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Original Article

Genetic mutations of hotspots in patients with nonsyndromic deafness in Fujian, China

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Abstract: In this study, comprehensive sequencing was performed not to provide appropriate genetic diagnosis testing and counseling to patients and family members, but to investigate the molecular etiology of nonsyndromic deafness in five typical areas from Fujian province in China. Subjects with hearing impairment (n=465) enrolled in special education schools in Fuzhou, Xiamen, Nanping, Longyan and Putian city which is in eastern, southern, western, northern and middle of Fujian were recruited for the study. Exon sequencing was performed to identify mutations in *GJB2*, *SLC26A4* and *mtDNA 12S rRNA* genes. This study included 291 males and 174 females with mean age of 13, 3 years, ranging from 5 to 34 years. Mutations in *GJB2* gene were observed in 27.5% (128/465) of the patients, with 13.3% (62/465) having two pathogenic alleles. The frequency of *SLC26A4* mutation was 16.8% (78/465) and two pathogenic alleles were found in 11.6% (54/465) of these patients. Three novel *SLC26A4* mutations were identified (c.1167G>A, c.1738_1739delAA, and c.1764_1765insAGGAAAATA). The mutation rate of *mtDNA 12S rRNA* was 5.4% (25/465), all carrying m.A1555G mutation. Our data revealed the spectra of mutations and special hotspots in the nonsyndromic deafness population of Fujian based on comprehensive sequencing analysis, which would be needed to further develop appropriate genetic testing protocols and characterize this disease entity.

Keywords: Nonsyndromic deafness, GJB2, SLC26A4, mitochondrial 12S rRNA, gene mutation

Introduction

Each year in China, approximately 0.8-1.2 million babies are born with congenital abnormalities, among which hearing impairment is the most common one. It has been estimated that 67.7% of newborns of hearing loss have a genetic etiology [1]. *GJB2*, *SLC26A4* and *mtDNA 12S rRNA* genes are known to be the most common causes of nonsyndromic deafness in China [2-4]. Gap junction beta 2 (*GJB2*) was the first gene implicated in the causation of hereditary hearing loss. The most common mutation of *GJB2* in China is reported to be c.235delC, with a 21% mutation detection rate [5, 6]. The reported frequency of homozygous and compound heterozygous pathogenic alleles of *SLC26A4* gene among the deaf population approaches 12% [7-9]. The prevalence of two mutations A1555G and C1494T in the *mtDNA 12S rRNA* gene which are maternally transmitted, are associated with hearing loss in Chinese population (A1555G 3.8% and C1494T 0.6%) [10].

It is known that China has the world's largest population. People from different regions of China have a diverse genetic background. Fujian province is located on the southeastern coast of China, with a long history as part of the "Maritime Silk Road" and at the time boasted of the world's largest commercial port during the Song dynasty era. With such a flourishing economy, people in Fujian had a close contact with the people from different parts of the world, which could have contributed to the genetic diversity within populations. However, no comprehensive genetic analysis of deaf patients in this region has been reported.

In this study, genetic screening was performed in 465 deaf patients from special education schools located in five major cities (Fuzhou,

