International Journal of Pediatric Otorhinolaryngology 95 (2017) 104–108

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: http://www.ijporlonline.com/

A novel missense mutation in the *SLC26A4* gene causes nonsyndromic hearing loss and enlarged vestibular aqueduct

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ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form
10 February 2017
Accepted 12 February 2017
Available online 14 February 2017

Keywords:
Hearing loss
Enlarged vestibular aqueduct
SLC26A4
Novel mutation

ABSTRACT

Objectives: We aimed to investigate the genetic causes of hearing loss in a Chinese proband with nonsyndromic hearing loss and enlarged vestibular aqueduct syndrome.
Methods: We conducted clinical and genetic evaluations in a deaf proband and his normal-hearing parents. Multiplex PCR technology combined with Ion Torrent™ next-generation sequencing technology was used to detect the pathogenic mutations. As a control, a group of 1500 previously studied healthy newborns from the same ethnic background were subjected to deafness gene screening using the same method as in our previous study.
Results: The proband harbored two mutations in the *SLC26A4* gene in the form of compound heterozygosity. He was found to be heterozygous for a novel mutation named c.1742 G > T (p.Arg581Met) in exon 13 and for the known mutation c.589 G > A (p.Gly197Arg). These variants were carried in the heterozygous state by the parents and therefore co-segregated with the genetic disease. The c.1742 G > T (p.Arg581Met) mutation was absent in 1500 healthy newborns. Protein alignment indicated high evolutionary conservation of the p.R581 residue, and this mutation was predicted by PolyPhen-2 and other online tools to be damaging.
Conclusion: This study demonstrates that the novel mutation c.1742 G > T (p.Arg581Met) in compound heterozygosity with c.589 G > A in the *SLC26A4* gene is the main cause of deafness in a family clinically diagnosed with enlarged vestibular aqueduct (EVA). Our study will provide a basic foundation for further investigations to elucidate the *SLC26A4*-related mechanisms of hearing loss.

1. Introduction

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, which can be progressive and usually ranges from severe to profound [1]. Its estimated prevalence varies from 3.7% to 11.4% in people with sensorineural hearing loss [2,3]. It is believed that *SLC26A4* gene mutations may cause NSHL associated with EVA, with hearing loss found at birth or during early childhood [4], and these gene mutations are regarded as the second most frequent cause of autosomal recessive nonsyndromic sensorineural hearing loss after mutations in *GJB2* [5].

The *SLC26A4* gene (*PDS*, NM_000441.1) is located on chromosome 7q22.3-7q31.1 and consists of 21 exons that encode a 780-amino-acid protein called pendrin [6]. Pendrin is mainly expressed in the thyroid gland, inner ear, and kidney [7,8]. Pendrin is a member of the solute carrier 26 family and functions as a chloride/iodide transporter in cell expression systems, playing an important role in maintaining the homeostasis of the endolymph in

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http://dx.doi.org/10.1016/j.ijporl.2017.02.013
0165-5876/

the inner ear [9]. It is reported that the mutant pendrins were retained in the endoplasmic reticulum, and the defects in the chloride/iodide and bicarbonate exchange activities of pendrin at the apical membrane of the inner ear epithelial cells is the key factor that causes EVA and deafness [10]. Mutations in the *SLC26A4* gene lead to the development of a variable clinical spectrum of hearing loss due to inner ear malformations, such as EVA or Mondini cochlea associated with goiter [1,9]. Approximately 200 mutations in *SLC26A4* have been identified so far, including missense, nonsense, frameshift and splice site mutations, and these mutations are distributed throughout the pendrin-coding region (https://research.cchmc.org/LOVD2/home.php?select_db=SLC26A4). *SLC26A4* mutations in Western countries and Asian populations show regional and ethnic diversity in their frequency and display mutational hot spots [11].

In this study, the *SLC26A4* gene was screened in a Chinese proband with EVAS, and clinical and molecular evaluations were performed. As a result, we identified compound heterozygous mutations in the *SLC26A4* gene: a novel mutation c.1742 G > A (p.Arg581Met) and a known mutation c.589 G > A.

