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Original Article

GJB2 and *SLC26A4* gene mutations in children with non-syndromic hearing loss in Southern China

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Abstract: Non-syndromic hearing loss (NSHL) is a major public health issue and affects a substantial proportion of newborns worldwide. Currently little information is available about the molecular etiology of hearing impairment in the Chinese population. Therefore, this study aimed to perform a comprehensive investigation on the genetic mutation patterns of non-syndromic deafness in Zhongshan City, a city located in Southern China. A total of 112 unrelated school children with NSHL in the Zhongshan city were enrolled in this study. Screening was performed for *GJB2*, *GJB3*, *SLC26A4* and *12S rRNA* using a microarray-based hybridization biochip assay. The incidence of genetic defects in the NSHL children was 38.39% (43/112) in this cohort. Among them, 20.54% of cases were caused by *GJB2* mutations (235delC, 299_300delAT and 35delG) and 15.18% of cases had pathogenic mutations in *SLC26A4* (IVS7-2A>G and 2168A>G). 0.89% of cases carried mutation in *12S rRNA* (1555A>G) and *GJB3* mutations (538C>T) were detected in 1.79% of the patients. Our results demonstrated that gene mutations played an important role in the pathogenesis of NSHL in children from the Zhongshan City. *GJB2* and *SLC26A4* mutations are two major causes contributing to NSHL.

Keywords: Non-syndromic hearing loss, *GJB2*, *GJB3*, *SLC26A4*, *12S rRNA*

Introduction

Hearing loss (HL) is the most common sensory impairment in humans, affecting about one in 1000 newborns [1]. Both genetic and environmental factors play important roles in the initiation and development of HL [2]. Most patients with congenital HL have a genetic etiology, and non-syndromic hearing loss (NSHL) comprised approximately 80% of genetic deafness [3]. More than 80 genes have been recognized to cause NSHL. However, due to the extreme ethnicity-specific variation and limited phenotypic variability, the genetic causes of inherited NSHL remain poorly unknown and it poses great challenges to the genetic counseling.

A variety of genetic alterations have been shown to contribute to the development of NSHL. Gap junction β2 (*GJB2*) encodes connexin26 (CX26) protein and mutations in *GJB2* accounts for about more than 50% of NSHL [4, 5]. To date, more than 200 different mutations have been identified in this gene. The most frequent pathogenic mutation in Caucasians is c.35delG, consisting of approximately 70% *GJB2*-related HL. However, c.235delC rather than c.35delG is commonly detected in NSHL patients in East Asian [6]. Gap junction β-3 protein (GJB3), also known as connexin 31 (Cx31), is encoded by the *GJB3* gene. Mutations in *GJB3* have been linked to various diseases such as NSHL and erythrokeratodermia variabilis [7, 8]. The *SLC26A4* gene is located at 7q22-q31 (*DFNB4*). It consists of 21 exons and encodes an anion transporter known as pendrin [9]. The mutations in *SLC26A4* gene have been identified in both Pendred syndrome and NSHL [10]. Although most cases with NSHL are due to abnormalities in nuclear genes, it has

Table 1. Genetic screening of 112 children with NSHL

Gene type	No. of patients (%)
GJB2 (235delC, 35delG, 299_300delAT)	
Two mutated *GJB2* alleles	15 (13.39)
One mutated *GJB2* allele	8 (7.14)
GJB 3 (538C>T)	
One mutated *GJB3* allele	2 (1.79)
SLC26A4 (IVS7-2A>G, 2168A>G)	
Two mutated *SLC26A4* alleles	10 (8.93)
One mutated *SLC26A4* allele	7 (6.25)
12S rRNA (1555A>G)	
One mutated *12S rRNA* allele	1 (0.89)
No mutation identified	69 (61.61)
Total	112 (100%)

become clear that mtDNA mutations can also lead to deafness. The m.1555A>G mutation in the mitochondrial *12S rRNA* gene has been demonstrated to play a major role in the development of aminoglycoside-induced NSHL [11, 12].

The goal of the present study was to reveal the common genetic mutations in NSHL patients in Zhongshan City. A DNA microarray-based analysis was conducted to profile the common deafness-related genes (*GJB2*, *GJB3*, *SLC26A4*, and *12SrRNA*) in 112 pediatric patients with NSHL from unrelated families from Zhongshan City in Southern China.

Materials and methods

Study population

One hundred and twelve children (8 months-12.5 years; mean age: 6.6±1.2) diagnosed with NSHL were enrolled in this cohort. The study was approved by the Ethics Committee of Boai Hospital of Zhongshan and informed consent was obtained from the guardians of the children before blood sample collection and genetic testing. All patients had a moderate to profound bilateral sensorineural hearing loss on Pure-tone audiometry and/or auditory brainstem response examination. The patients with syndromic related hearing loss were excluded from this study.

Molecular analysis

Approximately 3 mL of whole blood was drawn from the participants and a TIANamp Blood DNA Kit (TIANGEN Biotech Co., Beijing, China) was used to extract the genomic DNA from the peripheral blood based on the manufacture's protocol.

