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Mutation analysis of *SLC26A4* in patients with enlarged vestibular aqueduct in mainland China

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Abstract

OBJECTIVE—We have characterized the spectrum of *SLC26A4* mutations and clinical features in a mainland Chinese population with sensorineural non syndromic hearing loss (SNHL) and enlarged vestibular aqueduct (EVA).

STUDY DESIGN—Cross-sectional clinical genetic study.

SETTING—Tertiary care outpatient otolaryngology clinic.

METHODS—32 subjects identified with bilateral EVA using high resolution computed tomography (CT) were screened for mutations in *SLC26A4* by denaturing high-performance liquid chromatography (DPHLC) and direct sequencing methods.

RESULTS—A total of thirteen different mutations were identified in the *SLC26A4* gene, five of which are novel. A total of 88% of the patients harbored biallelic mutations, eleven patients were homozygotes, and seventeen were compound heterozygotes. Four patients were found to carry a single *SLC26A4* mutation. The IVS7-2A>G mutation was the most frequent, accounting for 60% of the mutant alleles. We have not found any correlations between the type of *SLC26A4* mutations and the type, degree and progression of hearing loss. There are significant proportions of patients with asymmetric (26%), progressive (32%), or fluctuating hearing loss (21%).

CONCLUSION—Our data confirm the high prevalence of *SLC26A4* mutations in Chinese patients with sensorineural hearing loss (SNHL) and EVA. We could not establish any relationship between genotype and phenotype. However, high incidence of asymmetric, progressive, and fluctuating hearing loss found in the current study indicates that patients with those features should be routinely screened for *SLC26A4* mutation in addition to diagnosis of EVA using CT or magnetic resonance imaging.

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INTRODUCTION

SLC26A4 is the gene responsible for both Pendred syndrome (SNHL, enlarged vestibular aqueduct-EVA and goiter) and non-syndromic recessive deafness DFNB4. Located on the long arm of chromosome 7, the *SLC26A4* gene is made up of twenty-one exons. The encoded protein, pendrin, is expressed in nonsensory cells in the cochlear scala media, thyroid, renal, uterine and placental tissue. Pendrin is thought to be involved in anion transport, although it remains unclear how this leads to cochlear dysfunction. Enlargement of the vestibular aqueduct is the most common anomaly associated with mutations of *SLC26A4* and is genetically characterized as a non-syndromic autosomal recessive disorder (DFNB4, OMIM 600791). The molecular genetic basis for the hearing loss associated with EVA was investigated in different ethnic populations. It is clear that EVA is associated with the mutations of the *SLC26A4* (PDS) gene in many racial populations. More than 150 *SLC26A4* mutations have been described in different populations.

In the present study, we screened DNA for *SLC26A4* mutations in a Chinese cohort of deaf probands with SNHL and EVA. Using the audiological classification criteria of genetic deafness proposed by the European Workshop on Genetic Hearing Loss,¹ we analyzed audiological data to characterize the clinical features and spectrum of *SLC26A4* mutations in the mainland Chinese patients.

