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Genetic analysis of auditory neuropathy spectrum disorder in the Korean population

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ABSTRACT

Auditory neuropathy spectrum disorder (ANSD) is caused by dys-synchronous auditory neural response as a result of impairment of the functions of the auditory nerve or inner hair cells, or synapses between inner hair cells and the auditory nerve. To identify a causative gene causing ANSD in the Korean population, we conducted gene screening of the *OTOF*, *DIAPH3*, and *PJVK* genes in 19 unrelated Korean patients with ANSD. A novel nonsense mutation (p.Y1064X) and a known pathogenic mutation (p.R1939Q) of the *OTOF* gene were identified in a patient as compound heterozygote. Pedigree analysis for these mutations showed co-segregation of mutation genotype and the disease in the family, and it supported that the p.Y1064X might be a novel genetic cause of autosomal recessive ANSD. A novel missense variant p.K1017R (c.3050A>G) in the *DIAPH3* gene was also identified in the heterozygous state. In contrast, no mutation was detected in the *PJVK* gene. These results indicate that no major causative gene has been reported to date in the Korean population and that pathogenic mutations in undiscovered candidate genes may have an effect on ANSD.

1. Introduction

Auditory neuropathy (AN) is a hearing disorder that consists of abnormal neural conduction of the auditory pathway and normal function from the external ear to outer hair cells (OHCs) (Starr et al., 1996). AN is also known as auditory dys-synchrony (AD) because patients with AN have very poor speech discrimination owing to the dys-synchronous auditory neural response (Berlin et al., 2003). AN can occur in response to dysfunction of the auditory nerve or inner hair cells (IHCs) or synapses between IHCs and auditory nerve terminals (Starr et al., 2000). This feature of AN has made it difficult to determine the exact etiological causes. For such a reason, in 2008, AN was named auditory neuropathy spectrum disorder (ANSD) (Manchaiah et al., 2011). The environmental factors causing ANSD are neonatal prematurity, hypoxia, and kernicterus; however, congenital ANSD is closely related to several genetic factors (Starr et al., 2000). The major causative genes of congenital ANSD have been identified as *OTOF*, *DIAPH3* and *PJVK*. Moreover, mutations of the *GJB2* and mitochondrial 12*S rRNA* gene in ANSD patients have seldom been reported (Santarelli et al., 2008; Wang et al., 2005).

The *OTOF* gene (OMIM: 603681) which contains 47 exons and spans 101,495 bp on 2p23.1, is known as the causative gene of autosomal recessive non-syndromic hearing impairment (DFNB9) associated with ANSD (Chaib et al., 1996; Rodriguez-Ballesteros et al., 2003; Varga et al., 2003; Yasunaga et al., 1999). Otoferlin, encoded by *OTOF*, belongs to the ferlin family and has six C2 domains and one transmembrane domain located near its C-terminus (Del Castillo and Del Castillo, 2012). Otoferlin plays an important role in exocytosis of synaptic vesicles at the auditory ribbon synapse (Roux et al., 2006).

The *DIAPH3* gene (OMIM: 614567) encoding diaphanous homologue 3 is associated with autosomal dominant auditory neuropathy 1, AUNA1 (Kim et al., 2004). The *DIAPH3* gene contains 29 exons and spans 498,402 bp on 13q21.2. It is affiliated with the diaphanous homolog family. Several genes included in this family encode diaphanous-related formins, which are known to play significant roles in diverse cellular functions such as cell shape, polarity, cytokinesis, and adhesion

Abbreviations: ANSD, auditory neuropathy spectrum disorder; AUNA1, autosomal dominant auditory neuropathy; *DIAPH3*, diaphanous homolog 3; *GJB2*, gap junction protein, beta 2; *OTOF*, otoferlin; OHCs, outer hair cells; *PJVK*, pejvakin; PTA, pure tone audiometry.

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(Goode and Eck, 2007). However, the exact expression patterns and functions of diaphanous-3 in the auditory pathway are still uncertain.

The *PJVK* gene (OMIM: 610219) was first reported in 2006 as DFNB59, which causes autosomal recessive auditory neuropathy in Iranian families (Delmaghani et al., 2006). It consists of 7 exons spanning 9950 bp on 2q31.2, and transcribes only a single type of mRNA. Pejvakin, which is encoded by the *PJVK* gene, is detected at the kinociliums in the vestibule, pillar cells and hair cells in the organ of corti, and cell bodies of neurons in the afferent auditory pathway (Del Castillo and Del Castillo, 2012). Moreover, pejvakin-mutated mice showed normal development and maintenance of hair cells (Schwander et al., 2007). Therefore, it is believed that pejvakin has an effect on the activity of neurons or hair cells, but not on structural development.

Although a number of previous studies of ANSD have been reported in various ethnic groups, no studies have investigated ANSD in Koreans. To investigate the major genetic causes of ANSD in the Korean population, gene screening of *OTOF*, *DIAPH3* and *PJVK* was conducted in 19 unrelated patients diagnosed with ANSD for the first time.