Table 1. Genotypes of patients in Fujian with mutations in the *GJB2* gene

Allele 1			Allele 2			
Nucleotide change	Consequence or amino acid change	Category	Nucleotide change	Consequence or amino acid change	Category	Number of patients
c.235delC	Frameshift	Pathogenic	c.235delC	Frameshift	Pathogenic	27
c.235delC	Frameshift	Pathogenic	299delAT	Frameshift	Pathogenic	6
c.235delC	Frameshift	Pathogenic	257C>G	p.T86R	Pathogenic	3
c.235delC	Frameshift	Pathogenic	512insAACG	Frameshift	Pathogenic	3
c.176del16	Frameshift	Pathogenic	257C>G	p.T86R	Pathogenic	3
c.235delC	Frameshift	Pathogenic	c.79G>A/c.341A>G	p.V27I, p.E146G	Polymorphism	3
c.235delC	Frameshift	Pathogenic	c.79G>A	p.V27I	Polymorphism	3
c.235delC	Frameshift	Pathogenic	c.608T>C	p.I203T	Pathogenic	3
c.235delC	Frameshift	Pathogenic	c.109G>A	p.V37I	Pathogenic	3
c.512insAACG	Frameshift	Pathogenic	c.109G>A	p.V37I	Pathogenic	3
c.11G>A	p.G4D	Polymorphism				3
c.109G>A	p.V37I	Pathogenic	c.109G>A	p.V37I	Pathogenic	12
c.109G>A	p.V37I	Pathogenic	c.79G>A/c.341A>G	p.V27I, p.E146G	Polymorphism	12
c.109G>A	p.V37I	Pathogenic	c.571T>C	p.F191L	Polymorphism	3
c.109G>A	p.V37I	Pathogenic	c.34G>T	p.G13D	Polymorphism	3
c.109G>A	p.V37I	Pathogenic				36
c.79G>A/c.341A>G	p.V27I, p.E146G	Polymorphism	c.79G>A/c.341A>G	p.V27I, p.E146G	Polymorphism	30
c.79G>A/c.341A>G	p.V27I, p.E146G	Polymorphism	c.608T>C	p.I203T	Polymorphism	15
c.79G>A/c.341A>G	p.V27I, p.E146G	Polymorphism				78
c.79G>A	p.V27I	Polymorphism	c.608T>C	p.I203T	Polymorphism	9
c.79G>A	p.V27I	Polymorphism	c.368C>A	T123N	Unknown	3
c.79G>A	p.V27I	Polymorphism				24
c.341A>G	p.E113G	Polymorphism				3
c.608T>C	p.I203T	Polymorphism				27
c.187G>T	p.V63L	Pathogenic				3
c.571T>C	p.F191L	Polymorphism				3

Xiamen, Nanping, Longyan and Putian) of Fujian province. Exon sequencing was conducted to determine the type and frequency of mutations in the three well-recognized deafness-related genes (*GJB2*, *SLC26A4* and *mtDNA 12S rRNA*), with the aim to understand the molecular etiology of deafness in this region.

Materials and methods

Study subjects

A total of 465 (291 males, 174 females; mean age 13.3 years, ranging from 5 to 34 years) deaf individuals from unrelated families were enrolled from special education schools located in the five cities of Fujian province (Fuzhou, Xiamen, Nanping, Longyan and Putian city). Ethnically, the patients consisted of 410 Han, 30 She, 10 Gaoshan, 10 Hui and 5 Man Chinese. All patients showed severe to profound hearing loss on audiograms.

Sample collection

This study protocol was approved by the Ethics Committee of the Ethics Committee of Fujian Provincial Hospital. All patients or their family members gave written informed consent. Parents were interviewed with regard to basic personal information, family history, mother's health during pregnancy, and patient's clinical history, including history of past infection, head or brain injury, and history of treatment with aminoglycoside antibiotics. After careful medical examination and hearing test, DNA was extracted from peripheral blood leukocytes of each subject according to standard protocols.

Genetic variant analysis

The coding exons plus approximately 50-100 bp of the flanking intronic regions of *GJB2*, *SLC26A4*, and *mtDNA 12S rRNA* gene were amplified using polymerase chain reaction

Table 2. Allele frequency of *GJB2* gene mutations among deaf population in Fujian province

	Gene mutation	Consequence or amino acid change	Number of alleles	Allele frequency (%)
GJB2	c.109G>A	p.V37I	86	9.25
	c.235delC	Frameshift	80	8.60
	c.257C>G	p.T86R	6	0.65
	c.512insAACG	Frameshift	6	0.65
	c.176del16	Frameshift	3	0.32
	c.187G>T	p.V63L	3	0.32
	c.299delAT	Frameshift	6	0.65

(PCR). The DNA sequencing was performed using ABI 3100 DNA sequencing machine (Applied Biosystems, Foster City, CA, USA) and ABI 3100 Analysis Software (version 3.7 NT). Patients carrying monoallelic mutation in the coding region of were further tested for the *GJB2* c.IVS1+1G>A mutation or defects in *GJB2* exon 1 and its basal promoter [4, 6].

Statistical analysis

Categorical variables were expressed as frequencies and percentages, and analyzed with chi-square test. All statistical analyses were performed using SPSS 20.0 software.

