

Original Article

# Screening of *SLC26A4* Gene Hotspots in 2673 Patients Associated with Sensorineural Hearing Loss in Northwestern China

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**BACKGROUND:** This study aimed to investigate the incidence of the hotspot mutations c.919-2A>G and c.2168A>G in *SLC26A4* in the northwestern Chinese population.

**METHODS:** A total of 2673 unrelated patients were recruited from northwestern China, and clinical information was obtained from all patients. Peripheral blood samples were acquired to detect the genotype of each patient by direct sequencing. Statistical analysis was conducted with Statistical Package for the Social Sciences 19.0 software.

**RESULTS:** Overall, 118 patients (4.4%) were identified with biallelic mutations, including 84 (3.14%) homozygotes and 34 (1.27%) compound heterozygotes. Moreover, significant differences between Han and Uighur were identified regarding the frequencies of c.919-2A>G homozygous and biallelic mutations.

**CONCLUSION:** This model for the rapid screening of hotspot mutations can identify the molecular cause for 4.4% of patients with severe to profound sensorineural hearing loss in northwestern China, and there may be distinctive hotspot mutations in different ethnic populations.

**KEYWORDS:** Enlarged vestibular aqueduct, ethnic groups, hotspot mutation, northwestern China, *SLC26A4*

## INTRODUCTION

Approximately 50% of deafness in childhood cases results from hereditary causes, with autosomal recessive deafness being the most common, accounting for 75-80% of nonsyndromic genetic hearing impairment.<sup>1</sup> Over 120 genes (e.g., *GJB2* and *SLC26A4*) display relationships to nonsyndromic hearing loss (<https://hereditaryhearingloss.org/>, updated on January 25, 2020). Among the genes, *SLC26A4* is considered to be the second most common cause of nonsyndromic deafness, with *GJB2* being the most common cause.<sup>2-4</sup> To date, over 200 *SLC26A4* mutations have been described,<sup>5</sup> and researchers have identified considerable differences in the hotspots of the *SLC26A4* mutation among populations from different regions or ethnic origins.<sup>3,6-13</sup> In China, large screenings of *SLC26A4* mutations in nonsyndromic deafness have been extensively conducted. As revealed from the results, c.919-2A>G was the most prevalent mutation, followed by c.2168A>G.<sup>3,10,13</sup>

Northwestern China refers to a large region with numerous ethnicities (e.g., Han, Hui, and Uighur). This area was once crossed by the Silk Road, which historically connected Chinese businesses with other countries. The population of this region was affected by immigration and national integration, thereby establishing a distinctive background regarding the economy, culture, and society. In this paper, 2673 patients subject to sensorineural hearing loss in northwestern China were recruited and screened by direct sequencing targeting the mentioned 2 hotspot mutations, c.919-2A>G and c.2168A>G, to detect the deafness gene of patients and to ascertain the cause of their deafness quickly and at low cost.

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## MATERIALS AND METHODS

A total of 2673 unrelated patients (1481 males and 1192 females; age range, 3 months-39 years; mean  $\pm$  SD,  $12.5 \pm 5.2$  years) were recruited from northwestern China. Although a number of families with more than 2 hearing impairments were recruited, this study only counted probands. These patients came from 11 different ethnicities, which were partitioned according to the same language, same region, same economy, and same psychology in China. There were Han, Hui, Uigur, and other ethnic groups (including Tibetan, Dongxiang, Kazak, Mongol, Tujia, Salar, Yugur, and Kirgiz) in this study. According to the protocol approved by the ethics committee of Lanzhou University Second Hospital (P 3, Line 6-7), the informed consent and blood samples of all subjects were obtained. The parents were interviewed to obtain the family history, age at diagnosis, and clinical history of subjects. A total of 472 neonates who passed the hearing screening at birth were also recruited as a control group.

