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Original Article

Gene Screening for Non-Syndromic Deafness in Hainanese Patients

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BACKGROUND: Hainan Province is the southernmost island in China, far from the mainland, and the resident population changes little. In order to understand the mutation spectrum in Hainan and provide effective genetic counseling for deaf people, we carried out genetic analysis on the non-comprehensive hearing impairment in this population. Therefore, in this study, 183 children with moderate sensorineural deafness in the northeast of Hainan were analyzed with susceptibility gene carrying and gene mutation, providing some reference for hainan to guide the prevention and treatment of deafness.

METHODS: Complete clinical evaluations were performed on 183 unrelated patients with a non-syndromic hearing impairment from Hainan Province. Each subject was screened for common mutations using the matrix-assisted laser desorption ionization-time of flight mass spectrometry, including *GJB2* c.35delG,c.235delC,c.299_300del AT,c.176_191del16,c.167delT; *GJB3* c.538 C>T,c.547G >A;*SLC26A4* IVS7-2 A>G,c.2168 A>G,c.1174A>T,c.1229 C>T,c.1226G>A,c.1975G>C,c.2027T>A,c.2162C>T,c.281C>T,c.589G>A,IVS15+5G>A; and mtRNA 1494 C>T,1555 A>G.

RESULTS: Genetic analysis showed that *GJB2*, *SLC26A4*, and mitochondrial M. 1555A > G mutations accounted for 7.10%, 8.74%, and 0.55% of the etiology of non-syndromic hearing impairment, respectively. Common mutations include *GJB2* C. 235delC, *SLC26A4* c.I vs7-2a →G, C. 2168A→G, and mitochondrial M. 1555A > G. The total mutation rate in Hainan was 16.39%.

CONCLUSION: Our study is the first one to carry out genetic analysis on non-syndromic hearing impairment in Hainan. The results show that in the cases of non-syndromic hearing impairment in these areas, there is a clear genetic cause accounted for 16.39%, and the mutation hot spots are mainly *GJB2* and *SLC26A4*, and *SLC26A4* is the most common mutation site. This study provides useful and targeted information for genetic counseling of deafness in people with non-syndromic hearing impairment in Hainan.

KEYWORDS: Children, sensorineural deafness, deafness gene

INTRODUCTION

At present, there are about 360 million deafness patients in the world, more than half of which are caused by genetic factors, among which *GJB2*, *SLC26A4*, mitochondrial 12S rRNA, and *GJB3* are the common deaf-causing genes.[1-3] According to the second National Sampling survey of persons with disabilities, in 2006, there were 21 million people with hearing disabilities in China, accounting for 33.51% of the total number of persons with disabilities, and about 30 000 new deaf children are born every year, of which about 50%-60% are caused by genetic factors.[4] A large number of epidemiological studies in China have shown that the common pathogenic genes of inherited deafness in China are *GJB2*, *SLC26A4*, mitochondrial 12S rRNA, and *GJB3*. The types and frequencies of deafness gene mutations are different in different regions and ethnic groups.[3,5-8]

Common pathogenic genes for non-syndromic hearing impairment (NSHI) are the same in different regions and ethnic groups. Regional and ethnic factors play an important role in the clinical diagnosis of non-syndromic hearing impairment. For example, in 2007, P Dai et al[3] tested the deafness gene of 3004 patients with a non-syndromic hearing impairment from 26 regions in China and found that *GJB2* gene mutations were the most common account for the etiology of patients with hearing loss. However,

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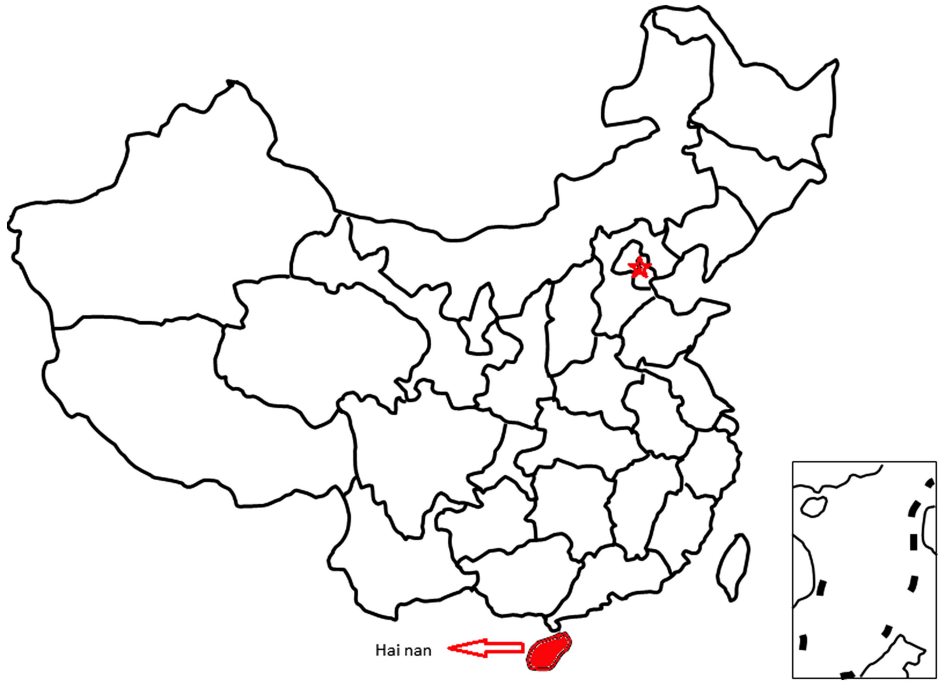

