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

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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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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		