Systematic clinical assessments and audiometric assessments were performed for all participants. The pure-tone audiometry (PTA) was conducted in a sound isolation room using a Madsen 622-type pure-tone audiometer (Nestor Medical Denmark Co. Ltd, Tastrump City, Denmark). The hearing thresholds of air conduction were measured in the frequency range of 125-8000 Hz and those of bone conduction were measured in the frequency range of 250-4000 Hz. The degree of hearing impairment was classified according to the air conduction hearing thresholds of the better ear at 500, 1000, 2000, and 4000 Hz. PTA  $\leq 25$  dB HL was classified as normal hearing, PTA  $\geq 26$  dB HL and  $\leq 40$  dB HL were classified as mild hearing loss, PTA  $\geq 41$  dB HL and  $\leq 60$  dB HL were classified as moderate hearing loss, PTA  $\geq 61$  dB HL and  $\leq 80$  dB HL were classified as severe hearing loss, and PTA  $\geq 81$  dB HL were classified as profound hearing loss.<sup>14</sup> If subjects could not respond to the PTA, ABR (Intelligent Hearing Systems, Miami, Fla, USA) was implemented in an electric shielding room. The distortion product otoacoustic emission (DPOAE, Eroscaan, MAICO, Berlin, Germany) and autoauditory brain-stem response (AABR, MB11, MAICO, Berlin, Germany) were used to test the newborn hearing.

Genomic DNAs were extracted from acquired peripheral blood samples. The software Primer 5.0 was used to design primers for the flanking sequences of intron or exon where c.919-2A>G and c.2168A>G were located. Polymerase chain reaction (PCR) was used to amplify subjects' DNA fragments, each PCR fragment was purified and directly sequenced, and the sequence data were compared with the NCBI (NT\_007933) reference sequences by Sequencher 5.4.5 software. The primers were synthesized by Invitrogen Company (Shanghai, China). All genotyping and sequencing were performed at Beijing Genomics Institute (Shenzhen, China).

Temporal bone high-resolution computed tomography (HRCT) was performed for randomly selected patients. Axial and coronal temporal bone CTs were carried out abiding by the recognized standards to define cochlea vestibular malformations. On axial sections, if the mid-vestibular aqueduct diameter between the common crus and the midpoint of external aperture  $\geq 1.5$  mm, it was defined as enlarged vestibular aqueduct (EVA),<sup>15</sup> or the coronal and axial width at the midpoint between labyrinth and operculum was greater than 1.5 mm on coronal sections.<sup>16</sup>

Statistical analysis was calculated using Statistical Package for the Social Sciences 19.0 software (SPSS Inc.; Chicago, IL, USA). Frequencies of c.919-2A>G and c.2168A>G mutations were compared among 3 different ethnic groups (Han, Hui, and Uighur). Assuming that  $\alpha = 0.05$ , if the *P* value is  $< 0.05$ , the difference was considered to be significant.

## RESULTS

All 2673 recruited subjects were nonsyndromic deafness, and all have bilateral severe to profound sensorineural hearing loss.

In the present study, 118 (4.4%, 118/2673) subjects with sensorineural hearing loss exhibited biallelic mutations of *SLC26A4*. The c.919-2A>G and c.2168A>G homozygous mutations were identified in 77 cases (2.88%, 77/2673) and 7 (0.26%, 7/2673), respectively. The compound heterozygous mutations were detected in 34 cases (1.27%, 34/2673). Among the 118 patients exhibiting biallelic mutations, 103 were Han, 12 were Hui, and 2 were Uighur, as shown in Table 1. Moreover, 5 subjects (1.06%, 5/472) with a heterozygous c.919-2A>G mutation were identified in 472 controls, whereas the other c.2168A>G mutation was not identified in this study.