Figure 1. Geographical distribution of Hainan province.

due to a large number of deaf populations and regional and ethnic differences, these results do not explain the spectrum of mutations in each region. For example, Yuan et al[9] reported in 2012 that common molecular causes are rare in non-syndromic Tibetan hearing impairment. No *GJB2* and *SLC26A4* gene mutations were found in 114 patients of Tibetan ethnicity, and 1.75% of hearing loss was related to mitochondrial 12S rRNA mutations.[6]

Therefore, in the research and consultation of deafness genes, it is necessary to fully consider the differences between regional and ethnic backgrounds and establish specific mutation databases for different populations in different regions. At present, there is a deafness gene mutation spectrum in northern China,[8] central China,[7] Xinjiang,[10] Tibet, and Yunnan.[3,6]

Hainan is China's southernmost island (Figure 1), far from the mainland, and its permanent population does not change much. At present, there is no research report on deafness gene of deaf patients in Hainan area. Therefore, in order to understand the genetic mutation spectrum of deaf people in Hainan and provide effective genetic counseling for deaf people, we conducted a genetic analysis of incomplete hearing impairment in this population.

MAIN POINTS

- The common pathogenic genes of deafness in China were *GJB2*, *SLC26A4*, and mitochondrial DNA. The most common deafness gene is *GJB2*.
- In this study, the mutation rate of deafness gene in Hainan was the highest in *SLC26A4* not *GJB2*. The detection rate of deafness genes is generally lower than that in other parts of China.
- Special geographical location and small population movement may be associated with a low mutation rate of deafness gene.

MATERIAL AND METHODS

Datasets

A total of 183 patients with moderate or above sensorineural hearing loss in Hainan province were recruited, including 106 males and 73 females. Age ranged from July to 18 years, with a mean age of 9.5 years. Approved by the Ethics Committee of Hainan Affiliated Hospital of Hainan Medical University (Approval No:Med-Eth-Re[2022] 342), this study all of the patients after informed consent signed by the guardian, collected by a professional Aurist, including general information of patients, the condition of the hearing-impaired time causes, such as family history, deafness and establish the medical records archive, to perfect the relevant examination and imaging examination, and hearing. Patients with all kinds of syndromic hereditary deafness and acute and chronic otitis media, Meniere's disease, acoustic neuroma, meningitis, and traumatic deafness were excluded. All patients were local residents of Hainan for more than 2 generations and all were of Han nationality. There were 130 patients (76 males and 54 females) with extremely severe hearing loss, 36 patients (21 males and 15 females) with severe hearing loss, and 17 patients (9 males and 8 females) with moderate hearing loss. Sixteen patients were diagnosed with large vestibular aqueduct syndrome (EVA) according to temporal CT examination.

Audiometry

All subjects underwent audiological examination at a professional audiology center. The examination contents included behavioral audiometry, pure-tone audiometry (RWM MICRO INC, TinniTest, Sichuan), acoustic resistance (Interacoustics A/S, AD235H, Denmark), auditory brainstem response (ABR) (Intelligent Hearing Systems, SmartEP, USA), and auditory steady-state response (ASSR) (Intelligent Hearing Systems, SmartEP, USA). The mean thresholds of binaural air conductance of 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz were calculated according to the hearing examination results of the subjects. According to the classification standard of hearing loss degree of WHO International Classification

Table 1. *GJB2* Mutation Spectrum in Patients with NSHI from Hainan Province

Detection of Gene	Mutation Site	Mutation Type	Mutation Cases	Proportion
GJB2	235delC	Homozygous	9	4.92% (9/183)
	235delC	Heterozygous	3	1.64% (3/183)
	235delC+299_300delAT	Complex heterozygous	1	0.55% (1/183)

