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Analysis of mutation spectrum of common deafness-causing genes in Hakka newborns in southern China by semiconductor sequencing

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Abstract

Hearing loss is a common neurosensory disorder, approximately half of the cases are caused by genetic factors, and approximately 70% of hereditary hearing impairments are nonsyndromic hearing loss (NSHL). The mutations of *GJB2* (gap junction beta-2 protein), *GJB3* (gap junction beta-3 protein), *SLC26A4* (solute carrier family 26 member 4), and *MT-RNR1* (mitochondrially encoded 12S RNA) are the most common inherited causes of NSHL. Because of different genetic backgrounds, the mutation spectrum of these common deafness-causing genes varies among different regions in China. Because no data are known on these mutations among the Hakka population of Southern China, we aim to investigate the mutation spectrum to add these to neonatal screening and genetic counseling. A total of 1252 blood samples from newborns have been detected by semiconductor sequencing for 100 mutations loci of 18 deafness-causing genes. Of the participants, 95 subjects carried deafness-causing genes mutations with the carrier rate of 7.59%. The mutation frequencies of *GJB2*, *SLC26A4*, *GJB3*, and mitochondrial genes were 3.04%, 3.51%, 0.16%, and 0.88%, respectively. We followed up subjects with single-gene homozygous or compound heterozygous mutations. Our study firstly analyzed deafness-causing genes mutation spectrum in Hakka population, providing evidence for future neonatal screening and genetic counseling in this area.

Abbreviations: *COCH* = cochlin, *DFNA5* = deafness, autosomal dominant 5, DIABLO = Diablo IAP-binding mitochondrial protein, DSPP = dentin sialophosphoprotein, *GJB2* = gap junction beta-2 protein, *GJB3* = gap junction beta-3 protein, *GPR98* = G-protein coupled receptor 98, *MT-CO1* = mitochondrially encoded cytochrome C oxidase I, *MT-RNR1* = mitochondrially encoded 12S RNA, *MT-TH* = mitochondrially encoded TRNA histidine, *MT-TL1* = mitochondrially encoded TRNA leucine 1 (UUA/G), *MT-TS1* = mitochondrially encoded TRNA serine 1 (UCN), *MYO15A* = unconventional myosin-15, *MYO7A* = unconventional myosin-VIIa, NSHL = nonsyndromic hearing loss, PDS = Pendred syndrome, *PRPS1* = phosphoribosyl pyrophosphate synthetase 1, *SLC26A4* = solute carrier family 26 member 4, *TECTA* = tectorin alpha, *TMC1* = transmembrane channel like 1.

Keywords: deafness-causing genes, *GJB*, *GJB3*, Hakka population, mitochondrial genes, semiconductor sequencing, *SLC26A4*

1. Introduction

Hearing loss is one of the most common neurosensory disorders, affecting approximately 1 to 3 newborns in every 1000 live births.[1–3] In China, there are approximately 800,000 children younger than 7 years who are hearing impaired, and this number continues to grow, with an increase of more than 30,000 deaf children every year.[3,4] According to whether other organ systems are abnormal, sensorineural hearing loss can be classified into nonsyndromic hearing loss (NSHL) and syndrome-induced hearing loss. If infants with profound hearing loss are not detected and treated within the first year of life, they may experience permanent hearing impairment with major and irreversible defects in linguistic and cognitive development. However, this situation can be improved if it is detected and intervention started before 6 months of age (cochlear implants can help patients with severe hearing loss to recover their hearing ability, implanting an electronic medical device that sends sound signals to the brain to replace the damaged inner ear.).

