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Carrier frequency of *SLC26A4* mutations causing inherited deafness in the Korean population

Hyogyeong Kim[1,†], Hwan-Sub Lim[2,†], Jae-Song Ryu[1], Hyun-Chul Kim[1], Sanghoo Lee[1], Yun-Tae Kim[1], Young-Jin Kim[1], Kyoung-Ryul Lee[1], Hong-Joon Park[3], and Sung-Hee Han[1,*]

[1]Department of Laboratory Medicine, Seoul Medical Science Institute, Seoul Clinical Laboratories, Seoul, Korea
[2]Department of Laboratory Medicine, Kwandong University College of Medicine, Gangneung, Korea
[3]Department of Otorhinolaryngology, Soree Ear Clinic, Seoul, Korea

Purpose: The mutation of the *SLC26A4* gene is the second most common cause of congenital hearing loss after *GJB2* mutations. It has been identified as a major cause of autosomal recessive nonsyndromic hearing loss associated with enlarged vestibular aqueduct and Pendred syndrome. Although most studies of *SLC26A4* mutations have dealt with hearing-impaired patients, there are a few reports on the frequency of these mutations in the general population. The purpose of this study was to evaluate the prevalence of *SLC26A4* mutations that cause inherited deafness in the general Korean population.

Materials and Methods: We obtained blood samples from 144 Korean individuals with normal hearing. The samples were subjected to polymerase chain reaction to amplify the entire coding region of the *SLC26A4* gene, followed by direct DNA sequencing.

Results: Sequencing analysis of this gene identified 5 different variants (c.147C>G, c.225G>C, c.1723A>G, c.2168A>G, and c.2283A>G). The pathogenic mutation c.2168A>G (p.H723R) was identified in 1.39% (2/144) of the subjects with normal hearing.

Conclusion: These data provide information about carrier frequency for *SLC26A4* mutation-associated hearing loss and have important implications for genetic diagnostic testing for inherited deafness in the Korean population.

Key words: *SLC26A4*, Hearing loss, Carrier frequency.

Introduction

Hearing loss is the most frequent sensorineural disorder in human. Approximately 1 in 1,000 children is born with hearing loss, and more than 50% of cases are caused by genetic defects [1,2]. Among them, 70% are accounted for by nonsyndromic hearing loss (NSHL); over 200 genes have been associated with NSHL. Furthermore, autosomal recessive hearing loss (ARHL) has been considered the main reason for NSHL (http://hereditaryhearingloss.org/). In patients with NSHL, some causative mutations in genes, such as *GJB2*, *SLC26A4*, *MYO15A*, *OTOF*, *CDH23*, and *TMC1* have been shown to be much more prevalent than other mutations [3]. In particular, *GJB2* and *SLC26A4* are the most frequent causative genes in genetic hearing loss among Koreans [4,5]. *GJB2* mutations are the most

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*Corresponding author: Sung-Hee Han, M.D., Ph.D.
Department of Laboratory Medicine, Seoul Medical Science Institute, Seoul Clinical Laboratories, 12 Jangmun-ro 6-gil, Yongsan-gu, Seoul, Korea.
Tel: +82-2-330-2290, Fax: +82-2-790-6509, E-mail: hansungh@scllab.co.kr
†Hyogyeong Kim and Hwan-Sub Lim contributed equally to this work.
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frequent cause of NSHL in most world populations, and can account for up to 50% of autosomal recessive NSHL cases [6]. Apart from mutations in *GJB2*, much research has shown that variants in *SLC26A4* are the second most frequent cause of ARHL [2,7].

In contrast to the *GJB2* gene, mutations in the *SLC26A4* gene have been identified as a major cause of ARHL associated with enlarged vestibular aqueduct (EVA) and Pendred syndrome. Pendred syndrome, which accounts for up to 10% of all hereditary hearing impairment, is characterized by bilateral hearing loss, EVA, and an iodine organification defect in the thyroid gland, which may lead to goiter [8,9]. In addition, in some patients Pendred syndrome leads to a congenital inner ear malformation, known as the Mondini malformation. Therefore, both nonsyndromic EVA and Pendred syndrome can be clinically associated with temporal bone abnormalities [10]. Pendrin, a protein encoded by the *SLC26A4* gene, exchange of iodide/chloride in thyroid cells [11], and is involved in secretion of bicarbonate into the endolymph in the inner ear [12]. Almost 200 variants in the *SLC26A4* gene have been identified in Pendred syndrome and NSHL patients with EVA. Variants have been detected throughout the entire gene (*SLC26A4* mutation database, http://www.healthcare.uiowa.edu/labs/pendredandbor), thus analysis of all exons and splice sites in each proband is necessary [13].

