INTRODUCTION

Hearing loss is a major cause of morbidity; it increases medical expenses and restricts economic development. Hearing loss is closely related to hereditary and environmental factors. Various types of late-presenting hearing loss are caused by gene defects or environmental susceptibility. Over 70% of hereditary deafness manifests only as hearing loss, called nonsyndromic hearing impairment (NSHI). According to the reported molecular epidemiology of NSHI, gene mutations causing deafness vary substantially across countries, regions, ethnic groups, and ages. A report of the Second National Sample Survey of Disabled People published in 2006 has stated that the carrier rate of gene mutations responsible for deafness in the Chinese population is approximately 6%. Thus, approximately 762,000 people in Guangzhou are carriers for gene mutations causing deafness because the resident population of this city is approximately 12.7 million. These carriers include individuals with hearing disabilities and a high-risk group that is yet to present with deafness. To determine the molecular etiology of NSHI in Guangdong, this study was conducted to screen and analyze the prevalent variations of patients with hearing impairment using a high-throughput, integrated, microminiaturized, automated, sensitive, and specific gene chip technology. Hot-spot mutations leading to deafness were identified to supply a theoretical basis for the risk of ototoxicity medicine in the high-risk group, as well as for awareness, education, and preventing hearing impairment in the community.

OBJECTIVES: To explore the characteristics of variations in patients with nonsyndromic hearing impairment (NSHI) in Southern China to supply a theoretical basis for screening, intervention, and prevention.

MATERIALS and METHODS: A total of 634 hearing-impaired students from the special schools for the deaf and 220 normal-hearing individuals in South China were tested using an allele-specific polymerase chain reaction-based universal array, and the screened SLC26A4 mutation carrier was examined using computed tomography. The pathogenesis of deafness was analyzed using pathography and objective hearing tests.

RESULTS: In total, 151 patients with NSHI carried pathogenic mutations in the screening chip, and the carrier rate was 23.82% (151/634) in the studied population. Of the 151 screened carriers, 65 (10.25%) patients harbored homozygous or homoplasmy mutated genes associated with autosomal recessive hearing loss; 36 (5.68%) patients with mutant alleles were homozygous for the GJB2 c.235delC mutation and 27 (4.26%) were heterozygous. Furthermore, 18 (2.84%) patients were homozygous with mutant alleles for the SLC26A4 c.919-2A>G mutation and 43 (6.78%) were heterozygous; 7 (1.10%) patients were homoplasmy mutation carriers of MT-RNR1 gene. There was 1 SLC26A4 c.919-2A>G and 1 GJB2 c.235delC heterozygous mutant allele in the group of 220 normal-hearing individuals.

CONCLUSION: GJB2 and SLC26A4 were much more prevalent than MT-RNR1 and GJB3 in South China according to this gene chip. Minuscule differences in the mutation spectrum or prevalence of GJB2 c.235delC and SLC26A4 c.919-2A>G were found in our study; furthermore, a relatively high incidence of variations was observed among these individuals with NSHI.

KEYWORDS: Nonsyndromic hearing impairment, gene chip, variation, molecular epidemiology, molecular etiology
MATERIALS AND METHODS

Participants
Patients with bilateral severe or profound hearing loss, suggested by pure-tone audiometry, whose hearing loss exceeded 70 dB according to the internationally used classification proposed by Stephens [3] in 2001 as well as those without any organic lesions or other genetic diseases were recruited after obtaining informed consent in accordance with the Ethics Committee regulations of our hospital. The hearing-impaired group comprised 634 students (354 males and 280 females; age, 7-24 years; median age, 15 years), all of Han ethnicity, of the special schools for deaf from Guangzhou, Zhaoqing, Zhongshan, and Zhuhai districts, Guangdong province. The onset of hearing loss was between 0 and 1 year of age. The demographic details as well as hearing impairment, family, and drug histories were provided by patients’ parents.

