temporal bone images of the family. (A): Temporal bone CT scan showed that the patient had a bilateral enlarged vestibular aqueduct (EVA). (B): Normal temporal bone CT scan of the father. (C): Normal temporal bone CT scan of the mother.