Results

GJB2 mutations

Among the 465 patients, 15 variants of *GJB2* gene were identified. Seven of these were pathogenic mutations, and included four frameshift mutations (c.235delC (6)), c.176del16 [6], c.299delAT [11], and c.512insAACG [6] and three missense mutations (c.109G>A (p.V37I) [12]), c.257c>G (p.T86R) [6], and c.187G>T (p.V63L) [11]. Eight were polymorphic mutations: c.79G>A [13], c.608T>C [13], 571T>C [14], c.11G>T [15], c.341A>G, c.34G>T [6], c.368C>A, and c.79G>A/341A>G [13]. The most common allele was 79G>A/341A>G (**Table 1**).

Of these patients, 13.3% (n=62) were confirmed as having *GJB2* deafness-causing mutations, followed by 39 homozygotes (27 c.235delC alleles and 12 c.109G>A alleles) and 21 compound heterozygotes (6 c.235delC/c.299DelAT alleles, 3 c.235delC/c.257C>G alleles, 3 c.235delC/c.512insAACG alleles, 3 c.176del16/c.257C>G alleles, 3 c.235delC/c.109G>A alleles and 3 c.109G>A/c.512insAACG alleles). In addition, 66 patients were carriers of only one heterozygous pathogenic mutation (**Table 1**). The *GJB2* gene mutation detection rate in the study population was 27.5% (128/465). The pathogenic allele frequency of *GJB2* was 20% (186/930) and c.109G>A was the most common mutation allele (9.0%, 84/930) (**Table 2**).

SLC26A4 mutations

A total of 22 variants of *SLC26A42* gene were identified in this study, including three novel variants (c.1167G>A, c.1738_1739delAA and c.1764_1765insAGGAAAATA). Of these variants, sixteen were pathogenic: c.754T>C [16], c.916_917insG [17], c.IVS7-2A>G [8], c.1079C>T [18], c.1229C>T [16], c.1692_1693insA [9], c.147C>G [19], c.1472T>C [8], c.1595G>T [9], c.IVS16-6G>A [20], c.1764_1765insAGGAAAATA, c.1738_1739delAA, c.21-68A>G [21], c.2086C>T [22], c.1336C>T, and c.2007C>G [23]. Three variants were polymorphic: c.IVS11+47T>C [24], c.IVS7-18T>G [25], and c.1790T>C [26]. Two were silent mutations: c.1167G>A and c.2283A>G (9). The significance of the variant c.2009T>C [19] is still under investigation (**Table 3**).

Of all the patients, 54 patients (11.6%) were confirmed as having *SLC26A4* deafness-causing mutations, including 18 homozygotes (15 with the c.IVS7-2A>G allele and 3 with the c.1079C>T allele) and 36 compound heterozygotes. And 24 patients carried only one heterozygous pathogenic mutation in *SLC26A4* gene (**Table 3**). The detection rate of *SLC26A4* mutation in the study was 16.8% (78/465). The pathogenic allele frequency of *SLC26A4* was 13.9% (129/930). c.IVS7-2A>G was found to be the most common mutation allele (6.1%, 57/930). In the present study, an otherwise rarely reported mutation c.1079C>T (p.A360V) (1.6%, 15/930) was identified as a hotspot mutation (**Table 4**).

Mitochondrial DNA 12S rRNA mutations

Twenty-five patients (5.4%) carried *mtDNA 12S rRNA* mutation, all of which were m.1555A>G

Table 3. Genotypes of deaf patients in Fujian province with mutations in *SLC26A4* gene