The normal-hearing individuals comprised 220 people (110 males and 110 females; age, 5-32 years; median age, 16 years), all of Han ethnicity, their hearing was confirmed to be normal on clinical health examination comprising normal physical examination and hearing tests to rule out organic lesions or any other genetic diseases. Basic information and past drug histories were obtained for all the participants.

Methods
The instruments included an Ultra-violet spectrophotometer (Thermo Co, Germany), polymerase chain reaction (PCR) instrument (ABI Co, America), thermostatic water bath (Changan Co, China), air bath oscillator (Changan Co, China), Jin Xin BioMixer™II chip hybridization station, Jin Xin SlidWaher™ chip clean-up station, Jin Xin LuxScan™10K-B Microarray Scanner and corresponding software system, and Jin Xin Deafness-Related Gene Mutation Detection Kit (Microarray, Product No.300065, CapitalBio Co, Beijing, China); these instruments were provided by the CapitalBio Corporation.

The follow-up procedure included drawing of peripheral venous blood, DNA extraction (TIANGEN Biotech Co. Ltd., Beijing, China), PCR, hybridization, chip clean-up, and finally scanning. A Jin Xin LuxScan™10K-B Microarray Scanner was used to scan the chip with an excitation wavelength of 532 nm, and it automatically interpreted the outcome.

In the result interpretation, the genetic locus was determined as wild type if, at a genetic locus, the W row (wild) was identified as positive and the M row (mutant) was identified as negative; the locus was determined as a heterozygous mutant if, at a genetic locus, if both the W row and M row were identified as positive; the locus was determined as a homozygous mutant if, at a genetic locus, the W row was identified as negative and the M row was identified as positive. The result was determined as a multiple-locus compound mutant if combinations of the above appeared at different loci.

Statistical Analysis
Statistical analyses were performed using statistical software Statistical Package for the Social Sciences (SPSS) 19.0 (IBM Corp.; Armonk, NY, USA). Chi-square test and unconditional logistic regression of deafness-related risk factors were used to analyze the data. The differences were considered statistically significant at p<0.05.

RESULTS
Family History Analysis Results
Of all, 124 (19.56%) were sporadic cases with a family history of deafness. Three patients had a definite drug history of large-dose aminoglycoside antibiotic intake. Although one patient carried the SLC26A4 (Solute Carrier Family 26 [Anion Exchanger], member 4) c.919-2A>G heterozygous mutation, the condition was considered to be ototoxic deafness due to a large-dose aminoglycoside injection and no relevant family history.

Hearing Examination Results
Among the 634 students with NSHI at the special schools for the deaf in Guangdong, 71 (11.20%) had severe sensorineural hearing loss

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Total</th>
<th>Percent</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJB2</td>
<td>c.235delC</td>
<td>36</td>
<td>5.68%</td>
<td>27</td>
<td>4.26%</td>
</tr>
<tr>
<td></td>
<td>c.35delG</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>c.299_300delAT</td>
<td>2</td>
<td>0.32%</td>
<td>1</td>
<td>0.16%</td>
</tr>
<tr>
<td></td>
<td>c.176del16bp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLC26A4</td>
<td>c.919-2A&gt;G</td>
<td>18</td>
<td>2.84%</td>
<td>43</td>
<td>6.78%</td>
</tr>
<tr>
<td></td>
<td>c.2168A&gt;G</td>
<td>2</td>
<td>0.32%</td>
<td>7</td>
<td>1.10%</td>
</tr>
<tr>
<td></td>
<td>c.919-2A&gt;G/c.2168A&gt;G</td>
<td>0</td>
<td>0</td>
<td>3*</td>
<td>0.47%</td>
</tr>
<tr>
<td>MT-RNR1</td>
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<td>7</td>
<td>1.10%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>m.1494C&gt;T</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GJB3</td>
<td>c.538C&gt;T</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*compound heterozygous mutations
GJB2: gap junction protein, beta 2; SLC26A4: Solute Carrier Family 26 [Anion Exchanger], member 4; MT: Mitochondrial DNA; GJB3: gap junction protein, beta 3
and 563 (88.80%) had profound hearing loss based on the pure-tone audiometry.

