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Genetic mutations in non-syndromic deafness patients in Hainan Province have a different mutational spectrum compared to patients from Mainland China

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ABSTRACT

Objectives: To provide appropriate genetic testing and counseling for non-syndromic hearing impairment patients in Hainan Province, an island in the South China Sea.
Methods: 299 unrelated students with non-syndromic hearing loss who attended a special education school in Hainan Province were enrolled in this study. Three prominent deafness-related genes (*GJB2*, *SLC26A4*, and mtDNA *12S rRNA*) were analyzed using Sanger sequencing.
Results: *GJB2* mutations were detected in 32.78% (98/299) of the entire cohort; however, only 5.69% (17/299) had two confirmed pathogenic mutations. The most common mutation observed in this population was c.109G > A in the *GJB2* gene, with an allelic frequency of 15.05% (90/598), which is significantly higher than that reported in previous cohorts. A total of 16 patients had two confirmed pathogenic *SLC26A4* gene mutations, and 16 patients had one. The IVS7-2A > G mutation was the most commonly observed, with an allelic frequency of 3.51% (21/598). Three patients had a m.1555A > G mutation in the mtDNA *12S rRNA* gene.
Conclusions: These results reveal that genetic etiology occurred in 11.71% (35/299) of patients, suggesting that Hainan province have a different mutational spectrum compare to Mainland China in non-syndromic deafness patients, which provide useful information to genetic counseling in Hainan province.

1. Introduction

Hearing impairment is the most commonly observed neurosensory disorder. Its reported incidence varies from 1/300 to 1/1000 children [1], and approximately half of all cases are caused by genetic defects. *GJB2*, *SLC26A4*, and mitochondrial DNA (mtDNA) *12S rRNA* genes are believed to be the most common causes of non-syndromic hearing loss [2].

In China, an estimated 30,000 babies are born each year with congenital hearing impairment per 20 million live births. Moreover, 27.80 million people in China have hearing and speech disabilities; of these, 20.04 million have simple hearing disabilities [3]. China is a large country, with 1.3 billion people representing 56 ethnicities. For effective genetic testing and accurate counseling, genetic studies have been conducted on deaf Chinese patients, but the mutation spectra differ by race and geographic location. For example, in typical areas of China, mutations in the *GJB2* gene account for 18.31% of patients with non-syndromic hearing loss, the mitochondrial *12S rRNA* gene mutation, m.1555A > G, accounts for 1.76%, and *SLC26A4* mutations account for 13.73% [4]. However, such molecular etiologies are rare in non-syndromic Tibetan Chinese patients with hearing impairment. No mutations in the *GJB2* or *SLC26A4* genes have been identified, whereas the mitochondrial m.1555A > G homogeneous mutation was associated with hearing loss in 1.75% of 114 Tibetan patients [3]. Thus, genetic counseling for deafness should take into account differences among patients from different geographical regions.

A previous study showed several common deafness-related mutations in the Chinese population: *GJB2* c.235delC, c.299-300delAT, c.176-191del16, and c.35delG; *GJB3* c.538C > T; *SLC26A4* IVS7-2A > G and c.2168A > G; and mitochondrial m.1555A > G and m.1494C > T [5]. However, given the heterogeneity of deafness, specific ethnic and regional factors are important components of this

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disorder. Although the mutation spectrum of non-syndromic hearing impairment (NSHI) has been studied in northern [6], central [4], southern [7], northwest [8], and southwest China [9], and in Xinjiang [10], Tibet [3], Yunnan [11], Gansu [12], Inner Mongolia Autonomous Region [13], Hebei [14], Tengzhou [15], and Xiamen Chinese [16], little is known with regard to Hainan Province, an island in the South China Sea. To extend the epidemiological data on common gene mutations among deaf individuals from different populations, we comprehensively analyzed three deafness-related genes (*GJB2*, *SLC26A4*, and mtDNA *12S rRNA*) in 299 patients from Hainan Province who experienced early-onset NSHI.

2. Materials and methods

2.1. Ethics statement

The study protocol was performed following approval by the ethics committee of the Chinese PLA General Hospital.

2.2. Patients and DNA samples

A total of 299 unrelated sporadic or familial cases of NSHI from the Hainan Special Education School in Hainan Province were enrolled in this study. The patient cohort consisted of 166 males and 133 females aged 1–31 years (average, 15.07 years).

Informed consent was obtained from all subjects prior to blood sampling. Parents were interviewed to determine the age of onset, family history, health of the mother during pregnancy, and clinical history of the patient, including the occurrence of infection, head or brain injury, and the use of aminoglycoside antibiotics. All subjects had moderate to profound bilateral sensorineural hearing impairment based on audiograms. Medical examinations revealed no clinical features other than hearing impairment. DNA was extracted from the peripheral blood leukocytes of all 299 patients with NSHI using a blood DNA extraction kit (TianGen, Beijing, China) according to the manufacturer's instructions.

2.3. Mutation analysis

The coding exons plus 50–100 base pairs (bp) of the flanking intronic regions of *GJB2*, mtDNA *12S rRNA*, and *SLC26A4* genes were amplified by polymerase chain reaction (PCR). DNA sequencing reactions were performed in both directions using the same primers as those used for PCR. Sequencing was performed using the Applied Biosystems (ABI) Prism BigDye Terminator v3.1 Cycle Sequencing Kit and was run on an ABI 3100 Genetic Analyzer. Patients with monoallelic *GJB2* coding region mutations were further tested for the *GJB2* IVS1 + 1G > A mutation and mutations in *GJB2* exon 1 and its basal promoter [5].