CapitalBio Deafness Gene Mutation Detection Array Kit (CapitalBio Corporation, Beijing, China), developed by Chinese State Food and Drug Administration, was applied to screen mutation carriers of deafness-associated genes (*GJB2*, *GJB3*, *SLC26A4* and *12S rRNA*) according to the manufacturer' protocol. Nine amplicons from these four genes were amplified with allele-specific PCR primers using APEX technology. After heat denaturation procedure, the hybridization mixture was applied to a microarray chip at 50°C for 1 h in the CapitalBio BioMixer™ II Microarray Hybridization Station (CapitalBio). The hybridization was stopped by washing the slide twice at 42°Cin 0.3% SSC/0.1% SDS and in 0.06% SSC. Finally, the chip was imaged with a LuxScan™ 10 K-B Microarray Scanner (CapitalBio).

Results

Mutant genes in sporadic NSHL children

The incidence of genetic defects was 38.39% (43/112) in this cohort using this DNA microarray screening method. Fifteen children carried two mutated alleles and eight children carried one mutated allele in *GJB2*. Two children carried one mutant allele in *GJB3*. Ten children carried two mutated alleles and seven children carried one mutated allele in *SLC26A4*. As regards to the gene *12S rRNA*, one child was identified carrying one mutated allele (**Table 1**). The representative mutation patterns of tested genes were shown in **Figure 1**.

Mutant allele frequencies of GJB2 in sporadic NSHL children

20.54% of the total sporadic NSHL children carried mutated alleles in *GJB2*. Among the 15 children with two mutated alleles in *GJB2*, 10 (43.48%) were homozygous for 235delC mutation, 5 (21.74%) were heterozygous for 235delC and 299_300delAT mutation. Among 8 children carrying one mutated allele in *GJB2*, 7 (30.43%) carried one 235delC mutant and 1 (4.35%) carried one 35delG mutant (**Table 2**).

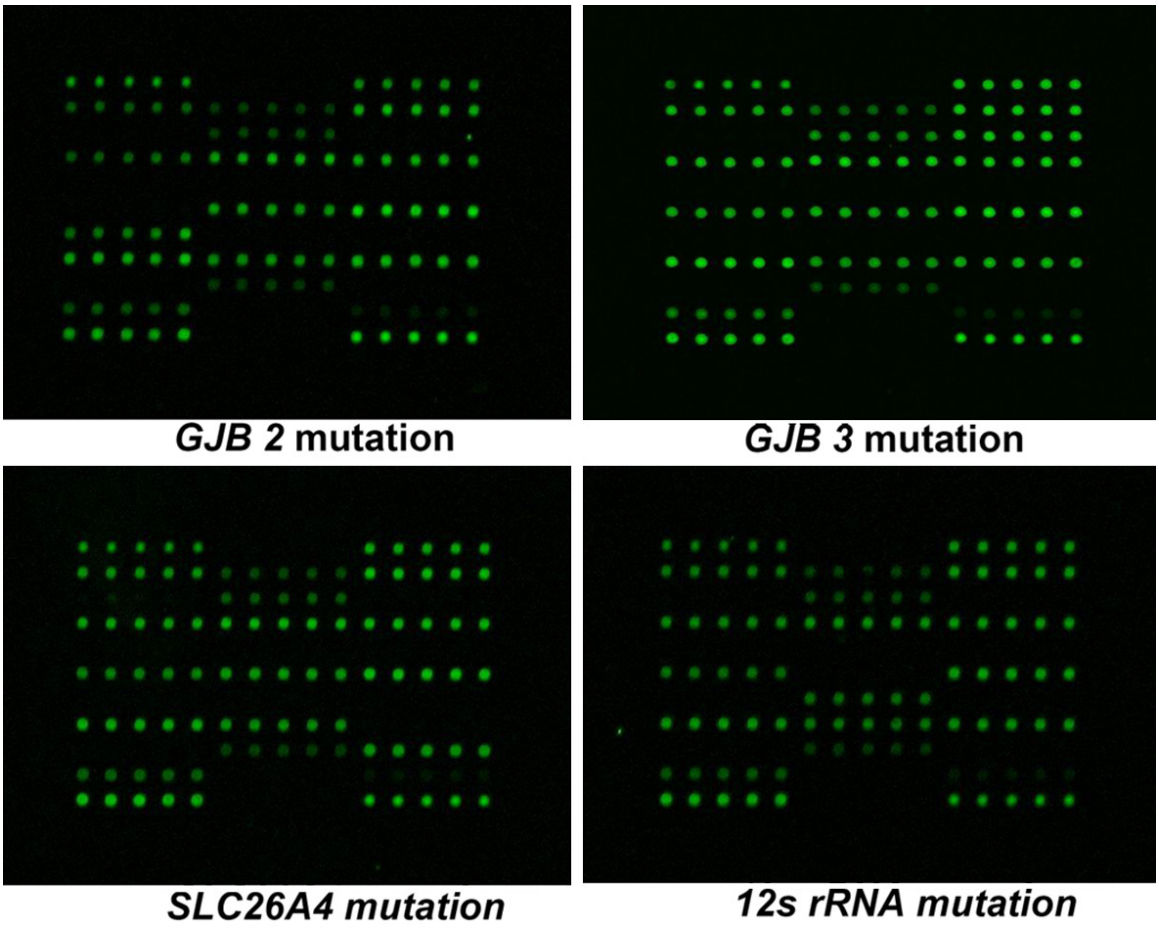

Figure 1. The representative mutation patterns of tested genes (*GJB2*, *GJB3*, *SLC26A4* and *12S rRNA*).