NSHI, non-syndromic hearing impairment.

of Impairments, Disabilities and Disablement, the degree of hearing loss of subjects over 5 years old was classified as normal hearing: ≤25 dB HL; mild hearing loss: 26-40 dB HL; moderate hearing loss: 41-60 dB HL; severe hearing loss: 61-80 dB HL; and extremely severe hearing loss: ≥81 dB HL. The diagnosis and degree of hearing loss under 5 years old were classified mainly by reference to the ABR response threshold. The ABR wave V response threshold ≤30 dBnHL was taken as the normal standard of response threshold between 2 kHz and 4 kHz, and the diagnosis of hearing loss greater than 30 dBnHL was classified as follows: mild 31-50 dBnHL, moderate 51-70 dBnHL, severe 71-90 dBnHL, and extremely severe ≥91 dBnHL. In the same person, hearing loss in both ears shall be judged on the less severe side.

Gene Detection

Peripheral venous blood was collected from the subjects and used the Matrix-assisted laser desorption ionization-time of flight-mass spectrometry, MALDI-TOF-MS (Huada Clinical Laboratory Center, Shenzhen, China) to detect common deafness genes, including 4 deafness genes and 20 common hot spot mutations, including C.35DELg, C.235DELC, C.299_300del AT, C.176_191DEL16, and C.167DELT in *GJB2* gene.C.538 C>T, C.547G > A mutation of *GJB3* gene; *SLC26A4* gene ivS7-2A >G, C.2168 A>G, C.1174A > T, C.1229C>T, C.1226G > A, C.1975G > C, C.2027T > A, C.2162C >T, C.281C >T, C.589G > A, IVS15+5G > A mutation; and 1494 C>T and 1555 A>G mutations of mitochondrial 12S rRNA.

Data Analysis

Then we set up an Excel database and used Statistical Package for Social Sciences version 19.0 (IBM SPSS Corp.; Armonk, NY, USA) statistical software for analysis and carried out a descriptive analysis of the situation of genetic testing.

RESULT

Total Detection of Deafness Gene in Hainan Province

In this study, 183 patients with hearing impairment were mainly distributed in the eastern, northern, and northwestern regions of Hainan province. All patients had preverbal deafness, with hearing loss ranging from moderate to very severe, ranging in age from 7 months to 18 years. Common deafness gene mutations were detected in 30 of 183 patients (16.39%, 30/183); 13 cases of *GJB2* gene mutation (7.10%); and 16 cases of *SLC26A4* gene mutation (8.74%) were detected among 30 cases of deafness gene mutation. Mitochondrial DNA mutation was detected in 1 case (0.55%). No *GJB3* gene mutation was detected.

Prevalent *GJB2* Mutations in Patients with NSHI From Hainan Province

GJB2 coding region sequencing identified 10 known pathogenic mutations (c.11G > A, c.35delG, c.109G > A, c.176-191del16, c.235delC, c.257C > G, c.299-300delAT, c.427C > T, c.512insAACG, and c.605ins46), 2 unclassified variants (c.187G > T, c.368C > A), and 5 polymorphisms (c.79G > A, c.341A > G, c.186C > T, c.558G > A and c.608 T > C). All *GJB2* mutations identified in this cohort were recessive. The dominant mutation was absent in this cohort.

Of these 183 patients, 13 (7.10%, 13/183) carried homozygous or complex heterozygous *GJB2* pathogenic mutations; there were 9 (4.92%, 9/183) 235delC homozygous mutations, 3 (1.64%, 3/183) 235delC heterozygous mutations, and 1 235delC heterozygous mutation; 299_300delAT heterozygous mutation) (0.55%, 1/183) (see Table 1 and Figure 2). The mutation sites of *GJB2* in Hainan were 235delC, 299_300delAT was rare, and other mutations of *GJB2* could not be detected.

Hereditary Heterogeneity of *SLC26A4* Mutations Correlated with EVA *SLC26A4*

Through genetic screening, we detected 16 (8.74%, 16/183) of 183 patients carrying *SLC26A4* CIVS.7-2A > G or C. 2168A > G allele. Temporal computed tomography (CT) examination of the temporal bone in 14 of these 16 patients indicated that the large vestibular aqueduct and CT examination of 2 temporal bones showed no abnormality. Among these 183 patients, CT examination of temporal bone indicated the large vestibular aqueduct in 2 patients, but no *SLC26A4* gene was detected. The *SLC26A4* gene is the highest detection rate in patients with non-syndromic deafness in Hainan (see Table 2 and Figure 3).