For all patients, 20 exons and flanking intronic sequences from the *SLC26A4* gene were directly sequenced as previously described [6,17].

DNA sequences were aligned using Genetool software and compared with published sequences from the NCBI database (*GJB2*: NM_004004.5; *SLC26A4*: NM_000441.1).

3. Results

3.1. GJB2

Sequence analysis of the *GJB2* gene identified eight previously identified pathogenic mutations (c.3G > T, c.23C > T, c.88A > G, c.107T > C, c.109G > A, c.176-191del16, c.235delC, and c.299-300delAT) and seven polymorphisms (c.79G > A, c.308A > G, c.341A > G, c.368C > A c.457G > A, c.571T > C and c.608T > C) (Table 1 and Table 2). All *GJB2* mutations identified were recessive, and dominant mutations were absent from this cohort.

Table 1
Genotypes of patients with pathogenic mutations in *GJB2*.

Allele 1		Allele 2		Number of patients
Nucleotide	AA change	Nucleotide	AA change	
c.235delC	Frameshift	c.235delC	Frameshift	7
c.109G > A	p.V37I	c.109G > A	p.V37I	7
c.235delC	Frameshift	c.299-300delAT	Frameshift	1
c.176-191del16	Frameshift	c.107T > C	p.L36P	1
c.3G > T	p.M1I	c.235delC	Frameshift	1
c.109G > A	p.V37I			76
c.235delC	Frameshift			3
c.88A > G	p.I30V			1
c.23C > T	p.T8M			1

In total, 17 patients (5.59%, 17/299) were homozygous (14 patients) or compound heterozygous (3 patients) for *GJB2* pathogenic mutations (Table 1). Additionally, 81 patients carried one heterozygous pathogenic mutation without an identified second mutant allele in *GJB2* (Table 1). Thus, *GJB2* mutant alleles accounted for 19.23% (115/598) of the total alleles in the 299 NSHI patients (Table 2). Unlike the case in most areas of China, the most prevalent mutation allele of *GJB2* in Hainan Province was not c.235delC, but c.109G > A, followed by c.235delC, c.3G > A, c.23C > T, c.88A > G, c.107T > C, c.176-191del16, and c.299-300delAT; the allele frequencies were 15.05% (90/598), 3.18% (19/598), 0.17% (1/598), 0.17% (1/598), 0.17% (1/598), 0.17% (1/598), 0.17% (1/598), and 0.17% (1/598) respectively. Patients with monoallelic mutations were also tested for the *GJB2* IVS1 + 1G > A mutation or defects in *GJB2* exon 1 and its basal promoter; however, no pathogenic mutations were identified.

In total, 83 patients were determined to carry at least one c.109G > A allele; 7 were homozygotes, and 76 were single heterozygotes. To identify the c.109G > A mutation in individuals with normal hearing in this area, we analyzed the *GJB2* gene in 97 subjects with normal hearing. The allelic frequency of c.109G > A was 17.53% (34/194), with one homozygote and 32 heterozygotes. The second most common mutation of *GJB2* in this group was c.235delC, with a mutation allelic frequency of 3.18% (19/598), including 7 homozygotes, 2 compound heterozygotes, and 3 heterozygotes.

3.2. SLC26A4

Of the 299 patients whose *SLC26A4* gene was sequenced, 16 patients carried two confirmed pathogenic mutations, 16 carried one confirmed pathogenic mutation, and 5 carried one unclassified variant (Table 3). Thus, mutations in *SLC26A4* were identified in 12.37% (37/299) of patients with hearing impairment.

In total, 11 different pathogenic mutations (IVS7-2A > G, c.259G > T, c.697G > C, c.754T > C, c.1086-1087insT, c.1225C > T, c.1229C > T, c.1983C > A, c.2009T > C, c.2086C > T, and c.2168A > G) and five unclassified variants (c.236G > A, c.1138G > A, c.2234C > T, IVS7-18T > G, and IVS7 + 35A > G) were identified (Table 3). The c.1086–1087insT mutation resulted in a premature stop codon and a truncated protein of only 375 amino acid residues. Mutations IVS7-2A > G, c.259G > T, c.697G > C, c.754T > C, c.1225C > T, c.1229C > T, c.1983C > A, c.2009T > C, c.2086C > T, and c.2168A > G were previously reported in patients with hearing loss [7,18–21], whereas mutations c.236G > A, c.1138G > A, c.2234C > T, IVS7-18T > G, and IVS7 + 35A > G were considered benign or of unknown significance in the Deafness Variation Database.

The mutation allelic frequency was 4.01% (24/598) for IVS7-2A > G, 1.00% (6/598) for c.2086C > T, 0.33% (2/598) for c.259G > T, 0.50% (3/598) for c.697G > C, 0.50% (3/598) for c.754T > C, 0.33% (2/598) for c.1229C > T, 0.67% (4/598) for c.1983C > A, and 0.17% (1/598) for each of the others (Table 4).