MATERIALS AND METHODS

Subjects and Audiology

From a Chinese cohort of patients with suspected genetic hearing loss, we identified 32 subjects (3 from multiplex and 29 from simplex families) with EVA using high-resolution computed tomography (HRCT) in the Department of Otolaryngology, Head and Neck Surgery, Chinese People's Liberation Army General Hospital (Beijing, China). The project was approved by the Ethic Committee of Chinese PLA General Hospital. Written informed consent was obtained from all of the participants prior to donation of a blood sample. Criteria for the diagnosis of EVA was a vestibular aqueduct diameter of >1.5 mm measured midway between operculum and the common crus.² All subjects were examined otoscopically, and pure tone audiometry was performed as well as immittance testing (tympanometry and acoustic reflexes) for assessment of the integrity and function of the middle ear. Air conduction thresholds were measured at 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, 6 kHz and 8 kHz and bone conduction thresholds at 0.5, 1, 2, 4 and 6 kHz. When output of the bone conduction audiometer is beyond vibration perception threshold in pure tone test, we are not able to determine whether what patients perceive is sound or vibration; for example, the vibration perception threshold on mastoid process at 250Hz is about 40dB HL. Therefore, we end the test when the output of the bone conduction reaches 40dB, 50dB, 60dB, 60dB and 60dB HL in 250, 500, 1000, 2000, 4000 Hz respectively. For this reason, the maximum output of the machine is always designed to be limited within 70dB and the air bone gaps (ABG) were not obtained for 1000Hz, 2000Hz and 4000Hz. The results of the pure tone audiograms were analysed using the audiological classification criteria of genetic deafness proposed by the European Workshop on Genetic Hearing Loss. The severity of hearing impairment was applied to the better hearing ear, averaged over 0.5, 1, 2, and 4 kHz and was categorized as follows: Mild, >20 dB and ≤40 dB; Moderate, >40 dB and <70 dB; Severe, >70 dB and <95 dB; Profound >95 dB. Asymmetry is defined by >10 dB difference between the ears in at least at two frequencies with a difference in the pure tone average between the ears of >20 dB; Progression – A deterioration of >15 dB in the pure tone average within a 10 year period. A comprehensive history inquiry and physical examinations were performed on all the subjects in order to exclude cases of the syndromic deafness or induced from environmental causes (e.g. aminoglycoside-induced or infections).

Mutation analysis

Genomic DNA sample of each subject was extracted from whole blood using a DNA Blood Kit (Watson Biotechnologies Inc, Shanghai, China). All 21 exons and the intron-exon boundaries of the *SLC26A4* gene were sequenced in each of the subjects with an EVA. In addition, a total of 200 matched controls of Chinese background with normal hearing were screened for *SLC26A4* mutation by high-performance liquid chromatography (DPHLC) followed by direct sequencing of amplicons with abnormal elution profiles. In brief, DNA sample were first amplified by polymerase chain reaction (PCR) using primers flanking each exon. PCR was conducted in a volume of 20µl which contained 40 ng of genomic DNA, 400 µM dNTP, 1.25 units of proTaq DNA polymerase, 0.2M intragenic primers and 2.5mM MgCl₂. Sequencing was performed on an Applied Biosystems 3100-Avant Genetic Analyzer. Sequence data were compared with the published sequence of *SLC26A4* gene (ENSG00000091137) using GeneTool Lite 1.0 software program.

RESULTS

A total of 32 SNHL patients with EVA including 19 males and 13 females were investigated (Table 1). Figure 1 shows a pedigree of a simplex case and the CT scan from a proband patient. All subjects had bilateral SNHL. One patient's audiometric data was not available among the patients (Table 1). Audiogram of ten patients (20 ears) shows a characteristic of conductive or mixed hearing loss in low frequencies (250Hz and 500Hz) and sensorineural hearing loss in middle and high frequencies. The air bone gaps (ABG) of 20 ears ranges from 15dB~90dB at 250Hz and from 20dB~60dB at 500Hz (Table 2). No mild hearing loss was found in the present study. A total of 48% (15/31) of patients with *SLC26A4* mutations had profound loss, 36% (11/31) had severe loss, and 16% (5/31) had moderate loss. Some patients with profound hearing loss may still have some measurable residual hearing, especially in low frequency. We consider the hearing loss progressive when the residual hearing continues to deteriorate in one or both ears. The symmetry of hearing loss detected in the 23 (74%) probands, is reported in Table 1. Twenty-two (69%) patients had a prelingual (≤ 2 year old) onset of hearing loss with a mean age-of-onset of 1.02 years. Ten subjects were found having progressive hearing loss. Average follow-up time was 9.5 years, with a range from 1 to 19 years. Six of the patients had fluctuating hearing loss.