Previous studies have confirmed that hearing loss can be congenital or caused by environmental factors, such as infection, trauma, or ototoxic drugs.[5,6] However, hearing loss is etiologically uneven. Some studies have shown that at least two-thirds of the cases of childhood-onset hearing loss have a genetic cause, and approximately 70% of hereditary hearing impairments are NSHL.[7–9] NSHL can be inherited by autosomal recessive, autosomal dominant, X-linked trait, or mitochondrial deafness.[10–12] At present, previous genetic screening studies have shown that a few genes mutations are

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known to cause hereditary hearing loss or deafness, such as *GJB2* (gap junction beta-2 protein; OMIM: 121011), *GJB3* (gap junction beta-2 protein; OMIM: 603324), *SLC26A4* (solute carrier family 26 member 4; OMIM: 605646), and the mitochondrial gene *MT-RNR1* (mitochondrially encoded 12S RNA; OMIM: 561000).[9,13–24] Knowledge of the gene mutation can help to identify hearing impairment at birth, and the educational programs for auditory stimulation and sufficient language exposure in early childhood can begin immediately. Furthermore, it can also provide warning to avoid taking certain types of aminoglycosides antibiotics, such as streptomycin, gentamicin, and tobramycin, which are known to cause deafness in children carrying certain mitochondrial gene mutations.

China is the most populous country in the world, consisting of 56 nationalities. Due to geographical separation, Chinese people from different regions may have different genetic backgrounds.[25,26] The Hakka population is a Han Chinese that mainly living in southern China with unique culture.[26] The city Meizhou with the most Hakka population in Guangdong Province locates in the south part of China. Because of its remote location, Meizhou city is a relatively conservative area with a less migration of population. Hearing loss in infancy is a common sensory disorder, of which about is hereditary, caused by known mutations such as *GJB2, SLC26A4, GJB3*, and mitochondrial genes. Although some genetic studies have been performed on Chinese patients with deafness,[8,9,27,28] the large-scale deafness population and racial differences require regional and individual genetic analysis; these data cannot simply be inferred from the conclusions of other groups. For example, 1 study with a comprehensive investigation of the molecular etiology of nonsyndromic deafness in 2 typical areas from northern and southern China (Chifeng City in Inner Mongolia and Nantong City in Jiangsu Province), *GJB2* gene mutations account for approximately 18.31% of patients with hearing impairment, *SLC26A4* gene mutations account for approximately 13.73%, and the mitochondrial m.1555A > G mutation accounts for 1.76%.[8] However, common molecular etiologies are rare in the Tibetan Chinese deaf population.[27] The prevalence of mutations varies among different regions in China. We aim to explore the prevalence of these mutations among the Hakka population in southern China, which might be helpful to neonatal screening and genetic counseling.

2. Materials and methods

2.1. Participants

This retrospective clinical study included 1252 newborns who born in Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University between May 2016 and January 2018. The inclusion and exclusion criteria are shown in Figure 1. All the blood samples from participants have been detected by semiconductor sequencing. Before blood sampling, informed consent was obtained from all participants' parents. The study was approved by the Committee of Ethics and Research of the Meizhou People's Hospital, Meizhou Hospital Affiliated to Sun Yat-sen University for experiments involving humans. Before participants recruited for the study, their guardians signed a written informed consent form according to the ethical guidelines of the Helsinki Declaration.

2.2. DNA extraction and detection of deafness-causing genes mutations

Peripheral blood samples were collected from the study participants and stored in 2-mL evacuated vacuum tubes containing ethylenediaminetetraacetic, or heel blood was collected to the blood spot card. These blood samples are stored at 4°C for not more than 3 days before testing. QIAamp DNA Blood Mini Kit (Qiagen, Germany) was used to extract genomic DNA from each blood sample following the manufacturer's instructions, and NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to evaluate the quantity and quality of extracted DNA. DNA from peripheral blood sample was used for library construction according to the Ion Plus Fragment Library Kit (Life Technologies, Carlsbad, CA), and semiconductor sequencing was performed using an Ion Proton instrument according to the manufacturer's instructions (Life Technologies). High-throughput sequencing technology and bioinformatics analysis methods were used to detect the presence of deafness-causing gene mutation in subjects. The detection contents include 18 deafness-causing genes, including *GJB2*, *SLC26A4*, *GJB3*, *MYO15A* (unconventional myosin-15), *TECTA* (tectorin alpha), *DIABLO* (Diablo IAP-binding mitochondrial protein), *COCH* (cochlin), *DSPP* (dentin sialophosphoprotein), *GPR98* (G-protein coupled receptor 98), *DFNA5* (deafness, autosomal dominant 5), *TMC1* (transmembrane channel like 1), *MT-CO1* (mitochondrially encoded cytochrome C oxidase I), *MT-RNR1* (mitochondrially encoded TRNA histidine), *MT-TH* [mitochondrially encoded TRNA serine 1 (UCN)], *MT-TS1* [mitochondrially encoded TRNA leucine 1 (UUA/G)], MT-TL1 [mitochondrially encoded TRNA leucine 1 (UUA/G)], *PRPS1* (phosphoribosyl pyrophosphate synthetase 1), *MYO7A* (unconventional myosin-VIIa), a total of 100 mutations loci, of these 91% (91/100) mutations loci are invariant, the other 9 mutations loci are updated based on pathogenicity.