Among different countries or regions, specific variants of the *SLC26A4* gene have been found. In Northern European populations, p.T416P and c.1001+1G>A were the most frequent mutations in the *SLC26A4* gene [14], whereas p.Q446R, p.V239D, and p.S90L were the most frequent mutations in Pakistanis [15]. Research on the *SLC26A4* gene is insufficient in the Korean population because most studies of *SLC26A4* mutations have dealt with hearing-impaired patients. There have been a few reports (case-control studies) on the prevalence of *SLC26A4* mutations [5,16-18]. In this study, we evaluated the carrier frequency of *SLC26A4* mutations that cause inherited deafness in the general Korean population.

Materials and Methods

1. Subjects

Samples were obtained from 144 unrelated individuals with normal hearing in the Korean population. Auditory capacity was established by evaluating pure-tone air-and bone-conduction threshold audiometry. Subjects gave written informed consent for genetic testing. Institutional review board (IRB) approval was obtained for the study from our IRB committee. Informed consent was confirmed by the IRB.

Genomic DNA was extracted from blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany).

2. Mutation analysis of *SLC26A4*

Mutations were detected by polymerase chain reaction (PCR) amplification, using primers of the coding exons 2-21 and the flanking intron sequences. Amplification was carried out in a C1000™ Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) from 25 ng target DNA in a final volume of 25 μL following the manufacturer's instructions. The reaction mixture consisted of 5 μL of each primer and Solg™ 2X Multiplex PCR Smart Mix (Solgent, Daejeon, Korea). The PCR conditions were denaturation at 95°C for 15 minutes, 35 cycles of denaturation at 95°C for 40 seconds, annealing at 56°C for 40 seconds, and extension 72°C for 1 minute. Final extension was at 72°C for 5 minutes. Unincorporated deoxyribonucleotide triphosphates (dNTPs) and primers were removed from the PCR products, which were then directly sequenced and analyzed using an ABI 3130XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA) and ABI Sequencing Analysis Software (v. 5.0; Applied Biosystems). The resultant sequences were compared with the coding sequence of *SLC26A4* (GenBank Accession No.NM_000441.1), and then the CLC Sequence Viewer 6 (http://www.clcbio.com/) was used to check whether the variation in the Pendrin protein structure was conserved between different species.

Results

Molecular analysis of the entire *SLC26A4* gene in 144 normal-hearing Korean individuals revealed 5 variants in a heterozygous state in the coding region, including 1 pathogenic mutation, c.2168A>G (p.H723R), 1 unclassified variant, c.147C>G (p.S49R), 2 polymorphisms, c.225G>C and c.2283A>G, and 1 novel variant, c.1723A>G (p.I575V) (Table 1). p.S49R and p.H723R had been previously reported [19,20]: p.H723R, which replaces histidine for arginine at c.2168A>G in exon 19, is the most prevalent mutation in various Asian populations and was observed in samples from 2 subjects (2/144, 1.39%) (Fig. 1A). The p.S49R variant was encodes serine at c.147C>G in exon 2 as a substitute for arginine. It was previously reported in Chinese

Table 1. *SLC26A4* sequence variants detected in 144 subjects with normal hearing

Nucleotide change	Amino acid change	Characterization of variant	Score		Carriers[c] (n)	Frequency (%)
			PolyPhen-2[a]	SIFT[b]		
c.147C>G	p.S49R	Unclassified	0.00	0.61	2	1.39
c.225G>C	p.(=)	Polymorphism			1	0.69
c.1723A>G	p.I575V[d]	Unclassified	0.00	0.47	1	0.69
c.2168A>G	p.H723R	Pathogenic	1.000	0.00	2	1.39
c.2283A>G	p.(=)	Polymorphism			1	0.69

[a]Big positive values may indicate that the studied substitution is rarely or never observed in the protein family.
[b]Amino acids with probabilities <0.05 are predicted to be deleterious.
[c]Total number=144.
[d]Novel variant.
PolyPhen-2, polymorphism phenotyping v2; SIFT, sorting intolerant from tolerant.