A total of thirteen different mutations were identified (Tables 3). The mutations were biallelic in 28 of the 32 subjects (88%). Of these, 11 subjects have homozygous mutations, 17 were compound heterozygotes. One case had complex allele with three variants. In four probands, only one *SLC26A4* mutation was detected. Clinical characteristics are provided in Table 1. The most common mutation was a splice site mutation in intron 7 (IVS7-2A>G) found in 26 of 32 patients which accounts for 60% (36 /60) mutant alleles. The IVS7-2A>G was detected in 10 homozygotes and 15 compound heterozygotes. Two cases were heterozygous with the second allele remaining unidentified. The ten homozygous patients with IVS7-2A>G had severe to profound deafness. Of the six patients with fluctuating hearing loss, five carried IVS7-2A>G mutation in a compound heterozygous state and one was homozygous for IVS7-2A>G. There was no clear relationship between genotype and phenotype identified.

The second most frequent mutation was p.H723R accounting for 15 % (9/60) mutant alleles. Overall, seven missense mutations, two splice site mutations, and four frameshift mutations were identified. Of these mutations, a total of 5 variants had not been previously reported, including two missense mutations (p.H135R and p.V545A) and three frameshift mutations (c.1555-1556delAA, c.1687-1692delA and c.2082delA). None of these variants was detected in any of the 200 normal hearing subjects of Chinese background. A schematic

distribution of the mutations identified in the present study and reported mutations among Chinese along the *SLC26A4* gene is shown in Figure 2.

Three different previously unreported mutations causing frameshifts with premature termination were detected in this study. The first new deletion is a two-base deletion of AA at nucleotide position 1555-1556 in exon 14. This mutation leads to a frameshift at codon 519, with a stop codon occurring at codon 539 or 20 amino acids residues downstream. This frameshift mutation was detected in a complex allele state with three variants (c.1548insC /c.1555-6delAA/p.H723R) in a proband. The second novel frameshift mutation consists of a deletion of A at nucleotide position 1687-92 in exon 15, which results in a frameshift, starting at codon 563, with a subsequent premature stop codon at the 572th amino acid residue. The mutation was identified in a compound heterozygous state with a splice site mutation IVS7-2A>G. Finally, the c.2082delA was also found in a compound heterozygous state with IVS7-2A>G mutation. This mutation caused a frameshift at codon 694 resulting in a premature stop at codon 699 in exon 18.

In the present study, five missense mutations and one frameshift mutation, including c.1173C>A/p.S391R, c.1174A>T/p.N392Y, c.1226G>A/p.R409H, c.1229C>T/p.T410M, c.2168A>G/p.H723R and c.1548insC have been previously described.³⁻⁶ Two novel missense mutations were identified in the present study. The c.404A>G/p.H135R mutation was detected in a compound heterozygous state with the c.2168A>G/p.H723R. The p.H135R change due to a A>G transition at nucleotide position 404 in exon 4 results in a replacement of histidine, that has a positively charged imidazole functional group with arginine, an amino acid with a positively charged guanidino group. This change occurs within the first extracellular domain of the *SLC26A4* protein. Another new mutation c.1634T>A/p.V545A was identified in a compound heterozygous state with IVS7-2A>G. The two missense changes might be pathogenic on the basis of the following criteria: 1) these substitutions were absent in 200 unrelated control DNA samples of Chinese background; 2) the two newly identified missense changes are located within functional domains of the *SLC26A4* protein; 3) sequence comparison across species demonstrates conservation of the p.H135 and p.V545 residues in human, rat, mouse and xenopus (Fig 3).