Low Frequency of Mitochondrial DNA 1555A > G Mutation, *GJB3* Gene Mutations Are Rare

One homozygous M. 1555A > G mutation (0.55%, 1/183) was detected in the 183 patients with non-syndromic deafness, but the *GJB3* mutation was not detected. These results

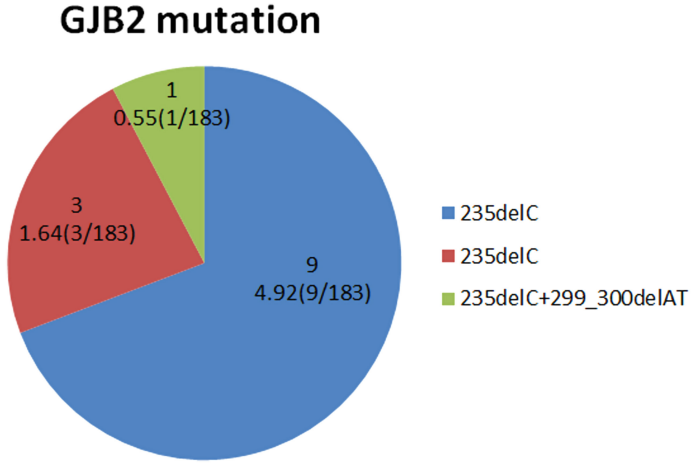

Figure 2. *GJB2* mutation spectrum in patients with NSHI from Hainan province. NSHI, non-syndromic hearing impairment.

Table 2. *SLC26A4* Mutation Spectrum in Patients with NSHI from Hainan Province

Detection of Gene	Mutation Site	Mutation Type	Mutation Cases	Proportion
SLC26A4	IVS7-2A>G+2168A>G	Complex heterozygous	2	1.09 (2/183)
	IVS7-2 heterozygous and 2168 heterozygous	Complex heterozygous	1	0.55 (1/183)
	IVS7-2A>G	Heterozygous	8	4.37 (8/183)
	2168A>G	Heterozygous	4	2.19 (4/183)
	IVS7-2A>G	Homozygous	2	1.09 (2/183)

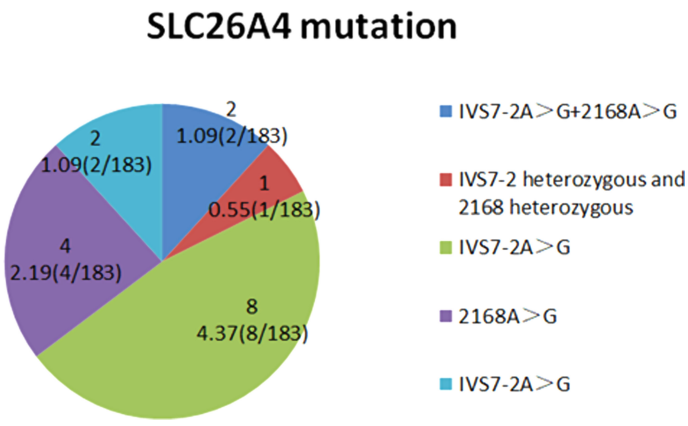

Figure 3. *SLC26A4* mutation spectrum in patients with NSHI from Hainan province. NSHI, non-syndromic hearing impairment.

suggest that m.1555A > G mutation and *GJB3* mutation are relatively rare in Hainan.

The Relationship Between Deafness and Gene Mutation in Patients

There were 20 cases of moderate sensorineural hearing loss, 33 cases of severe sensorineural hearing loss, and 130 cases of very severe sensorineural hearing loss. Among the 30 mutations detected, 23 cases had very severe sensorineural hearing loss, 5 cases had severe sensorineural hearing loss in both ears, and 2 cases had moderate sensorineural hearing loss (all were 235delC homozygous mutations). The greater the hearing loss, the greater the detection rate of the mutation. The more severe the hearing loss, the higher the detection rate of *GJB2*, *SLC26A4* gene mutation (see Table 3).

Detection of Deafness-Mutated Gene in Patients with a Family History of Deafness

Of 183 cases, 14 had a family history, among the 14 cases, 8 cases were brothers (sisters/siblings), but only 1 of the 4 pairs of brothers (sisters/siblings) detected *GJB2* 235 delC homozygous mutation, and the other 3 pairs did not detect common deafness gene mutation sites. Blood samples from parents were collected for high-throughput gene testing to determine whether there were new deafness gene mutation sites. There was 1 homogenous mutation of mtRNA1555A > G and 1 homozygous mutation of *GJB2* 235 delC in the remaining 6 patients with family history. There were no common mutations in the remaining 4 patients.