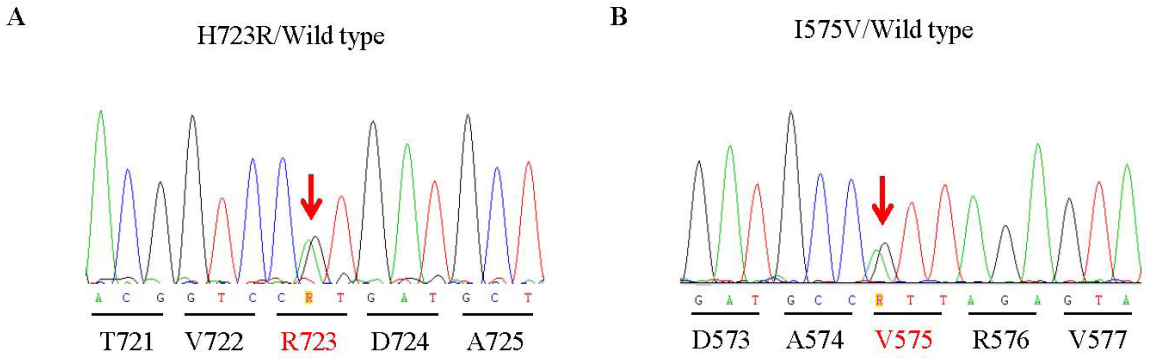

Fig. 1. Nucleotide sequence showing a heterozygous nucleotide change at the mutated location (arrows). (A) The H723R mutation encodes a histidine to arginine change at position 723. This mutation has been known as the most prevalent mutation in various Asian populations; in this study it was detected in samples from 2 subjects (2/144, 1.39%) in a heterozygous state. (B) A novel variant, A→G, at nucleotide 1723 in exon 16, which results in an isoleucine to valine substitution at amino acid 575.

normal populations but has remained unclassified [21,22]; it was observed in 2 subjects (2/144, 1.39%) in this study (Table 1). A novel variant, p.I575V, which has an A→G change at nucleotide 1723 in exon 16 and results in an isoleucine to valine substitution at amino acid 575, has never been reported and was detected in 1 subject (1/144, 0.69%) (Fig. 1B). We also detected 2 polymorphisms, c.225G>C and c.2283A>G, from each subject in a heterozygous state.

In order to determine how conserved the novel variant p.I575V was at the substitute location, we used the CLC Sequence Viewer 6 (Fig. 2). We checked the conservation scores of the alignments generated form pendrin protein sequences of the following species: chimpanzee (*Pan troglodytes*, XP_519308), monkey (*Macaca mulatta*, XP_001094049), orangutan (*Pongo abelii*, XP_003780614), sheep (*Ovis aries*, XP_004007900), cattle (*Bos taurus*, XP_002686849), wild boar (*Sus scrofa*, XP_003357559), dolphin (*Tursiops truncates*, XP_004315995), mouse (*Mus musculus*, NP_035997), and rat (*Rattus norvegicus*, NP_062087). As shown in Fig. 2, we found each variant to be

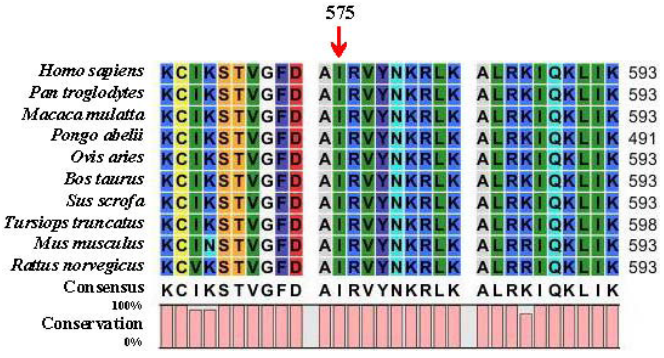

Fig. 2. Alignment of human pendrin protein sequence with its homologs. The red arrow indicates that isoleucine at amino acid 575 is highly conserved.

highly conserved. We also investigated the pathogenicity of the p.I575V variants using the Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant from Tolerant (SIFT; http://sift.bii.a-star.edu.sg/) prediction software. Both prediction tools indicated the results were benign even though the substitute location was highly conserved (Table 1).

Table 2. Carrier frequency of the p.H723R mutation in different normal Asian populations

Population	Subjects tested	p.H723R Heterozygote	Carrier frequency (%)	95% CI binomial distribution	References
Chinese	173	1	0.58	0-3.18	Chen et al. [23]
Japanese	96	1	1.04	0-5.67	Tsukamoto et al. [24]
Korean-1	120	2	1.67	0.2-5.89	Park et al. [5]
Korean-2	3,057	41[a]	1.34	0.96-1.82	Song et al. [17]
Korean-3	144	2	1.39	0.17-4.93	Present study

[a]This value was obtained by targeted mutation analysis for p.H723R.
CI, confidence interval.