DISCUSSION

Mutations in *SLC26A4* are the major genetic cause of Pendred Syndrome (PS) and DFNB4 in many racial populations. To date, more than 100 mutations causing Pendred syndrome and nonsyndromic (EVA) sensorineural hearing loss have been reported in the *PDS* gene (<http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm>). Moreover, a significant difference in the frequency and distribution of the mutations has been observed in different populations. Among Asian populations, a distinct spectrum of *SLC26A4* mutations in patients associated with EVA with highly prevalent founder mutations has been observed, such as p.H723R in Japan and Korea and IVS7-2A>G in China.^{3,6-9} Park et al performed a mutation screening of the seven exons of the *SLC26A4* gene in 274 East Asians and 318 South Asians deaf probands with sporadic or familial severe to profound prelingual deafness, including 86 Chinese affected subjects. They demonstrated mutations in *SLC26A4* in about 5.5% in both groups and identified 3 mutations (p.S252P, IVS7-2 A>G, p.N392Y) in 5 out of 86 (5.8%) Chinese probands.⁶ Tsukamoto et al reported that causative mutations have been identified in 90% of typical Pendred families, and in 78% of families with sensorineural hearing loss associated with EVA in Japan. Of the mutations detected, 53% were the p.H723R mutation.³ Campbell et al stated that p.L236P (16%), p.T416P and IVS8+1G>A were the three frequent mutations in the Caucasian population¹⁰. In the Spanish population the most frequent mutation is p.Q514K (17%).¹¹

To date, more than 40 mutations have been reported among Chinese (Fig 2). IVS7-2A>G mutation was the most common mutation accounting for 22 -58% of all the mutant alleles in Chinese patients with hearing loss associated with EVA or both EVA and Mondini dysplasia (MD).⁷⁻⁹ Wang et al⁷ reported that biallelic mutations were identified in 88% of patients from the simplex families. Overall, up to 98% patients were found to have at least one possible pathogenic variant in *SLC26A4*. The IVS7-2A>G mutation accounted for 58 % (102/177) of all the mutant alleles. The IVS7-2A>G mutation caused skipping of exon 8 with a resulting stop codon at position 311, leading to a predicted truncated protein. The mutation was first reported in a Turkish family¹² and subsequently detected in deaf subjects from Japan, Korea, and China. IVS7-2A>G has not been found in western populations, including deaf patients in the United States (<http://www.healthcare.uiowa.edu/labs/pendredandbor//slcMutations.htm>). Dai et al⁸ have recently reported a comprehensive study of the prevalence of IVS7-2A>G mutation in an analysis of 3271 deaf subjects from 27 regions of 24 provinces of China. The detection rate was found to range from 28% to below 1% based on ethnicity. The results showed that genetic test for the IVS7-2A>G mutation alone would identify the molecular cause in up to 8–12% of deaf patients in a few eastern and central regions of China. However, the IVS7-2A>G mutation occurs at a much lower frequency (0.000-0.019) among ethnic groups in the southwest and northwest regions of China. The data suggest that for subjects that are negative for the IVS7-2A>G mutation, further mutational analysis of the full *SLC26A4* gene or other deafness-related genes will be necessary.

In the present study, the prevalence of *SLC26A4* mutation was 100% (32/32) in this cohort of Chinese patients with bilateral EVA. Biallelic mutations were detected in 88% (28/32) of patients, eleven of whom were homozygous. Four subjects were found to carry a single *SLC26A4* mutation. We identified *SLC26A4* mutations in 60 of the 64 alleles tested (94%) (Table 3). The IVS7-2A>G mutation was the most common form accounting for 60% (36/60) of all the mutant alleles, which supports the view that the IVS7-2A>G mutation is indeed the most common mutation found in genetic deafness patients with EVA of Chinese background. Thus, if screening for mutations in *SLC26A4* is considered, it is reasonable to screen for the IVS7-2A>G mutation first. A total of 5 new mutations were identified in this study including two missense mutations and three frameshift mutations. The distribution of the five novel mutations described in the present study along the *SLC26A4* gene is shown in Figure 1: the c.404A>G/p.H135R mutation occurs in the first extracellular region; two of the mutations (c.1555-6delAA; c.1634T>A/p.V545A) are located in the cytoplasmic domain; the c.1687-92delA is detected in the transmembrane domain; and the c. 2082delA mutation was found within the STAS domain (sulfate transporter and antisigma antagonist) at the C-terminal region of the protein, which seems to be involved in the binding of nucleotides and/or the interaction with the cAMP-dependent chloride channel CFTR (cystic fibrosis transmembrane conductance regulator).¹³ STAS domain is proposed to be important for proper localization to the plasma membrane¹⁴ and regulation of anion transport activity.^{13,15}