DISCUSSION

The main causes of sensorineural deafness are genetic factors. According to epidemiological investigation, the common pathogenic genes of deafness in China are *GJB2*, *SLC26A4*, and mitochondrial DNA. The most common deafness gene is *GJB2*, which is carried by 19.41% of deafness patients.[7] The common mutant genotype of *GJB2* in Chinese deafness patients is as follows: among the 3 genotypes, 235delC, 176-191DEL16, and 299-300DELAT, 235delC has the highest carrying frequency.[7,8] The detection rate of *SLC26A4* gene responsible for large EVA in deaf people is more than 15%.[3,11,12] However, the carrying rate of mitochondrial DNA 1555A>G mutation site was 3.43%.[12] In this study, the deafness gene was detected in 30 cases (16.39%, 30/183) of 183 deafness patients. The detection rate of *GJB2* gene mutation was 7.10% (13/183), *SLC26A4* gene mutation was 8.74% (16/183), and *mtRNA* gene mutation was 0.55% (1/183). The results were generally lower than those reported in other studies in China. This may be due to the small number of subjects and the limited number of subjects (moderate-to-severe hearing impairment). At the same time, the gene detection method adopted in this paper is Kappe diversion hybridization DNA detection, which has few detection sites, which may also be the possible reason for the low detection rate of pathogenic genes. In addition, the sensitivity of the kit, the operation of the gene tester, and the extraction of DNA may also affect the detection rate.

Our country is a multinational country with a vast, large population, different regions, different nationalities in the long historical process, long time may be more fusion, increase the difficulty of abnormal gene research, to a certain extent, affected the stability and accuracy of the objective data, test results may also is not the same in

Table 3. Detection of Common Deafness Gene Mutations in Patients with Different Hearing Types

Hearing loss level	Detection cases	Mutation cases	Mutation ratio	Mutation genes		
				GJB2	*SLC26A4*	*mtDNA*
Moderate	20	2	1.1% (2/183)	2	0	0
Severe	33	5	2.7% (5/183)	1	4	0
Extremely severe	130	23	12.6% (23/183)	10	12	1

NSHI, non-syndromic hearing impairment.

each region. Hainan province is an island province. Due to the barrier of the Strait and inconvenient transportation, Hainan has been relatively close to some extent in the long history, with little communication with the inland and slower population flow and change than other provinces. As a result, the genetic test results in Hainan are different from those in mainland of China.

In this paper, 13 mutation loci of 4 common deafness-related genes were detected in 183 deaf patients from 6 cities and counties in the northeast of Hainan Province. The results showed that the detection rate of *SLC26A4* gene mutation was higher than that of *GJB2* gene mutation, which was inconsistent with other reports in China. On the one hand, it may be due to the insufficient number of cases detected. On the other hand, it may be due to the small population flow caused by the special geographical position of Hainan Island. The results showed that IVS-2A>G was the most common mutation in *SLC26A4* gene, and *235delC* was the most common mutation in *GJB2* gene, which was basically consistent with domestic literature reports. The detection rate of mitochondrial DNA gene mutation was much lower than that reported in China. In the future, we will continue to expand the screening scope, carry out the full sequence detection of related genes for those with family history but no common deafness gene mutation, clarify the characteristics of deafness gene mutation in Hainan area, improve the deafness gene map, and provide a reference for prevention and treatment of deafness in Hainan area.

We conducted screening and genetic analysis of common deafness genes in patients with non-syndromic deafness in Hainan for the first time. Compared with the common deafness gene mutations in other regions of mainland China, *GJB2* and *SLC26A4* were the main mutation hotspots in patients with deafness in Hainan, and *SLC26A4* was the most common mutation site. It provides useful and targeted information for genetic counseling of deafness in Hainan population with non-syndromic hearing impairment.

Ethics Committee Approval: This study was approved by Ethics Committee of Hainan Affiliated Hospital of Hainan Medical University (Approval No: Med-Eth-Re[2022] 342).

Informed Consent: Informed consent was obtained from the patients' guardians who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Z.Z., Y.F.; Design – J.Z., Y.F.; Supervision – Z. Z., J.Z.; Resources – Z.Z., L.S.,Y.Z.; Materials – Y. F., Y.Z.; Data Collection and/or Processing– Y.F., Y.Z.; Analysis and/or Interpretation – Y.F., Y.Z., .S.; Literature Search – Y.F.; Writing – Y.F.; Critical Review – Z.Z.

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

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