

## Novel compound heterozygous mutations in *SLC26A4* gene in a Chinese family with enlarged vestibular aqueduct

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### Summary

In order to investigate the genetic causes of hearing loss in a Chinese proband with nonsyndromic hearing loss and enlarged vestibular aqueduct (EVA), we conducted clinical and genetic evaluations in a deaf proband and her parents with normal hearing. 20 exons and flanking splice sites of the *SLC26A4* gene were screened for pathogenic mutations by PCR amplification and bidirectional sequencing. As a control, a group of 400 healthy newborns from the same ethnic background were subjected to *SLC26A4* gene screening using the same method. The proband harbored two mutations in the *SLC26A4* gene in the form of compound heterozygosity. She was found to be heterozygous for a novel mutation c.574delC (p.Leu192Ter) in exon 5 and for the known mutation c.919-2A>G(c.IVS7-2A>G). Her mother was a heterozygous carrier of the c.919-2A>G mutation, and her father was a heterozygous carrier of the c.574delC and therefore co-segregated with the genetic disease. The c.574delC mutation was absent in 400 healthy newborns. The frameshift mutation causes the leucine (Leu) at amino acid position 192 to become a termination codon, leading to termination of protein sequence coding. This study demonstrates that the novel frameshift mutation c.574delC (p.Leu192Ter) in compound heterozygosity with c.919-2A>G in the *SLC26A4* gene is the main cause of deafness in a family. Our study will expand the spectrum of known *SLC26A4* mutations in the Chinese population, providing more information on genetic counseling, and diagnosis in hearing loss with EVA.

**Keywords:** *SLC26A4*, novel mutation, enlarged vestibular aqueduct

### 1. Introduction

*SLC26A4* (OMIM 605646, also named PDS gene, NM\_000441.1) maps on 7q22-31(DFNB4 locus) (1). Domestic epidemiological data shows that *SLC26A4* is the second most common gene that causes nonsyndromic hearing loss (NSHL), accounting for 14.5% (2), which encodes a 780-amino-acid protein called pendrin, a member of the solute carrier 26 protein family that functions as a chloride iodide transporter in cell expression systems (3). There are two clinical phenotypes from mutations in the *SLC26A4* gene: (1) the syndromic

form, called Pendred Syndrome (PS) (OMIM 274600), characterized by hearing loss, goiter and eventually hypothyroidism, with/without EVA or other inner ear malformations; (2) the nonsyndromic form, called DFNB4 or non syndromic EVA (OMIM 600791) (when EVA is present), characterized by hearing loss with/without EVA or other inner ear malformations (4-6). The common mutations of the *SLC26A4* gene show regional and ethnic diversity. To date, about 539 mutations have been identified. (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SLC26A4>)

Enlarged vestibular aqueduct (EVA) is known as an inner ear malformation of the temporal bone that predisposes patients to hearing loss from childhood as well as vestibular symptoms. It is a congenital abnormality that can be diagnosed radiographically in the hearing loss population. Nonsyndromic hearing loss (NSHL) with EVA is typically characterized by congenital, bilateral sensorineural hearing loss (SNHL),

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which can be progressive and usually ranges from severe to profound (7). It is believed that *SLC26A4* gene mutations may cause NSHL associated with EVA, with hearing loss found at birth or during early childhood. According to domestic studies, about 62%-88.4% of patients with EVA can be due to bi-allelic mutations (including homozygous mutations and compound heterozygous mutations). Among those mono-allelic mutations of *SLC26A4* accounts for about 7.4%-24% (8,9).

In this study, we investigated the *SLC26A4* gene in 3 members of a Chinese family associated with EVA. As a result, novel compound heterozygous mutations of *SLC26A4* were identified. This will expand the spectrum of *SLC26A4* mutations in the Chinese population.

## 2. Materials and Methods

Written informed consent was obtained from parents. The protocol was approved by the Declaration of Helsinki principles and Ethics Committee of Beijing Tongren Hospital, Capital Medical University.

### 2.1. Subjects and clinical evaluation

A Chinese family associated with EVA was recruited from the Department of Otolaryngology, Head and Neck Surgery, Beijing Tongren Hospital (Beijing, China). The proband performed with c.919-2A>G single-allele mutation detected by deafness genetic screening (9 variants in 4 genes, including *GJB2* c.235delC, c.299delAT, c.176del6, and c.35delG; *GJB3* c.538C>T; *SLC26A4* c.919-2A>G and c.2168A>G; and Mt *12SrRNA* m.1555A>G and m.1494C>T). Clinical evaluation was conducted including description of family history and detailed medical history, and a physical examination, including thyroid sonography, and a high-resolution computed tomography (CT) scan of the temporal bone. Four hundred unrelated Chinese newborns with normal hearing were recruited as normal controls.

### 2.2. Mutational analysis

Genomic DNA was extracted from 2 ml of whole blood from each patient, using the Blood DNA kit (Tiangen Biotech, Beijing, China). 20 exons and flanking splice sites of the *SLC26A4* gene were screened for mutations by PCR amplification and bidirectional sequencing. ACMG guidelines were used for variant interpretations (10).