The identification of the role of *SLC26A4* in non-syndromic deafness with EVA may allow us to investigate any possible genotype-phenotype relation by combining molecular genetic study and an audiological analysis. In the present study, the following features of the *SLC26A4*-related deafness were observed. The hearing impairment in 69% of the patients has a prelingual onset. The degree of hearing loss varies greatly ranging from moderate to profound, but a higher prevalence of severe and profound hearing loss was found. We have not found any correlations between the type of *SLC26A4* mutations and the audiogram. There are significant proportions of patients with asymmetric (26%), progressive (32%), or fluctuating hearing loss (21%). These audiological features are not common in other recessive forms of non-syndromic hearing loss. Clearly more cases and comprehensive audiological studies are needed to address any potential genotype and phenotype correlation.

CONCLUSION

Our data confirm the high prevalence of *SLC26A4*-related deafness in Chinese SNHL and EVA patients and support the view that a unique spectrum of the *PDS* gene in Chinese, which is distinct from that found in the other ethnic populations. All categories of hearing loss severity except for mild loss were found. The *SLC26A4*-related deafness appears to be prevalent in severe to profound loss. Although no clear genotype and phenotype correlation was found, high incidence of asymmetric, progressive, and fluctuating hearing loss detected in the current study warrants further investigations to define any molecular basis/ environmental factor of these uncommon features in non-syndromic forms of recessive deafness. The high detection rate of *SLC26A4* mutations in Chinese with SNHL and EVA indicates that screening *SLC26A4* mutations should be offered to Chinese deaf patients with EVA.

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REFERENCES

1. European Concerted Action Project on Genetics of Hearing Impairment. Study group on terminology, definition and hearing assessment. Nov. 1996 Newsletter No 2 http://hear.unife.it/he_infol.htm
2. Lasak JM, Welling DB. The enlarged vestibular aqueduct syndrome. *Curr Opin Otolaryngol Head Neck Surg.* 2000; 8:380–3.
3. Tsukamoto K, Suzuki H, Harada D, et al. Distribution and frequencies of PDS (*SLC26A4*) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. *Eur J Hum Genet.* 2003; 11:916–22. [PubMed: 14508505]
4. Coyle B, Reardon W, Herbrick JA, et al. Molecular analysis of the PDS gene in Pendred syndrome (sensorineural hearing loss and goitre). *Hum Mol Genet.* 1998; 7:1105–12. [PubMed: 9618167]
5. Van Hauwe P, Everett LA, Coucke P, et al. Two frequent missense mutations in Pendred syndrome. *Hum Mol Genet.* 1998; 7:1099–104. [PubMed: 9618166]
6. Park HJ, Shaikat S, Liu XZ, et al. Origins and frequencies of *SLC26A4* (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness. *J Med Genet.* 2003; 40:242–8. [PubMed: 12676893]
7. Wang QJ, Zhao YL, Rao SQ, et al. A distinct spectrum of *SLC26A4* mutations in patients with enlarged vestibular aqueduct in China. *Clin Genet.* 2007; 72:245–54. [PubMed: 17718863]
8. Dai P, Li Q, Huang D, et al. *SLC26A4* c.919-2A>G varies among Chinese ethnic groups as a cause of hearing loss. *Genet Med.* 2008; 10:586–92. [PubMed: 18641518]
9. Hu H, Wu L, Feng Y, et al. Molecular analysis of hearing loss associated with enlarged vestibular aqueduct in the mainland Chinese: a unique *SLC26A4* mutation spectrum. *J Hum Genet.* 2007; 52:492–7. [PubMed: 17443271]
10. Campbell C, Cucci RA, Prasad S, et al. Pendred syndrome, *DFNB4*, and PDS/*SLC26A4* identification of eight novel mutations and possible genotype-phenotype correlations. *Hum Mutat.* 2001; 17:403–11. [PubMed: 11317356]
11. Pera A, Villamar M, Viñuela A, et al. A mutational analysis of the *SLC26A4* gene in Spanish hearing-impaired families provides new insights into the genetic causes of Pendred syndrome and *DFNB4* hearing loss. *Eur J Hum Genet.* 2008; 1–9.
12. Coucke PJ, Van Hauwe P, Everett LA, et al. Identification of two different mutations in the PDS gene in an inbred family with Pendred syndrome. *J Med Genet.* 1999; 36:475–7. [PubMed: 10874637]