**Test Results of Gene Mutations Leading to Deafness**

Among the 634 patients with NSHI, 151 were found to carry gene mutations leading to deafness, resulting in a mutation carrier rate of 23.82% (151/634). The carrier rate of GJB2 was 11.20% (71/634), comprising 36 (5.68%) c.235delC homozygous mutations and 27 (4.26%) heterozygous mutations. The carrier rate of c.299_300delAT homozygous mutations was 0.32% (2/634), with heterozygous mutations comprising 0.16% (1/634). The carrier rate of c.235delC/c.299_300delAT compound heterozygous mutations was 0.79% (5/634). The carrier rate of SLC26A4 was 11.51% (73/634), of which 18 (2.84%) were c.919-2A>G homozygous mutations and 43 (6.78%) were heterozygous mutations. There were two (0.32%) c.2168A>G homozygous, seven (1.10%) heterozygous, and three (0.47%) c.919-2A>G/c.2168A>G compound heterozygous mutations. The carrier rate of the MT-RNR1 m.1555A>G homogeneous mutation was 1.10% (7/634). No GJB3 (c.538C>T)s or MT-RNR1 m.1494C>Ts mutations were detected (Table 1, Figures 1 and 2).

**SPSS Analysis Results**

Among the 220 normal-hearing individuals, one was found to carry the SLC26A4 c.919-2A>G heterozygous mutation and another carried a GJB2 c.235delC heterozygous mutation. The carrier rate of genes causing deafness in normal-hearing individuals was 0.91% (2/220), which is statistically significant (χ²=58.28, P<0.05) compared with that of the high-risk group (Figure 3).

Deafness was found to be a dependent variable, along with gender, age, native place, and gene mutation. The risk of deafness reduced by 9% when age increased by 1 year (OR=0.91, 95%CI=0.88-0.94). The risk of deafness in individuals with gene mutations was 34.13-fold more than no gene mutations (Table 2).

**HRCT Results**

Among the 73 NSHI patients who carried SLC26A4 gene mutations, 2 patients with c.919-2A>G heterozygous mutations were found to have no enlarged vestibular aqueducts or inner ear malformations on imaging. Furthermore, 33 patients (including 12 with c.919-2A>G homozygous mutations, 13 with c.919-2A>G heterozygous mutations, 2 with c.2168A>G homozygous mutations, 4 with 2168A>G heterozygous mutations, and 2 with c.919-2A>G/c.2168A>G compound heterozygous mutations) were found to have enlarged vestibular aqueducts and Mondini malformations bilaterally. In addition, 38 patients (including 6 with c.919-2A>G homozygous mutations, 28 with c.919-2A>G heterozygous mutations, 3 with c.2168A>G heterozygous mutations, and 1 with a c.919-2A>G/c.2168A>G compound heterozygous mutation) exhibited only enlarged vestibular aqueducts bilaterally (Figures 4 and 5).

One normal-hearing individual with a SLC26A4 c.919-2A>G heterozygous mutation had no inner ear malformation or enlargement of the vestibular aqueduct.
DISCUSSION

GJB2 (gap junction protein, beta 2) gene exon coding Connexin26 (Cx26) protein is expressed in the organ of Corti and is part of the potassium ion circulation channel. GJB2 mutation decreases Cx26 expression, leading to the production of abnormal connexins. The number of potassium ions that enter into the lymph circulation in the cochlea therefore decreases, causing the organ of Corti to retain potassium and become poisoned with it and eventually leading to hearing loss. In an epidemiological study on the students of schools for the deaf in 18 provinces of China, Dai found that the detection rate of c.235delC (the most common mutation locus among the Chinese) homoyzygous mutations was 8.81% and that of the heterozygous mutations was 9.35% with an overall detection rate of 18.15%. The detection rates also varied across regions; in white families, approximately 49% of the autosomal recessive hearing losses were induced by GJB2 mutations. The most common GJB2 mutation reported in the Caucasian population is c.35delG, which presents with a frame shift, leading to the production of abnormal connexins. The GJB2 mutation is heterogeneous, another mutation may have been located in the control region of the GJB2 gene or other genes may have jointly participated in the production of the deafness phenotype.