Allele 1			Allele 2			Number of patients
Nucleotide change	Consequence or amino acid change	Category	Nucleotide change	Consequence or amino acid change	Category	
c.754T>C	p.S252P	Pathogenic	c.1738_1739delAA	FS580, P606*	Pathogenic	3
c.916_917insG	FS306, P329*	Pathogenic	c.2168A>G	p.H723R	Pathogenic	3
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.IVS7-2A>G	Aberrant splicing	Pathogenic	15
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.1079C>T	p.A360V	Pathogenic	9
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.2086C>T	p.Q696*	Pathogenic	6
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.1336C>T	p.Q446*	Pathogenic	3
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.2007C>G	p.D669E	Pathogenic	3
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.2168A>G	p.H723R	Pathogenic	3
c.1079C>T	p.A360V	Pathogenic	c.1079C>T	p.A360V	Pathogenic	3
c.1229C>T	p.T410M	Pathogenic	c.2168A>G	p.H723R	Pathogenic	3
c.1692_1693ins	FS565, P573*	Pathogenic	c.2168A>G	p.H723R	Pathogenic	3
c.147C>G	p.S49R	Pathogenic				3
c.IVS7-2A>G	Aberrant splicing	Pathogenic				3
c.1472T>C	p.I491T	Pathogenic				3
c.1595G>T	p.S532I	Pathogenic				3
c.IVS16-6G>A	Aberrant splicing	Pathogenic				6
c.IVS16-6G>A	Aberrant splicing	Pathogenic	c.IVS11+47T>C	Aberrant splicing	Polymorphism	3
c.1764_1765insAGGAAAATA	Frameshift	Pathogenic				3
c.2009T>C	p.V670A	Unknown				6
c.IVS7-18T>G	Aberrant splicing	Polymorphism				9
c.IVS7-18T>G	Aberrant splicing	Polymorphism	c.IVS11+47T>C	Aberrant splicing	Polymorphism	3
c.1167G>A	p.G389G	Silent mutation				3
c.IVS11+47T>C	Aberrant splicing	Polymorphism	c.IVS11+47T>C	Aberrant splicing	Polymorphism	12
c.IVS11+47T>C	Aberrant splicing	Polymorphism				69
c.1790T>C	p.L597S	Polymorphism				3
c.2283A>G	p.T761T	Silent mutation				12

*indicates stop codon.

mutation. Among the 20 carriers, 15 patients had a confirmed history of treatment with aminoglycosides. Five patients had a history of treatment with gentamicin, three used streptomycin alone, five combined gentamicin and streptomycin, while two combined streptomycin and neomycin.

Discussion

The *GJB2* gene codes for GJB2 protein (connexin 26) is a member of the connexin protein family. Connexin proteins form gap junction channels that permit the transport of nutrients, ions and small signaling molecules across neighboring cells [27]. The connexin 26 coding gene, *GJB2* contains 2 exons, and is located on chromosome 13q11-q12 (28). More than 220 mutations have been discovered in this gene, which were detected in 10%-50% of nonsyndromic deafness patients [28].

In this study, 27.1% of the nonsyndromic hearing impairment patients carried *GJB2* mutation. The two prevalent variants c.235delC and c.109G>A, accounting for 12.9% of all mutations of *GJB2* identified in Fujian. The most common GJB2 mutation was c.109G>A (9.0%, 84/930) rather than the c.235delC (8.4%, 78/930) in Fujian province, which is different to other areas of China.

Previous studies reported allele frequency of c.109G>A mutation with reported was 4.3% in Thailand [29], 0.6% in Korea [30], 1.0% in Japan [31], and 6.2% in Shanghai [12]. A recent study revealed an association of the homozygous c.109G>A allele with diverse hearing phenotypes among Chinese Han population, of whom some patients had severe hearing loss [32]. Our study showed 12 patients with this homozygous mutation. A previous investigation of a large number of Chinese patients with nonsyndromic hearing impairment reported that the allele frequency of c.109G>A was 4.2% [6], while in the present study the corresponding allele frequency was much higher at 9.0%. The c.109G>A mutation has been detected in East

Table 4. Allele frequency of *SLC26A4* gene mutations in Fujian province

	Gene mutations	Consequence or amino acid change	Number of alleles	Allele frequency (%)
SLC26A4	c.IVS7-2A>G	Aberrant splicing	57	6.13
	c.1079C>T	p.A360V	15	1.61
	c.2168A>G	p.H723R	12	1.29
	c.IVS16-6G>A	Aberrant splicing	9	0.97
	c.1472T>C	p.I491T	3	0.32
	c.2086C>T	p.Q696*	6	0.65
	c.1336C>T	p.Q446*	3	0.32
	c.2007C>G	p.D669E	3	0.32
	c.1229C>T	p.T410M	3	0.32
	c.1692_1693ins	FS565, P573*	3	0.32
	c.754T>C	p.S252P	3	0.32
	c.1764_1765insAGGAAAATA	Frameshift	3	0.32
	c.916_917insG	FS306, P329*	3	0.32
	c.1595G>T	p.S532I	3	0.32
	c.1738_1739delAA	FS580, P606*	3	0.32

*indicates stop codon.