13. Ko SB, Zeng W, Dorwart MR, et al. Gating of CFTR by the STAS domain of SLC26 transporters. *Nat Cell Biol.* 2004; 6:343–50. [PubMed: 15048129]
14. Chernova MN, Jiang L, Shmukler BE, et al. Acute regulation of the SLC26A3 congenital chloride diarrhoea anion exchanger (DRA) expressed in *Xenopus* oocytes. *J Physiol.* 2003; 549:3–19. [PubMed: 12651923]
15. Zalzal GH, Tomaski SM, Vezina LG, et al. Enlarged vestibular aqueduct and sensorineural hearing loss in childhood. *Arch Otolaryngol Head Neck Surg.* 1995; 121:23–8. [PubMed: 7803018]

Figure 1.

A. The pedigree of one EVA subject with simplex family history. B. Appearance of temporal bone with EVAS in CT image. The arrow indicates the enlarged vestibular aqueduct (right).

Figure 2.

A schematic representation of the relative linear location of mutations of *SLC26A4* gene identified in present study and all *SLC26A4* mutations among Chinese (refs 11, 14, 15). Novel mutations from this study were in bold italic.

	H135R
	▼
SCL26A4_HUMAN	GTSR H I SVGPFPVV
Slc26a4_MOUSE	GTSR H I SVGPFPVV
Slc26a4_RAT	GTSR H I SVGPFPVV
slc26a4_XENTR	GTSK H I SVGPFPVV
	****.*****
	.
	V545A
	▼
SCL26A4_HUMAN	GVK I LRFSS
Slc26a4_MOUSE	GVK I LRFSS
Slc26a4_RAT	GVK I LRFSS
slc26a4_XENTR	GVK I I RHTS
	****. * .*
	. . .

Figure 3.
Multiple sequence alignment showing conservation of residues H135 and V545.

Table 1

Characteristics of 32 patients screened for *SLC26A4* mutations.

Subjects number	Age (Years)	Sex	Onset	Severity(L/R)	Evolution	Family history	CT scan (mm)
1	9	F	4Y	Profound/ Profound	fluctuating	simplex	EVA(3)
2	3.1	F	birth	severe/profound	NA	simplex	EVA(2.5)
3	13	F	1Y6M	Profound/ Profound	NA	simplex	EVA(3.5)
4	3.6	F	3Y6M	Severe/severe	No change	simplex	EVA(4)
5	1.4	F	1Y3M	Severe/severe	No change	simplex	EVA(3)
6	10	F	4Y	NA	NA	simplex	EVA(3.5)
7	1.6	M	4M	Severe/severe	progressive	simplex	EVA(3)
8	7.5	M	4Y	Profound/ Profound	No change	simplex	EVA(4)
9	4.5	F	2Y	severe/profound	No change	simplex	EVA(3.5)
10	12	M	11M	Profound/ Profound	progressive	simplex	EVA(3.5)
11	4.5	M	4Y	moderate/severe	fluctuating	simplex	EVA(3)
12	20	M	2Y	Severe/severe	fluctuating	simplex	EVA(2.5)
13	11	M	4Y	Severe/severe	progressive	simplex	EVA(3.5)
14	23	F	4Y	Profound/ Profound	progressive	multiplex	NA(3.5)
15	12	F	10M	Profound/ Profound	progressive	simplex	EVA(4)
16	3.5	M	2Y	Severe/severe	fluctuating	simplex	EVA(3)
17	7	M	1Y	Profound/ Profound	progressive	simplex	EVA(4)
18	5	M	11M	severe/moderate	No change	simplex	EVA(2.5)
19	2	M	2Y	profound/severe	fluctuating	simplex	EVA(4)
20	0.5	M	3M	Profound/ Profound	No change	multiplex	EVA&CD (3.5)
21	3	M	3Y	severe/moderate	No change	simplex	EVA(3)
22	13	M	10Y	profound/moderate	progressive	simplex	EVA(4)
23	13	M	2Y	moderate/profound	No change	simplex	EVA(5)
24	7	F	birth	Profound/ Profound	No change	simplex	EVA(4)
25	33	F	birth	Profound/ Profound	No change	simplex	EVA(3.5)