As shown in Table 2, the carrier frequency of p.H723R was calculated to be 1.39% with a 95% binomial confidence interval of 0.17-4.93. Compared to other Asian populations, this frequency proved to be higher than that of the Chinese (0.58%) and Japanese populations (1.04%) [23,24], and similar to that found in previously studied Korean populations (Table 2) [5,17].

Discussion

SLC26A4 gene screening is widely used in the diagnosis of Pendred syndrome and EVA. Previous studies indicated that the spectrum of *SLC26A4* mutations vary based on the patients's ethnic background [5,21,22,24]. The *SLC26A4* gene variants present in the Asian population are significantly different from that of ethnic groups from European ancestry [5,24]. Park et al. [18] found that *SLC26A4* mutations occurred in 81% of unrelated Korean deaf patients with EVA. Among these *SLC26A4* mutations, p.H723R [c.2168A>G] has been shown to be the most frequent mutation in East Asian populations, especially in Japan and Korea [5,16-18,23]. In the present study, p.H723R was detected as the most frequent mutated allele, as expected (2/144, 1.39%). To determine the significance of the p.H723R frequency in our study, we compared the carrier frequency of p.H723R to the normal hearing population of Asia (Table 2). The frequency that we found (1.39%) proved to be similar to that of Park et al. [5] (1.67%) and Song et al. [17] (1.34%) for Korean populations. Our results showed that the prevalence of *SLC26A4* mutations was similar with previous Korean reports even though our study screened all exons and splice sites [5].

In this study, we evaluated the prevalence of *SLC26A4* gene mutations that cause inherited deafness in a Korean population with normal hearing and identified 5 different variants. One mutation, p.I575V, has not been previously identified as a variant. We confirmed the pathogenicity of this novel variant through alignment and prediction programs. Comparison of the pendrin protein (encoded by the *SLC26A4* gene) sequences between species showed that the position of this mutation is highly conserved (Fig. 2); however, pathogenic prediction programs unexpectedly indicated that the mutation is estimated to be benign (Table 1).

Among the detected variants, p.S49R, c.225G>C, and c.2283A>G have previously been reported in normal Chinese or Inner Mongolian populations [19,25]. In fact, c.919-2A>G is the most frequent mutation in the Chinese population; it is a splice site mutation in intron 7, which causes skipping of exon 8 resulting in a premature stop codon and leading to a predicted truncated protein [26,27]. However, this variant was not found in our study even though it was detected in hearing deficient populations from Japan, Korea, and China [5,16,24]. Choi et al. [28] and Shin et al. [29] reported p.H723R mutation frequencies of 63% and 61.5%, respectively, which were higher than their reported 18.5% and 30.8%, respectively, for c.919-2A>G in Korean patients with EVA. Likewise, the mutations p.L236P, p.T416P, and c.1001+1G>A, which, account for nearly half of all *SLC26A4* mutant alleles in Caucasian populations, were not detected in this study [7,21].

Despite hearing loss being the most frequent neurosensory disorder, deafness can remains undetected until it is too late to prevent undesirable or irreversible damage in many patients. Evidence shows that identification and habilitation of deaf infants before 6 months of age improves language outcomes [30,31]. These data support the time-critical nature of newborn hearing diagnosis and treatment, and they have provided the impetus for newborn hearing screening programs throughout the world. However, prior to the implementation of such a program, the carrier prevalence in a population and the availability of an effective screening test should be evaluated. In a Korean study by Han et al. [4], the diversity of *GJB2* mutations identified in the Korean population pointed to the importance of genetic testing by direct sequencing of the entire coding

region, not by targeted mutation analysis. Unlike the *GJB2* gene, *SLC26A4* is a large-scale gene, but until now, 5 variants, p.H723R, c.919-2A>G, c.1149+3A>G, p.M147V, and c.365_366insT have been considered hot-spot mutations of the *SLC26A4* gene in Korean. These 5 mutations should be sufficient, as evidenced by our results, which are in agreement with those previously reported [18].

Through genetic testing of considered to ethnic background the *GJB2* gene and mutational hot-spots of the *SLC26A4* gene, early diagnosis enables genetic counseling and early effective risk assessment for hearing loss patients and their families. In conclusion, the *SLC26A4* pathogenic mutations in a Korean population were identified with a carrier frequency of 1.39%, which is similar to that in other East Asian populations. We identified 5 variants in a heterozygous state found throughout the coding region of *SLC26A4* in the general Korean population. These results provide a fundamental basis for predicting a spectrum of the *SLC26A* mutation and for the design and interpretation of cost-efficient mutation detection algorithms in Korea.

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