The patients carrying mutations differed considerably in ethnic group. Most of the participating subjects were Han, accounting for 77.1% of the 2673 patients, followed by Hui at 12.0%, and Uighur at 7.8%. The percentages of total biallelic mutations in Han, Hui, and Uighur reached 5.00%, 3.73%, and 0.96%, respectively. The Pearson's chi-square test was performed to analyze the 2 according to 2 contingency tables for c.919-2A>G homozygous mutation in 3 different ethnic groups; the identical method was adopted to compare the frequencies of c.2168A>G homozygous mutation, compound heterozygous mutations, as well as the total biallelic mutations in those 3 groups. All *P* values in the 3 different ethnic groups are listed in Table 2. Notably, significantly different mutation frequencies of c.919-2A>G homozygous and total biallelic mutations were identified between Han and Uighur. However, no statistically significant difference was reported in the frequencies of c.919-2A>G homozygous, c.2168A>G homozygous, compound heterozygous mutations, and biallelic mutations between Han and Hui. Furthermore, no significant difference between Hui and Uighur was observed in the frequencies of all the mentioned mutations.

### Axial Temporal Bone Computed Tomography Is Helpful for Confirming the Presence of Enlarged Vestibular Aqueduct

A total of 112 patients exhibiting homozygous or compound heterozygous *SLC26A4* mutations were randomly selected to undergo HRCT scans. As a result, EVA was confirmed by HRCT in all 112 patients with biallelic *SLC26A4* mutations. Extrapolating to the larger population,

**Table 1.** Number of Patients Exhibiting Biallelic Mutations and Percent of Each Biallelic Mutation in the Different Ethnic Groups

Ethnicity	c.919-2A>G/ c.919-2A>G		c.2168A>G/ c.2168A>G		c.919-2A>G/ c.2168A>G		Total	
	n	f (%)	n	f (%)	n	f (%)	n	f (%)
Han	69	3.35	5	0.24	29	1.41	103	5.00
Hui	6	1.86	2	0.62	4	1.24	12	3.70
Uigur	1	0.48	0	0	1	0.48	2	0.96
Others	1	1.20	0	0	0	0	1	1.20

**Table 2.** Results of Pearson's Chi-Square Test ( $P^{\#}$ ) for the Different Mutations in 3 Different Ethnic Groups

Different Ethnicities	c.919-2A>G Homozygous Mutations	c.2168A>G Homozygous Mutations	compound Heterozygous Mutations	Total Biallelic Mutations
Between Han and Uigur	0.023 <sup>#</sup>	1.000	0.517	0.008
Between Han and Hui	0.156	0.242	1.000	0.322
Between Hui and Uigur	0.255	0.522	0.653	0.053

<sup>#</sup>We have assumed that  $\alpha=0.05$  in advance, if the  $P < .05$ , a statistical difference was considered significant.

these data indicate that the majority of patients with biallelic *SLC26A4* mutations will suffer from EVA.

## DISCUSSION

In the present study, the direct sequencing containing the hotspot mutations c.919-2A>G and c.2168A>G was employed to detect the genetic etiology of 2673 subjects experiencing severe to profound sensorineural hearing loss. By screening out the above 2 hotspot mutations of *SLC26A4*, this study verified that 4.4% of patients were identified with biallelic mutations in northwestern China. It is estimated that about 800 000 children under the age of 7 have inherited sensorineural hearing loss and approximately 30 000 neonates suffer from congenital hearing loss in China each year.<sup>17</sup> This study revealed that several patients would be clearly diagnosed exhibiting biallelic mutations of *SLC26A4* at early stages of disease development by direct sequencing. Note that for babies with congenital hearing loss, early identification would establish a foundation for positive and effective clinical guidance. Implementation of hearing aids, cochlear implants, and speech training may elevate these patients' quality of life.