Subjects number	Age (Years)	Sex	Onset	Severity(L/R)	Evolution	Family history	CT scan (mm)
26	0.2	F	2M	Profound/ Profound	No change	simplex	EVA(2.5)
27	12	M	2M	Profound/ Profound	No change	simplex	EVA(4)
28	9	F	2Y	Profound/ Profound	progressive	simplex	EVA(3.5)
29	4.5	M	3Y	Profound/ Profound	NA	simplex	EVA(3)
30	21	M	2Y	Severe/severe	fluctuating	simplex	EVA(3.5)
31	10	M	4Y	Severe/severe	progressive	simplex	EVA(4.5)
32	2.5	M	birth	Profound/ Profound	No change	multiplex	EVA(3)

CD, bilateral cochlear dysplasia; EVA, bilateral enlarged vestibular aqueduct; NA, not available

Table 2
Bone conduction and air conduction thresholds at 250Hz and 500Hz for the right and left ears*

Subjects number	250Hz (left)			500Hz (left)			250Hz (right)			500Hz (right)		
	BC	AC	A-B gap	BC	AC	A-B gap	BC	AC	A-B gap	BC	AC	A-B gap
5	20	80	60	50	80	30	20	80	60	50	80	30
10	45	70	25	65	105	40	40	65	25	60	85	25
13	25	70	45	40	70	30	25	80	55	40	80	40
15	35	100	65	50	90	40	35	100	65	50	100	50
16	15	70	55	55	85	30	15	60	45	40	85	45
18	10	60	50	40	70	30	10	55	45	40	60	20
28	0	50	50	40	70	30	10	55	45	40	60	20
29	0	50	50	25	70	45	0	75	75	25	85	60
30	25	65	40	25	70	45	20	35	15	20	50	30
31	5	60	55	20	50	30	5	60	55	10	40	30

* BC = bone conduction; AC = air conduction; A-B gap = air bone gap.

Table 3Details of genotypes of *SLC26A4* detected in 32 SNHL associated with EVA patients in a Chinese population

Genotypes	Number of individuals
IVS7-2A>G/IVS7-2A>G	10
IVS7-2A>G/p.S391R	1
IVS7-2A>G/p.N392Y	1
IVS7-2A>G/p.R409H	3
IVS7-2A>G/p.V545A ^a	1
IVS7-2A>G/c.1687-92delA ^a	1
IVS7-2A>G/IVS15+5G>A	1
IVS7-2A>G/p.H723R	5
IVS7-2A>G/c.2082delA ^a	1
IVS7-2A>G/WT	2
p.H135R ^a /p.H723R	1
p.N392Y/p.T410M	1
c.1548insC /c.1555-6delAA ^a /p.H723R	1
IVS15+5G>A/WT	2
p.H723R/p.H723R	1

^aNovel changes identified in the present study