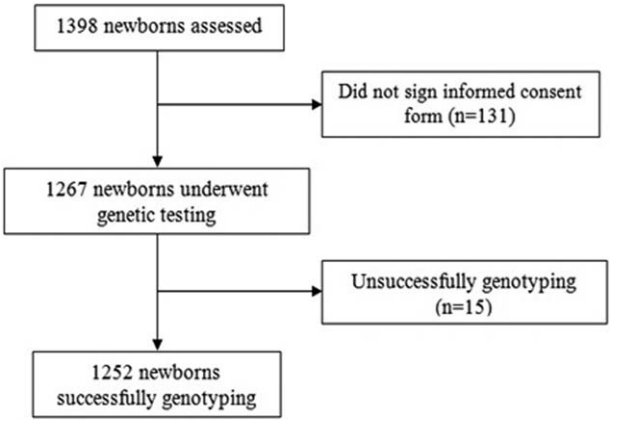

Figure 1. Flow chart of participant inclusion and exclusion.

3. Results

Among the 1252 participants enrolled in this study (669 men and 583 women), 95 subjects carried common deafness-causing genes mutations, total carrier rate was 7.59% (Table 1). A total of 38 participating subjects carried mutations in *GJB2*, and the carrier rate was 3.04% in the population. *SLC26A4* mutations were detected in 44 participants; the carrier rate was 3.51%. Two participants carried mutations in *GJB3*. Mitochondrial gene mutations carrier rates were *MT-RNR1* (0.64%), *MT-TL1*

Table 1

Deafness gene mutation carrier rates detected in 1252 Hakka newborns.

Sex	Subjects (n)	Gene	Positive (n)	Detection rate, %	Number of mutated alleles	Allele frequency, %
Male	669		59	4.71		
		GJB2	25	2.00	27	1.08
		GJB3	2	0.16	2	0.08
		SLC26A4	25	2.00	26	1.04
		MT-RNR1	5	0.40		
		MT-TL1	0	0.00		
		MT-CO1	2	0.16		
Female	583		36	2.88		
		GJB2	13	1.04	14	0.56
		GJB3	0	0.00	0	0.00
		SLC26A4	19	1.52	20	0.80
		MT-RNR1	3	0.24		
		MT-TL1	1	0.08		
		MT-CO1	0	0.00		
Total	1252		95	7.59		

(0.08%), and *MT-CO1* (0.16%). Mutations of *GJB2* and *SLC26A4* are major ones (86.32% of total).

3.1. Mutations in GJB2 gene

Four variants were identified in this cohort. They were 2 frameshift deletions (c.235delC, c.299_300delAT), 1 frameshift insertion (c.511_512insAACG) and 1 missense mutations (c.109G>A) (Table 2). All of the variants were pathological mutations, which have been determined in previous studies. The mutant alleles of *GJB2* accounted for 1.64% (41/2504) of the total alleles in all subjects (Table 2). Like most areas of China, the most common mutation allele of *GJB2* in Hakka area was c.235delC, the allele frequency was 0.80% (20/2504), followed by 0.68% (17/2504) for c.109G>A, c.511_512insAACG for 0.12% (3/2504), c.299_300delAT for 0.04% (1/2504).