Asia, North Africa and the Middle East. Population migrations is the likely the result of the wide variability of c.109G>A allele frequencies. It would be interesting to further explore the founder effect and the probable migration route of the *GJB2* gene mutations in representative areas of China, which may help explain the marked regional differences in the c.109G>A allele frequency.

The c.235delC variant, which is known to disrupt the connexin protein and cause hearing impairment through frameshift effect, has been reported to be the most common mutation of *GJB2* in East and Southeast Asia, while the c.235delG mutation was responsible for nonsyndromic hearing impairment in Europe and Oceania [27-32]. The c.235delC allele frequency of 2063 Chinese nonsyndromic deafness patients was 12.3% (509/4126) in a nationwide study [6]. In the present study, the frequency of c.235delC allele in Fujian (8.4%, 78/930) is not significantly different from the nationwide result ($P>0.05$), indicating the consistency of c.235delC frequency in Fujian with that of the rest of the country. Though c.235delC mutation is reported to be the most common mutation among nonsyndromic deafness population in China, it was found to be the second most common mutation in Fujian, which might be due to the lower frequency of c.235delC allele among the indigenous population of Fujian.

Mutations in *SLC26A4* gene can cause either nonsyndromic hearing loss or Pendred syndrome (enlarged vestibular aqueduct with sensorineural hearing loss and goiter). Over 170 mutations in *SLC26A4* have been identified, and the hotspot mutations show clear regional and ethnic differences. In a study performed by Wang *et al.*, *SLC26A4* gene was screened in 107 Chinese patients from 6 multiplex and 95 simplex families, with hearing loss associated with enlarged vestibular aqueduct. Our data showed the most common *SLC26A4* mutation was c.IVS7-2A>G with an allele frequency of 6.1% (57/930), which is in line with other reports [7, 17].

The second most common mutation of *SLC26A4* in Fujian was found to be c.1079C>T, with an allele frequency of 1.6% (15/930). c.1079C>T has been rarely reported, and was first reported in 2007 by Lai *et al.* in Taiwan. One patients with a compound heterozygous mutation of c.1079C>T/c.IVS7-2A>G was diagnosed with Pendred syndrome and tested positive for both the perchlorate discharge test and anti-thyroid peroxidase antibodies [18]. In our study, 3 homozygous c.1079C>T mutations and 9 compound heterozygous mutations of c.1079C>T/IVS7-2A>G were identified; however, none of the patients showed thyroid dysfunction. The frequency of c.1079C>T mutation in Fujian and Taiwan was higher when compared to the rest of China, indicates that the

mutation is associated with the distribution of ethnic groups. The concentration of c.1079C>T mutation in Fujian and Taiwan might be explained by the historical migrations from southern part of Fujian to Taiwan.

Deafness induced by aminoglycoside antibiotics causes bilateral high-frequency sensorineural hearing loss, and the patients usually have a confirmed history of treatment with aminoglycoside antibiotics. Studies have proven the association of *mtDNA 12S rRNA* mutations with this type of deafness. The mutation frequency of *mtDNA 12S rRNA* among nonsyndromic hearing impairment population has been reported to be 0.6-5% in Caucasians [33-37], 3% in Japanese [33], 5.3% in Indonesians [34], and 3.4%-7.7% in Chinese [4, 10] population. Our data showed 3.9% patients (20/465) had *mtDNA 12S rRNA* mutation, all of which were m.1555A>G. Fifteen patients had a confirmed history of treatment with aminoglycoside antibiotics. The mothers of 9 patients also suffered from hearing loss. As a preventive strategy, maternal screening for mitochondrial gene mutation in suspected cases, and targeted maternal health education on the subject of aminoglycoside toxicity may be of benefit.

Conclusion

We performed exon sequencing for the three most common deafness-associated genes in a cohort of 465 deaf patients who were enrolled in special education schools in three major cities of Fujian province. Almost half of the patients had mutations in *GJB2*, *SLC26A4* or *mtDNA 12S rRNA*. The genetic screening helped in generating valuable data on molecular epidemiology of deafness in Fujian province, which would be helpful in designing appropriate genetic testing protocols and preventive strategies in the area.

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Disclosure of conflict of interest

None.

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