2. Materials and methods

2.1. Subjects and clinical evaluations

The proband, a 6-year-old boy with hearing loss of congenital origin and early childhood onset, and his normal-hearing parents were recruited to participate in this study. All three came from Hunan Province and are part of the southern Han Chinese population.

A clinical evaluation of the family was conducted, including a description of the family history and detailed medical history and a physical examination including thyroid sonography, thyroid function tests and a high-resolution computed tomography (CT) scan of the temporal bone. Auditory evaluations consisted of otoscope examination, pure-tone audiometry (PTA), tympanometry audiometry, acoustic stapedial reflex, auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs). The degree of hearing loss was estimated based on PTA results as previously described. Severity level was classified as mild (35–55 dB HL), moderate (56–70 dB HL), severe (71–90 dB HL), or profound (>91 dB HL). Temporal bone imaging was performed using computed tomography (CT). All CT images were scanned with 0.625-mm contiguous increments in both axial and coronal sections. EVA was defined as a diameter greater than 1.5 mm at a midway point between the common crus and the external aperture.

This study was approved and conducted in accordance with the protocol of the Institutional Medical and Ethics Committee of Dongguan Children's Hospital. Written informed consent was obtained from the parents or legal guardians of the subjects.

2.2. Mutational analysis

Genomic DNA was extracted from 200 to 400 μl of peripheral blood samples from the subjects using the Blood DNA Kit (TIANGEN BIOTECH, Beijing, China), following the manufacturer's protocol. DNA yield and quality were determined using a NanoDrop 8000 ultraviolet-visible spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and 1% agarose gel electrophoresis was

Table 1
The 100 mutations in eighteen NSHL associated gene covered in deafness diagnostic screening panel.

Gene	Mutation	Gene	Mutation	Gene	Mutation
GJB2	c.235delC	*SLC26A4*	c.679G > C	*GJB3*	c.538C > T
	c.299-300delAT		c.IVS14-2A > G		c.547G > A
	c.35delG		c.919-18T > G		c.423delATT
	c.176-191del16		c.920C > T		c.497A > G
	c.167delT		c.109G > T		c.421A > G
	c.512insAACG		c.1160C > T	*MT-RNR1*	m.1555A > G
	c.456C > G		c.1181_1183delTCT		m.1494C > T
	c.456C > G		c.1318A > T		m.827A > G
	c.427C > T		c.1336C > T		m.961delTinsC
	c.416G > A		c.1555_1556delAA	*MT-CO1*	m.7444G > A
	c.257C > G		c.1586T > G	*MT-TL1*	m.3243A > G
	c.253T > C		c.1594A > C	*MT-TS1*	m.7445A > G
	c.109G > A		c.1634T > C		m.7505T > C
	c.99delT		c.1673A > T		m.7511T > C
	c.94C > T		c.1717G > T	*MT-TH*	m.12201T > C
SLC26A4	IVS7-2A > G		c.1746delG	*DSPP*	c.52G > T
	c.2168A > G		c.2054G > T	*GPR98*	c.10088_10091delTAAG
	c.1229C > T		c.2082delA	*DFNA5*	IVS8+4A > G
	c.1174A > T		c.2107C > G	*TMC1*	c.150delT
	c.1975G > C		c.227C > T		c.1334G > A
	c.2027T > A		c.230A > T	*MYO7A*	c.652G > A
	c.2162C > T		c.269C > T		c.731G > C
	c.589G > A		c.334C > T	*TECTA*	c.4525T > G
	c.1226G > A		c.349delC	*DIABLO*	c.377C > T
	c.281C > T		c.387delC	*COCH*	c.1535T > C
	IVS15+5G > A		c.404A > G		c.1625G > A
	c.2086C > T		c.439A > G	*MYO15A*	c.8183G > A
	c.754T > C		c.697G > C		c.8767C > T
	c.1079C > T		c.812A > G	*PRPS1*	c.193G > A
	c.259G > T		IVS10-12T > A		c.259G > A
	c.1343C > T		IVS13+9C > G		c.869T > C
	c.1540C > T		IVS14+1G > A		c.916G > A
	c.1919G > A		IVS14-1G > A		
	c.2000T > C		IVS16-6G > A		