Thirty-five newborns carried monoallelic variants in the heterozygous form: 19 with c.235delC, 12 with c.109G>A, 3 with c.511_512insAACG, and 1 with c.299_300delAT. Two newborns carried homozygous mutation for c.109G > A and 1 with c.235delC heterozygote compound c.109G > A heterozygote. Totally, 38 participants had molecular defects in *GJB2* gene (Table 3).

3.2. Mutations in SLC26A4 gene

Eleven variants were identified in this cohort, including 5 missense mutations (c.589G > A, c.2168A > G, c.1229C > T, c.697G > C, c.1160C > T), 2 splice site mutations (c.IVS7-2A > G, c.IVS16-6G > A), 2 frameshift mutations (c.1975G > C, c.387delC), 1 nonsense mutation (c.2086C > T), and 1 variant in intron (c.919-18T > G). The mutant alleles of *SLC26A4* accounted for 1.84% (46/2504) of the total alleles in all subjects (Table 4). The most common mutation allele of *SLC26A4* in Hakka area was c.919-18T > G with a mutant frequency of 0.56% (14/2504). The second common mutation allele was c.IVS7-2A > G, the allele frequency was 0.52% (13/2504).

Forty-two newborns carried monoallelic variants in the heterozygous form. One newborns carried homozygous mutation for c.IVS7-2A > G and 1 with c.2168A > G heterozygote compound c.1229C > T heterozygote. Totally, 44 subjects had molecular defects in *SLC26A4* gene (Table 5).

Table 2

Allele frequencies of *GJB2* mutations in 1252 Hakka newborns.

Mutations	Consequence	Number of alleles	Allele frequency, %
c.109G>A	Missense	17	0.68
c.235delC	Frameshift	20	0.80
c.299_300delAT	Frameshift	1	0.04
c.511_512insAACG	Frameshift	3	0.12

3.3. Mutations in GJB3 and mitochondrial genes

Two neonates carried mutations in *GJB3*, one was heterozygous mutation for c.538C > T and another one with c.547G > A heterozygote. In addition, 11 subjects were detected to be mitochondrial gene mutation carriers, accounting for 0.88% (11/1252) of the group. Eight newborns carried mutations in *MT-RNR1*, containing 1 heteroplasmic mutation for m.827A > G, 2 homoplasmic mutation for m.827A > G, 4 homoplasmic mutation for m.1555A > G, and 1 homoplasmic mutation for m.1494C > T. One newborns carried a heteroplasmic mutation for m.3243A > G in *MT-TL1*. Another 2 newborns carried a homoplasmic mutation for m.7444G > A in *MT-CO1*. Since the mutation of mitochondrial gene *MT-RNR1* is important mechanism of genetic susceptibility to aminoglycoside ototoxicity, screened carriers were provided with detailed drug using guide.

4. Discussion

Previous studies have reported that *GJB2*, *SLC26A4*, *GJB3*, and mitochondrial genes are the most common causes NSHL in Chinese people.[14,29–31] Here we studied retrospectively 100 loci of 18 genes known to cause hearing impairment. The actual hearing tests of the subjects were not complete in our study. This is a limitation due to our study design.

4.1. GJB2 mutation analysis

In the present study, *GJB2* mutations were detected in 3.04% (38/1252) of all subjects. Like most areas of China,[8,32,33] the c.235delC was the most prevalent mutation in Hakka population

Table 3

Identified *GJB2* genotypes in the studied 1252 Hakka Chinese newborns.