2. Materials and methods

2.1. Subjects

A total of 19 unrelated Korean patients diagnosed with ANSD were recruited from the Department of Otorhinolaryngology-Head and Neck Surgery, Kyungpook National University Hospital, Daegu, Soonchunhyang University Hospital, Bucheon, and Severance Hospital, Seoul, Korea. The diagnosis of ANSD was made when the auditory brainstem response (ABR) was severely abnormal in the presence of normal or partially normal otoacoustic emissions. The hearing levels in all of the participants were examined by audiological tests, including pure-tone audiometry (PTA) and speech audiometry. PTA hearing level was calculated as an average of the threshold measured at 0.5, 1.0, 2.0, and 3.0 kHz and classified as follows: normal hearing, below 20 dB HL; mild hearing impairment, 21–40 dB HL; moderate hearing impairment, 41–70 dB HL; severe hearing impairment, 71–95 dB HL; profound hearing impairment above 95 dB HL. A clinical questionnaire excluded some history of ototoxic drugs use or environmental factors, and physical examinations previously ruled out other syndromic hearing loss. An unrelated Korean normal control group composed of 180 normal hearing subjects was also evaluated for comparison by audiological tests including PTA. Written informed consent was obtained from all individuals, and the local ethics committee approved this study.

2.2. Genetic analysis

Genomic DNA (19 subjects and 180 controls) was extracted from blood using a FlexiGene DNA extraction kit (QIAGEN, Hilden, Germany). The primers were designed using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) to amplify each coding region of *OTOF* (47 exons), *PJVK* (7 exons) and *DIAPH3* (29 exons), respectively (Table S1–S3). First, amplification of the coding regions was performed by polymerase chain reaction (PCR) using H-Taq DNA polymerase (Solgent, Daejeon, Korea). To confirm the PCR products, gel electrophoresis was carried out using 2% agarose gel with ethidium bromide (EtBr). Shrimp alkaline phosphatase (USB, Cleveland, OH, USA) and exonuclease I (USB, Cleveland, OH, USA) were used for purification of the amplified products. Sequencing reactions were conducted using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Following ethanol precipitation, PCR products were sequenced using a 3130xl Genetic Analyzer (Applied Biosystems, Forster City, CA, USA). SeqScape Software v2.5 (Applied Biosystems, Foster City, CA, USA) and ChromasPro 1.6 (Technelysium Pty Ltd., Tewantin, QLD, Australia) were used as genetic analysis tools to identify the nucleotide sequence. The sequencing data were then compared with the wild-type *OTOF* (NG_009937.1, NM_194248.2), *PJVK* (NG_012186.1, NM_001042702.3), and *DIAPH3* (NG_032693.1, NM_001042517.1) sequences registered in the NCBI database. dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and the 1000 genome database (http://www.1000genomes.org/) were used as references to investigate the novelty and probable pathogenicity of detected variations. Comparison of protein conservations among species was conducted using CLC sequence viewer 6 (CLC Bio, Aarhus, Denmark). To predict the functional pathogenic effects of the variations, four types of bioinformatics tools were used: Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), PMut (http://mmb.pcb.ub.es/PMut/PMut.jsp), Mutation Taster (www.mutationtaster.org), SIFT (http://sift.jcvi.org/), and SNPs&GO (http://snps-and-go.biocomp.unibo.it/snps-and-go/). *GJB2* and the mitochondrial 12*S rRNA* genes, which are known to be mostly associated with non-syndromic hearing loss (NSHL), were also examined by Sanger sequencing for whole exons of the genes in all patients.

3. Results

To investigate genetic causes of ANSD in the Korean population, all exons and exon–intron boundaries of genes related to ANSD were sequenced in the 19 Korean ANSD patients who have no pathogenic mutations in the *GJB2* and mitochondrial 12*S rRNA* genes. As a result, 16, 10, and 5 variants were identified in each of the genes (*OTOF*,

Table 1
Variants of the *OTOF* gene identified in this study.

Location	Nucleotide change	Amino acid change	Allele frequency in patients (2n = 38)	Reference
Exon 2	c.129 C>T	p.D43D	1/38	Matsunaga et al. (2012)
Exon 3	c.157 G>A	p.A53T	1/38	Wang et al.(2010)
	c.158 C>T	p.A53V	8/38	Varga et al. (2006)
Exon 4	c.244 C>T	p.R82C	3/38	Migliosi et al. (2002)
Exon 5	c.372 A>G	p.T124T	22/38	Migliosi et al. (2002)
Exon 20c[a]	c.−63_−62delCC	-	38/38	rs140808607[b]
	c.−2 T>C	-	6/38	This study
Exon 20c	c.62 C>T	p.P21L	38/38	rs4665855
Exon 23	c.2580 C>G	p.V860V	9/38	Migliosi et al. (2002)
	c.2613 C>T	p.L871L	1/38	Matsunaga et al. (2012)
Exon 24	c.2736 G>C	p.L912L	38/38	Houseman et al. (2001)
Exon 27	c.3192 C>G	p.Y1064X	1/38	This study
Exon 39	c.4677 G>A	p.V1559V	8/38	Varga et al. (2006)
	c.4767 C>T	p.R1589R	1/38	Varga et al. (2006)
Exon 44	c.5418 C>T	p.I1806I	1/38	Wang et al. (2010)
Exon 48	c.5816 G>A	p.R1939Q	1/38	Matsunaga et al. (2012)

[a] Otoferlin isoform c mRNA (NM_001258366.1), but this region is an intron in otoferlin isoform a.
[b] Registered variations in the dbSNP database.