performed according to routine methods. A deafness diagnostic screening panel developed by our laboratory group was used for the gene mutation screening. This panel covered 100 mutational hotspots of 18 common deafness genes (Table 1) and the neighboring sequencing regions of mutational hotspots. Multiplex PCR technology combined with Ion Torrent™ next-generation sequencing technology was used to explore the causative mutations in the proband with hearing loss. The screening panel could simultaneously detect 53 mutational hotspots of the *SLC26A4* gene. As a control, a group of 1500 previously studied healthy newborns from the same ethnic background were subjected to deafness gene screening, using the same method as in our previous study.

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 the DNA Star 5.0 software. The 1000 Genomes Project database (http://www.1000genomes.org/) and the dbSNP database of the NCBI (http://www.ncbi.nlm.nih.gov/) were used as references to assess the novelty of the mutations found in this study. The evolutionary conservation of the mutated amino acids was examined by multiple sequence alignment. Online tools including MutationTaster, SIFT and PolyPhen-2 were used to predict the functional outcome of the novel variant.

3. Results

3.1. Clinical features

A hearing-impaired subject and his normal-hearing parents were enrolled in our study (Fig. 1 A). Hearing loss in the proband was noticed by his parents during his early childhood at the age of eight months. He showed significant delays in speech development and was diagnosed with binaural large vestibular aqueduct syndrome when first seen by a medical doctor at one year and eight months old. As shown in Fig. 1 B, PTA revealed that the proband suffered from moderate sensorineural hearing loss with 62 dB in the right ear and profound sensorineural hearing loss with 96 dB in the left ear. Both the physical examination and otoscopy were normal. Thyroid goiter was excluded based on normal thyroid function tests. DPOAE showed no response from the patient in both ears. The proband had a tympanogram result of “A”, and the bilateral acoustic stapedial reflex was not elicited. A temporal bone CT scan of the proband showed bilateral EVA with the width of the vestibular aqueduct greater than 1.5 mm (Fig. 1 C). His hearing loss progressed gradually, and he received a cochlear implant (CI) at the age of two years.