Allele 1			Allele 2			
Mutation	**Consequence**	**Category**	**Mutation**	**Consequence**	**Category**	**Positive detection**
c.109G>A	Missense	Pathogenic	Wild type	–	–	12
c.109G>A	Missense	Pathogenic	c.109G>A	Missense	Pathogenic	2
c.235delC	Frameshift	Pathogenic	Wild type	–	–	19
c.235delC	Frameshift	Pathogenic	c.109G>A	Missense	Pathogenic	1
c.299_300delAT	Frameshift	Pathogenic	Wild type	–	–	1
c.511_512insAACG	Frameshift	Pathogenic	Wild type	–	–	3

with a carrier rate of 1.60% (20/1252), this result was similar with one study that c.235delC mutation carrier rate in the Chinese hearing population is 1.76% (198/7263).[34] The second frequent mutation was c.109G > A, with a carrier rate of 1.20% (15/1252). We followed up 2 newborns with homozygous mutation in c.109G > A, the result was that they have normal hearing at the time of 9 months after birth. Another infant who with c.235delC heterozygote compound c.109G > A heterozygote also has normal hearing (Table 6). The mutation in c.109G > A is common in East Asians. The mutation frequency of c.109G > A in deaf population had been reported to be 4.2% in China,[9] 4.3% in Thailand,[35] 1.0% in Japan,[36] and 0.6% in Korea.[37] At present, the pathogenicity of this mutation site is controversial, because some individuals with normal hearing also carry the homozygous mutation.[38,39]

4.2. SLC26A4 mutation analysis

In this study, *SLC26A4* with higher mutation rate 3.51% (44/1252) compared to *GJB2* 3.04% (38/1252). The mutation hotspots of *SLC26A4* differed among different nations and areas. In our group, the hotspot mutation of *SLC26A4* was c.919-18T > G, with a carrier rate of 1.12% (14/1252), but this variant has been proposed to as benign variation according to the reported data (http://deafnessvariationdatabase.org/). The carrier rate of c.IVS7-2A > G was 0.96% (12/1252), was similar with the study that c.IVS7-2A > G mutation carrier rate in the Chinese hearing population is 1.24% (90/7263).[34] We followed up 1 subject with homozygous mutation in c.IVS7-2A > G; the result was that the infant has normal hearing at the time of 7 months after birth. Another infant with c.2168A > G heterozygote compound c.1229C > T heterozygote also has normal hearing (Table 6). Pendred syndrome (PDS) is classically described as bilateral sensorineural hearing loss and thyroid enlargement, and PDS is caused by mutations of *SLC26A4* gene, disease may occurs at any age from birth to adolescence, inducement including colds, fever, mild craniocerebral trauma, barotrauma, or other causes of increased intracranial pressure.[40] We have informed the parents of the subject with homozygous mutation in c.IVS7-2A > G, to closely observe the child's behavior and its response to sound. If in doubt the parents should make a new hospital appointment, and we will follow-up this case next time.

Table 4

Allele frequencies of *SLC26A4* mutations in 1252 Hakka Chinese newborns.

Mutations	Consequence	Number of alleles	Allele frequency, %
c.IVS7-2A>G	Splice site	13	0.52
c.1975G>C	Frameshift	1	0.04
c.919-18T>G	Variant in intron	14	0.56
c.589G>A	Missense	1	0.04
c.2168A>G	Missense	3	0.12
c.1229C>T	Missense	2	0.08
c.IVS16-6G>A	Splice site	6	0.24
c.387delC	Frameshift	2	0.08
c.697G>C	Missense	2	0.08
c.1160C>T	Missense	1	0.04
c.2086C>T	Nonsense	1	0.04

4.3. GJB3 and mitochondrial genes mutation analysis

In our study, 2 subjects were found carrying mutation in *GJB3*, one was heterozygous mutation for c.538C > T and another one was heterozygous mutation for c.547G > A. This result indicates that deafness-associated variation in *GJB3* was considered not common in Hakka population. The mutations of m.1555A > G and m.1494C > T for the *MT-RNR1* gene are considered as the most common mutations of mitochondrial genes. The mutation frequency of m.1555A > G was observed in 2.9% in China, Japan 3%,[41] and Indonesia 5.3%.[42] In our study, the mutation carrier rate of m.1555A > G and m.1494C > T accounted for 0.32% (4/1252) and 0.08% (1/1252), respectively, was similar with the study that m.1555A > G and m.1494C > T mutation carrier rate in the Chinese hearing population are 0.25% (18/7263), 0.04% (3/7263), respectively.[34] Since the mutation of mitochondrial gene *MT-RNR1* is important mechanism of genetic susceptibility to aminoglycoside ototoxicity, screened carriers should be provided with detailed drug using guide. In our study, we have given out a warning to these carriers' parents that these children may be prone to ototoxic effects of aminoglycoside, and informed the physician.