Table 2. Genotypes of children with mutations in the *GJB2* gene

Allele 1			Allele 2			No. (%)
Nucleotide change	Consequence	Category	Nucleotide change	Consequence	Category	
c.235delC	Frameshift	Pathogenic	c.235delC	Frameshift	Pathogenic	10 (43.48%)
c.235delC	Frameshift	Pathogenic	c.299_300delAT	Frameshift	Pathogenic	5 (21.74%)
c.235delC	Frameshift	Pathogenic		---		7 (30.43%)
c.35delG	Frameshift	Pathogenic		---		1 (4.35%)

Mutant allele frequencies of SLC26A4 in sporadic NSHL children

Seventeen children (15.18%) were found carrying the mutant *SLC26A4* sequences. Among the 10 children with two mutated alleles in *SLC26A4*, six (35.29%) were homozygous for IVS7-2A>G, and four (23.53%) were heterozygous for 2168A>G. Among 7 children carrying one mutated allele in *SLC26A4*, five (29.41%) carried only one IVS7-2A>G and 2 (11.76%) carried only one 2168A>G (**Table 3**).

Discussion

Hereditary hearing loss, caused by genetic mutation, can be categorized into NSHL and syndromic hearing loss based on the clinical manifestations. The genes associated with NSHL are involved in many important biological functions such as hair bundle morphogenesis, extracellular matrix formation and cochlear ion homeostasis maintenance [13]. Therefore, understanding the genetic basis leading to NSHL is not only important for early diagnosis,

Table 3. Genotypes of children with mutations in the *SLC26A4* gene

Allele 1			Allele 2			No. (%)
Nucleotide change	Consequence	Category	Nucleotide change	Consequence	Category	
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.IVS7-2A>G	Aberrant splicing	Pathogenic	6 (35.29%)
c.IVS7-2A>G	Aberrant splicing	Pathogenic	c.2168A>G	H723R	Pathogenic	4 (23.53%)
c.IVS7-2A>G	Aberrant splicing	Pathogenic	---			5 (29.41%)
c.2168A>G	H723R	Pathogenic	---			2 (11.76%)

prevention and treatment, but also help provide genetic counseling and future perspective to probands.

In the current study, our results showed that 38.39% of NSHL children had genetic mutations using the DNA microarray based screening method. Among them, *GJB2* and *SLC26A4* mutations were the common types of mutations and a few cases carried mutations in *12S rRNA* and *GJB3*. Three frameshift (235delC, 299_300delAT, and 35delG) pathogenic mutations of GJB2 genes were found in this cohort and 235delC was the most common form of mutation. Consistent with previous findings. Qu et al reported that 235delC mutation accounted for about 80% of all *GJB2* mutations among Chinese children with NSHL [14]. Similarly, Shi et al showed that 235delC and 299delAT mutations of *GJB2* comprised of 91% Chinese NSHL populations in the Northern China [15]. *GJB3* mutation has been demonstrated to be associated with the high-frequency hearing loss. Chen et al revealed that 538C>T mutation of *GJB3* is only detected in Han Chinese patients, but not in Uyghur patients [16]. *SLC26A4* gene mutations were detected in nearly 15% of our NSHL patients, with IVS7-2A>G being the most prevalent mutation. IVS7-2A>G mutation seems to the most common form of Chinese mutation spectrum of *SLC26A4*, accounting for 57.63% of all the mutant alleles [17]. However, H723R is the most prevalent mutation of *SCL26A4* in the Japanese population, indicating the mutation patterns of *SLC26A4* might be various due to the ethnic differences. The mitochondrial *12S rRNA* is another mutation gene closely correlated with both aminoglycoside-induced NSHL. It is known that nucleotides 1494 and 1555 are located in a highly conserved region of the *12S rRNA*, Two mutations (1555A>G, 1494C>G) consist of a large proportion of aminoglycoside ototoxicity. Lu et al demonstrated that the frequency of 1555A>G mutation was 3.96% in a large cohort of Han Chinese pediatric subjects with aminoglycoside-induced and NSHL [18].

One possible limitation of the current study is the relative small sample size. Large scale cohort study is needed to further confirm mutation pattern of NSHL in the Zhongshan City in the future. Another limitation is that the DNA microarray based screening method can only identify a few common mutation spots of four genes. Future studies should focus on developing more advanced screening techniques to reveal more or novel NSHL related genetic mutations.

In conclusion, a total of 38.39% of patients with NSHL showed evidence of genetic involvement based on the DNA microarray based screening method. Mutations in the *GJB2* and *SLC26A4* are two major genetic causes of NSHL in Zhongshan City.

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Disclosure of conflict of interest

None.

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