3.2. Genetic analysis

The sequence analysis of *SLC26A4* indicated that the proband

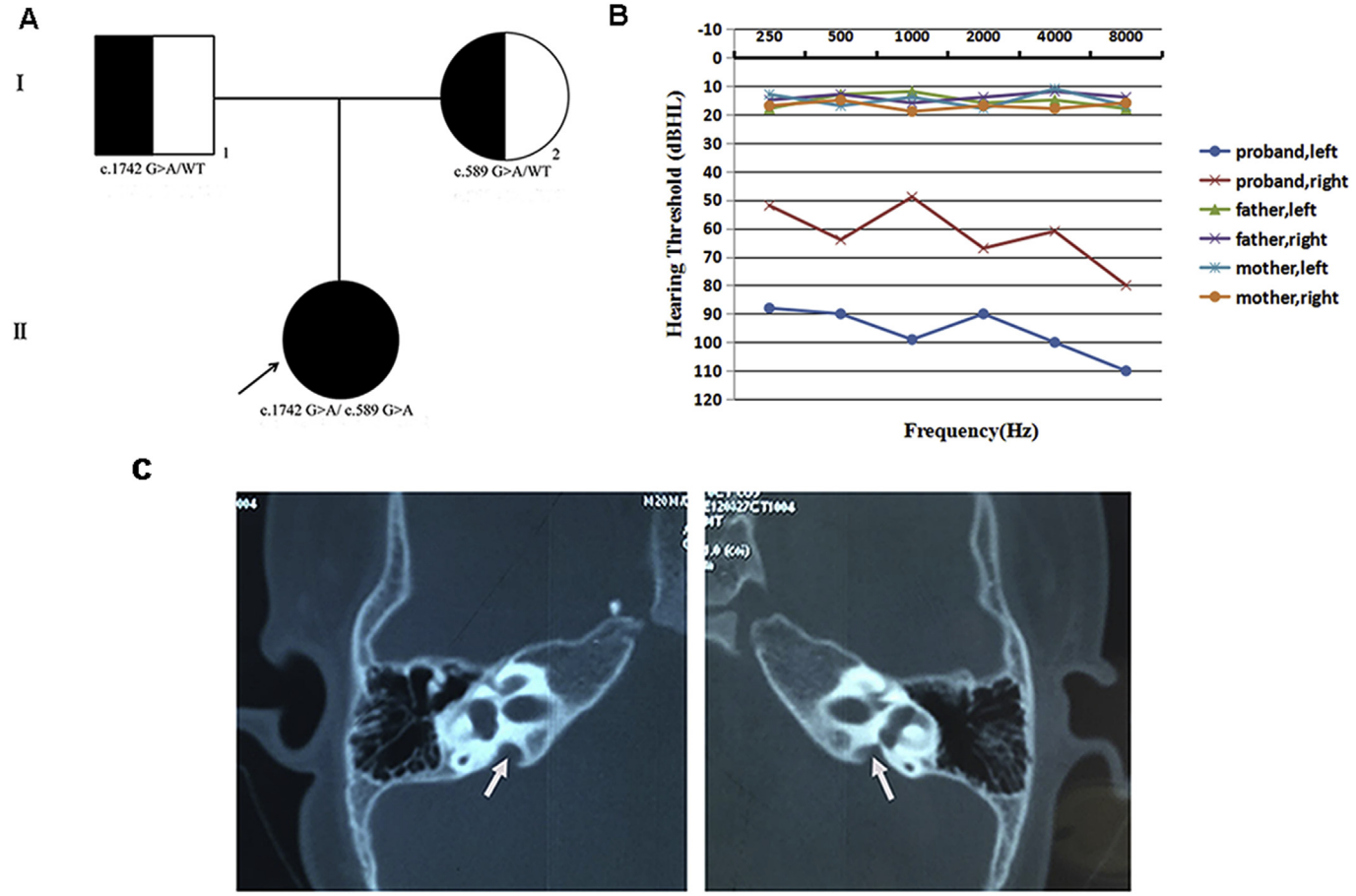

Fig. 1. Genotypes and clinical phenotype presentations of the pedigree. (A) Pedigree map of the consanguineous family with hearing loss. Squares and circles denote males and females, respectively. (B) Pure-tone audiograms of the family members. Frequency in hertz (Hz) is plotted on the x-axis and the 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).

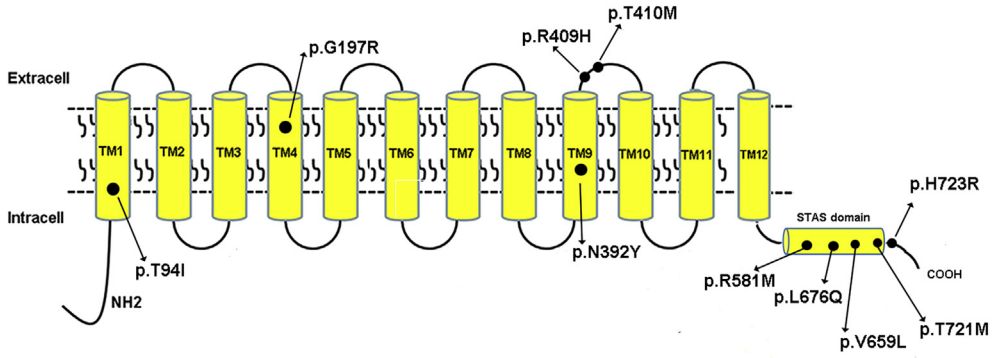

Fig. 2. A schematic representation of the location of mutations of *SLC26A4* gene identified in this study and common SLC26A4mutations among Chinese. TM represents the transmembrane domain. The extracellular loop and cytoplasmic loop are represented as black lines.

presented compound heterozygosity of a c.589 G > A mutation in exon 4 and a c.1742 G > T (p.Arg581Met) missense mutation in exon 13. Additionally, the mother was a heterozygous carrier of the c.589 mutation, and the father was a heterozygous carrier of the c.1742 G > T (p.Arg581Met) mutation.