5. Conclusions

Considering that 1 to 3 newborns in every 1000 could be hearing impaired, screening for these mutations causing genetic hearing loss is relevant and may be applied if the gene mutation spectrum of the Hakka Chinese population is known. With the results of our study, the basis for neonatal screening is laid: of the 1252 participants, 95 subjects carried deafness-causing genes mutations with the carrier rate 7.59%. The mutation frequencies of *GJB2*, *SLC26A4*, *GJB3*, and mitochondrial genes were 3.04%, 3.51%, 0.16%, and 0.88%, respectively.

Table 5

Identified *SLC26A4* genotypes in 1252 Hakka Chinese newborns.

Allele 1			Allele 2			
Mutation	**Consequence**	**Category**	**Mutation**	**Consequence**	**Category**	**Positive detection**
c.IVS7-2A>G	Splice site	Pathogenic	Wild type	–	–	11
c.IVS7-2A>G	Splice site	Pathogenic	c.IVS7-2A>G	Splice site	Pathogenic	1
c.1975G>C	Frameshift	Pathogenic	Wild type	–	–	1
c.919-18T>G	Variant in intron	Benign	Wild type	–	–	14
c.589G>A	Missense	Likely pathogenic	Wild type	–	–	1
c.2168A>G	Missense	Pathogenic	Wild type	–	–	2
c.1229C>T	Missense	Pathogenic	Wild type	–	–	1
c.2168A>G	Missense	Pathogenic	c.1229C>T	Missense	Pathogenic	1
c.IVS16-6G>A	Splice site	Pathogenic	Wild type	–	–	6
c.387delC	Frameshift	Pathogenic	Wild type	–	–	2
c.697G>C	Missense	Uncertain significance	Wild type	–	–	2
c.1160C>T	Missense	Likely pathogenic	Wild type	–	–	1
c.2086C>T	Nonsense	Likely pathogenic	Wildtype	–	–	1

Table 6

Hearing in 5 subjects with single-gene homozygous/compound heterozygous mutations from the 1252 Hakka Chinese newborns studied.

Gene mutation type	Amount of positive	Follow-up results
GJB2 homozygous c.109G>A	2	Normal hearing
GJB2 heterozygous c.235delC compound heterozygous c.109G>A	1	Normal hearing
SLC26A4 homozygous c.IVS7-2A>G	1	Normal hearing
SLC26A4 heterozygous c.2168A>G compound heterozygous c.1229C>T	1	Normal hearing

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Author contributions

Pingsen Zhao conceived and designed the experiments; Pingsen Zhao and Liubing Lan recruited subjects and collected clinical data. Pingsen Zhao and Lifang Lin conducted the laboratory testing and prepared the manuscript.
Conceptualization: Pingsen Zhao.
Data curation: Pingsen Zhao, Lifang Lin, Liubing Lan.
Formal analysis: Pingsen Zhao.
Funding acquisition: Pingsen Zhao.
Investigation: Pingsen Zhao.
Methodology: Pingsen Zhao, Lifang Lin, Liubing Lan.
Project administration: Pingsen Zhao.
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Software: Pingsen Zhao, Lifang Lin.
Supervision: Pingsen Zhao.
Validation: Pingsen Zhao, Lifang Lin, Liubing Lan.
Visualization: Pingsen Zhao.
Writing – original draft: Pingsen Zhao, Lifang Lin.
Writing – review and editing: Pingsen Zhao.

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