### 2.3. Bioinformatics and validation of the variants

Sequence data were analyzed by alignment with the National Center for Biotechnology Information (NCBI) reference sequence of *SLC26A4* (NT\_007933) with

the assistance of DNA Star 5.0 software. The 1000 Genomes Project database (<http://www.1000genomes.org/>), ClinVar (<https://www.st-va.ncbi.nlm.nih.gov/clinvar/>) dbSNP database of NCBI (<http://www.ncbi.nlm.nih.gov/>), and the Deafness Variation Database (<http://deafnessvariationdatabase.org/>) were used as references to assess the novelty of mutations found in this study.

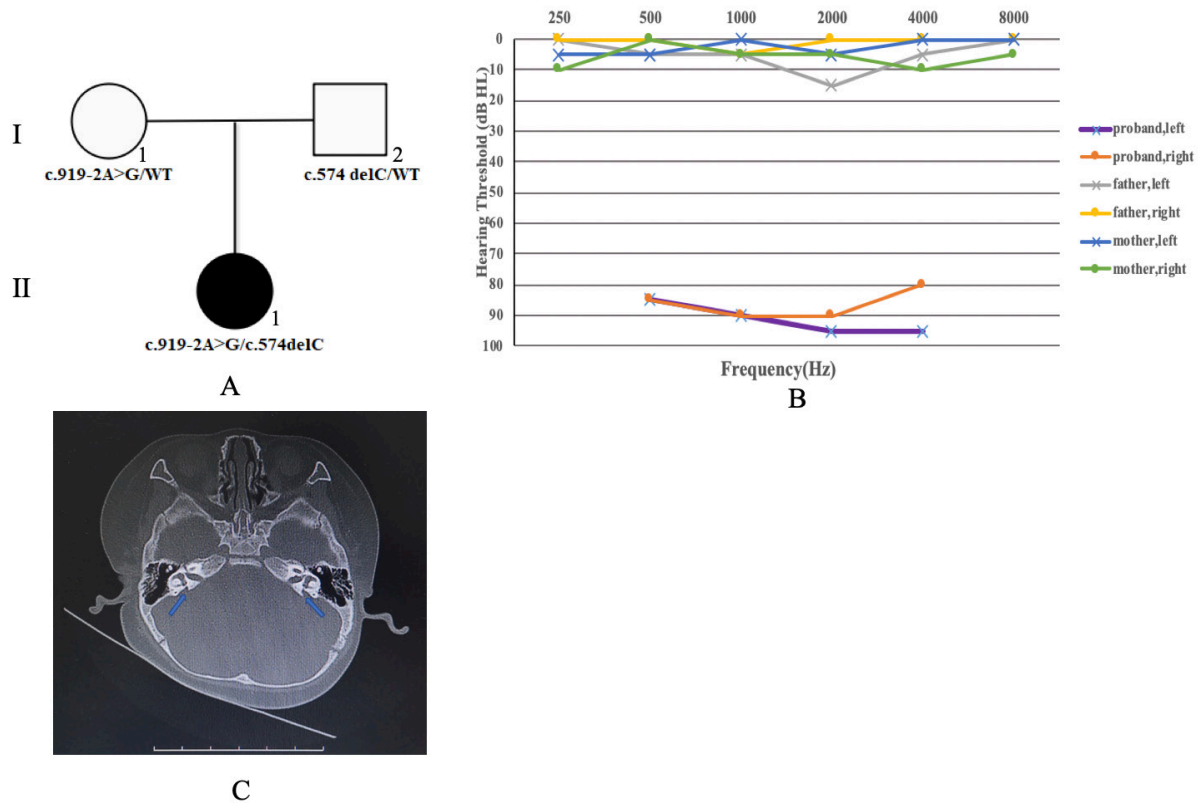
### 2.4. Auditory evaluation

The subject underwent universal newborn hearing screening and had specific results. Comprehensive audiological evaluation included pure tone audiometry (PTA), auditory brainstem response (ABR), 40Hz auditory event-related potential, distortion product otoacoustic emission (DPOAE), auditory steady-state response (ASSR), acoustic immittance, and pediatric behavioral audiometry. The hearing threshold was calculated as the average hearing level at 0.5, 1.0, 2.0, and 4.0 k Hz according to the 1997 World Health Organization standard. The severity of hearing impairment was defined as mild (26-40 dB), moderate (41-60 dB), severe (61-80 dB), or profound (> 80 dB). Owing to the subjects' young age, the ABR threshold and/or ASSR were recorded, and mean thresholds at frequencies in the 0.5-4 k Hz range were averaged to obtain an approximation for directional conditioned reflex (11,12).