Existing studies have reported that each ethnic population has its own unique hotspot of *SLC26A4* variants. In east Asia, c.2168A>G is considered the most prevalent mutation in Japanese and Koreans,<sup>8-9,11</sup> whereas c.919-2A>G refers to the most common mutation in mainland Chinese and Taiwanese.<sup>3,7,10,12-13</sup> However, the p.T416P and IVS8+1G>A were reported as the 2 most frequent mutations in northern European populations.<sup>13,18</sup> This study identified a significant difference in frequencies of c.919-2A>G homozygous and biallelic mutations between Han and Uighur, as evidenced by the fact that the Uighur population contains both European and East Asian motifs,<sup>19</sup> which is in contrast to the Mongoloid subspecies, to which the Han population belongs. No significant difference was reported in the frequencies of c.919-2A>G homozygous, c.2168A>G homozygous, and biallelic mutations between Han and Hui. Moreover, no significant difference between Hui and Uighur was found regarding the frequencies of all the mentioned mutations. This finding is probably the result of the different origins of Han, Hui, and Uighur population. As revealed from human mtDNA analysis, the region did not significantly impact the matrilineal gene pool of Hui and Han population.<sup>20</sup> In this study, the frequencies of c.919-2A>G homozygous and biallelic mutations reached 3.35% and 5.00%, respectively, in the Han population, while the frequencies were 0.48% and 0.96%, respectively, in the Uighur population. Thus, the incidence of Uighurs was considerably lower than that of Hans regarding these 2 mutations. Accordingly, this study speculates that there are unique hotspot mutations in the Uighur population, and this possibility warrants further research.

At present, the diagnosis of EVA primarily relies on temporal bone HRCT. However, CT examination requires patients who can achieve an acceptable level of cooperation, and some children fail to

cooperate. Furthermore, a temporal CT scan costs more than \$47 in China, which remains a large expense for some poor patients, while the cost of the above-mentioned genetic testing does not exceed \$15. Due to those limitations, it is unlikely that CT scans will be performed on each patient with hearing loss during the diagnosis of EVA. Compared with CT examination, genetic diagnosis of deafness possesses a unique advantage in the etiological diagnosis of sensorineural deafness. For some children unable to cooperate with CT examination or some sensorineural patients facing difficulty in CT diagnosis, gene detection can elevate the diagnostic level.<sup>21</sup> Furthermore, 97.9% of patients with EVA were reported to carry at least one possible pathogenic variant in *SLC26A4* in China.<sup>10</sup> In this study, patients with biallelic *SLC26A4* mutations who underwent testing for EVA had EVA. These findings confirmed that *SLC26A4* mutations in patients with hearing loss indicate a high possibility of EVA.

Note that *SLC26A4* contains 20 exons. It is expensive and time-consuming to identify the genotype of each deaf patient by scanning all exons of *SLC26A4*; therefore, this approach serves as an economical and convenient method to initially screen the hotspot mutations. According to the distinctive hotspot mutations in different ethnic populations, hotspot mutation screening of c.919-2A>G will be conducted in the Han population, while other hotspot mutation screening methods will be employed in the Uighur population. For detected subjects with biallelic mutations of *SLC26A4*, normal speech may be achieved using early cochlear implants, even in patients with profound hearing loss, which may also reduce the consequences for family members affected by EVA.

## CONCLUSION

The hotspot mutations c.919-2A>G and c.2168A>G was employed to quickly detect the genetic etiology of 2673 subjects exhibiting severe to profound sensorineural hearing loss, and 4.4% of patients were identified as exhibiting biallelic mutations. The 3 major ethnic groups exhibited different frequencies of the c.919-2A>G and c.2168A>G mutations. Significant differences between Han and Uighur populations were identified in c.919-2A>G homozygous and biallelic mutation frequencies. This model for the rapid screening of hotspot mutations can identify the molecular cause for patients with severe to profound sensorineural hearing loss in northwestern China, and there may be distinctive hotspot mutations in different ethnic populations.

**Ethics Committee Approval:** This study was conducted in accordance with the protocol approved by the ethics committee of Lanzhou University Second Hospital (P 3, Line 6-7).

**Informed Consent:** The informed consent and blood samples of all subjects were obtained.

**Peer-review:** Externally peer-reviewed.

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**Declaration of Interests:** The authors have no conflict of interest to declare.

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