A schematic distribution of the mutations identified in this study and reported common mutations among Chinese along the *SLC26A4* gene is shown in Fig. 2. To our knowledge, this paper is the first report of the c.1742 G > T (p.Arg581Met) mutation. The novel missense mutation c.1742 G > T (p.Arg581Met) led to a substitution of an AGG codon with an ATG codon at position 581 (Fig. 2). We also analyzed this amino acid region among *Homo, Ailuropoda, Sus, Canis, Oryctolagus, Myotis, Equus, Rattus, Bos* and *Loxodonta* species. The results indicated that p.R581M is located in the conserved region of the protein sequence. This mutation was not found in 1500 healthy newborns and was predicted to be “disease causing” by MutationTaster (disease causing), to “affect protein function” by SIFT (Score: 0.044), and to be “probably damaging” by PolyPhen-2 (Score: 1.000), which strongly suggests that this novel mutation is closely associated with deafness.

4. Discussion

In this study, we identified the compound heterozygous mutations c.589 G > A and c.1742 G > T (p.Arg581Met) in the proband. These mutations were located in exon 4 and exon 13 of *SLC26A4*, respectively. Neither of these exons are mutation hotspots in East Asian populations, and mutations in these regions are especially rare in the Chinese population [12–14]. To rule out the possible role of other untested mutations (not included in the targeted panel), the whole exome of *SLC26A4* in the proband and his parents was analyzed by Ion Proton sequencing. According to the bioinformatics results, no other possible pathogenic mutation was found.

The c.589 G > A mutation was first reported by Wang [15] based on studying the spectrum of *SLC26A4* mutations in Chinese patients with hearing loss associated with EVA or with both EVA and Mondini dysplasia (MD). A patient heterozygous for c.589 G > A showed mild hearing impairment. The frequency of the c.589 G > A mutation in patients with nonsyndromic EVA is approximately 2% [14,16].

Another mutation, c.1742 G > T (p.Arg581Met), was reported for the first time in this study. This particular variant identified in our study is located in the highly conserved domain of the *SLC26A4* gene and is predicted to be pathogenic. This mutation leads to the p.R581M transition at amino acid position 581 in the sulfate transporter and anti-sigma factor antagonist (STAS) domain of SulP-like sulfate transporters. The STAS domain found in members of the SLC26A family regulates the stability, tracking and anion transport function of the SLC26 family proteins [17,18]. The structural significance of this domain has been substantiated by the disease-causing nature of mutations therein among SLC26A family proteins [17]. Therefore, it is possible that the novel mutation discovered in our study may impair the activity of pendrin as an anion transporter by altering the structure of the pendrin protein and causing increased randomness in its conformation.

It is currently known that EVA is associated with mutations of the *SLC26A4* gene that confer high risk in multiple racial populations. In China, 97.9% of the EVA patients in simplex families exhibited either biallelic or monoallelic mutations [15], which is different from the rates in other ethnic populations, such as 92% in Korean [19], 78.1% in Japanese [20] and only 40% in Caucasian populations [21]. These data indicated that the cause of genetic EVA showed regional and ethnic diversity. As EVA is genetically characterized as a non-syndromic autosomal recessive disorder, there would be a more severe phenotype for biallelic *SLC26A4* mutations. The presence of these two variations in this Chinese proband corresponded to the clinical diagnosis of EVAS. Considering the youth of the proband, it is possible that the boy will develop Pendred syndrome in the future, and thus, monitoring of the thyroid by echography and TSH should be recommended.

In conclusion, we reported a patient with EVAS carrying biallelic mutations (c.589G > A/c.1742 G > T). This finding of a novel mutation further expands the spectrum of known *SLC26A4* mutations in the Chinese population.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

This work was supported by the Dongguan Bureau of Science and Technology for the City Key Program of Science and Technology (Project Number: 2013108101018) and the Science and Technology Planning Project of Guangdong Province (Project Number: 2014A020213001). We also thank all the neonates and their parents for their cooperation during this work.

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