## 3. Results and Discussion

All the members were negative for systemic and thyroid disease, and physical examination and otoscopy were also normal. There was one subject aged nine months old with hearing loss in this family (II-1), and pure-tone audiometry revealed normal hearing in two family members (I-1, I-2). The proband (II-1) referred UNHS with two ears and then was diagnosed with bilateral profound SNHL when first seen by a doctor in our hospital at three months old. The air-conduction and bone-conduction of ABR of the proband showed both sides were not elicited as a reproducible wave at 100 dB nHL and 50 dB nHL, respectively. The proband had a tympanogram result of "A", and the bilateral acoustic stapedial reflex was not elicited. DPOAE showed no response from the patient in both ears. A temporal bone CT scan of the proband showed bilateral EVA with the width of the vestibular aqueduct greater than 1.5 mm and she was diagnosed with bilateral large vestibular aqueduct syndrome at seven months old (Figure 1C). The results of pediatric behavioral audiometry in the proband and PTA in her parents are demonstrated in Figure 1B

The sequence analysis of *SLC26A4* indicated that the proband presented compound heterozygosity of a c.919-2A>G (IVS7-2A>G) (rs111033313) mutation in intron7 and a c.574delC (p.Leu192Ter) frameshift mutation in exon 5. Additionally, the mother was a



**Figure 1. Genotypes and clinical phenotype presentations of the pedigree.** (A) Pedigree map of the three families. Squares and circles denote male and female patients, respectively. Abbreviations: WT, wild type. (B) The audiograms of pediatric behavioral audiometry in the proband and PTA in her parents. Frequency in hertz (Hz) is plotted on the x-axis and the hearing level in decibels (dB HL) on the y-axis. (C) The temporal bone CT scan of the proband shows the bilateral enlarged vestibular aqueduct (arrows).



**Figure 2. Sequence electropherograms of abnormal sequences from three members of this family.**

heterozygous carrier of the c.919-2A>G mutation, and the father was a heterozygous carrier of the c.574delC (p.Leu192Ter) mutation. Figure 1A and Figure 2

demonstrate a pedigree map of the three families and sequence electropherograms of abnormal sequences from three members of this family, respectively. The variant c.574delC (p.Leu192Ter) was not reported in ClinVar, Pubmed, Deafness Variation Database, dbSNP, the 1000 Genomes Project database and HGMD, and has never been described in a clinical report. The novel mutation was not found in 400 healthy newborns. The part of *SLC26A4* amino acid sequence alignment demonstrated that the encoded amino acid sequence of c.574delC is only the part before the asterisk, the theoretical amino acid sequence is given from the missing position (arrow), shown in Figure 3. The frameshift mutation causes the leucine (Leu) at amino acid position 192 to become a termination codon, leading to termination of protein sequence coding.

EVA is a genetically autosomal recessive disorder. Subjects with bi-allelic mutations have earlier age of onset, more severe deafness, more fluctuating hearing loss, and larger vestibular aqueduct size than those without mutations (13,14). It is currently known that EVA is closely linked to the *SLC26A4* mutations and the variants have high heterogeneity and ethnic differences. p.V138F (c.412G>T) is the most common mutation in the Czech population (15). p.L236P (c.707T>C), p.T416P (c.1246A>C), and IVS8+1G>A (c.1001+1G>A) are mainly detected in Caucasian (16), and p.H723R



Figure 3. *SLC26A4* amino acid sequence alignment between normal and c.574delC (Part), Asterisk: termination codon.

(c.2168A>G) in Korean (17). The deaf population in South America and North America is dominated by p.V609G (c.1826T>G) mutation and IVS8+1G>A (c.1001+1G>A) mutation, respectively (18). Along with increasing research related to genes, more and more novel mutations have been reported in Chinese patients. c.919-2A>G (IVS7-2A>G) and p.H723R (c.2168A>G) account for the majority of mutations in China (19).

In this study, we found the proband's father and mother (the heterozygous carrier of c.574delC and c.919-2A>G mutation, respectively) both demonstrate normal hearing, while the proband with compound heterozygous mutations c.919-2A>G and c.574delC has profound SNHL, as well as EVA. Therefore, gene mutations transmitted from the parents to offspring indicate the segregation of genotype and phenotype. The splice-site mutation of c.919-2A>G mentioned above is the most prevalent pathogenic mutation of *SLC26A4* in China. Another mutation, c.574delC (p.Leu192Ter), has not been reported in other countries and ethnicities, which causes the leucine (Leu) at amino acid position 192 to become a termination codon, leading to termination of protein sequence coding and therefore, leads to early translational termination at amino acid position 515 in the sulfate transporter and anti-sigma factor antagonist (STAS) domain. Meanwhile, SLC26 STAS domain amino acid position 673 indicates human disease associated with a frameshift (20). However, the STAS domain included in members of the SLC26A family regulates the stability, trafficking, and anion transport function of SLC26A family proteins (20). The structural significance of this domain has been substantiated by the disease-causing nature of mutations among SLC26A family proteins (21). Therefore, it is possible that the novel mutation discovered in our study is closely related to hearing loss. Meanwhile, according to ACMG guidelines, c.574delC is pathogenic.

#### 4. Conclusions

This study demonstrates that the novel frameshift mutation c.574delC (p.Leu192Ter) in compound

heterozygosity with the c.919-2A>G in *SLC26A4* gene is the main cause of deafness in a family. Our study will expand the spectrum of known *SLC26A4* mutations in the Chinese population, providing more information on genetic counseling, and diagnosis of hearing loss with EVA.

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