Among the 634 deaf children enrolled in this study, the overall carrier rate of GJB2 was 11.20%; among them, 5.68% (n=36) were c.235delC homoyzygous mutations and 4.26% (n=27) were heterozygous mutations. The carrier rate of c.299_300delAT homozgygous mutations was 0.32% (2/634), with heterozygous mutations comprising 0.16% (1/634) and the carrier rate of c.235delC/c.299_300delAT compound heterozgyugous mutations being 0.79% (5/634). No 35delG or c.176del16bp mutations were detected. Due to the apparent racial differences in the GJB2 mutation, the number of people carrying GJB2 mutations may be lower in Guangdong region. Due to the limited hot spots in this kind of gene chip and the polymorphism of genetic mutations, another mutation may have been located in the control region of the GJB2 gene or other genes may have jointly participated in the production of the deafness phenotype.
mutations. The remaining 71 deaf children were all found to have bilateral enlarged vestibular aqueducts with or without bilateral Mondini malformations. A correlation between deaf people with normal inner ear imaging findings and the SLC26A4 gene cannot be ruled out. It is generally held that dialleles or homozygous mutations can lead to abnormal phenotypes. For some changes in phenotypes caused by single heterozygous mutations, we believe that an undetected mutation locus may exist in promotor regions or in hidden splicing sites. Alternatively, there may be other unknown genes playing a joint role. The role of environmental factors cannot be ruled out.

**MT-RNR1 Gene Analysis**

Mitochondrial DNA (mtDNA) is independent of the chromosomes in the cell nucleus and is the only DNA molecule that exists in the cytoplasm. The mode of inheritance of mtDNA is maternal. Mechanisms of hearing loss include large dose-induced ototoxicity and small dose-induced mtDNA mutations \(^{21,22}\).

Among the 634 students at these special schools for the deaf in Guangdong, the carrier rate of the MT-RNR1 m.1555A>G homogeneous mutation was 1.10% (7/634). The authors believe that this may be related to the small sample size. There is also a certain degree of sampling error. The preliminary result may also be related to the regional characteristics of the hereditary composition in Guangdong, Han ethnicity, cultural background, and economic development. China has a vast territory and large population, the education and economy development are various. We infer that being a coastal city with a relatively prosperous economy, Guangdong is experiencing a continuous increase in the per capita income and consumption level of its residents. The government is also paying increasing attention to the market, and aminoglycoside antibiotics are gradually exiting the drug market. But the aminoglycoside antibiotics are also frequently used in some remote villages. Most of the participants of this study were Han children born after 1990 with severe hearing loss. Therefore, the present study investigated individuals with a reduced range of ethnicity, age of onset, and degree of hearing loss compared with similar studies. Among the three children who had a definite drug history of large-dose aminoglycoside antibiotics, one carried the SLC26A4 c.919-2A>G heterogeneous mutation. Because this patient came from a rural area of our province, which is relatively remote with a small economy and incomplete medical services, as well as no relevant family history, the authors consider it to be sensorineural hearing loss induced by large-dose aminoglycoside antibiotic injection. It cannot be ruled out that chondriogenes are not at the mutation locus in the rest of the coding region of this test (such as tRNA\(^{\text{mt}}\)), that the SNP of the locus has changed, or that there is an unknown gene with functions similar to those of this gene. Environmental factors, a background of nuclear genes, or the mtDNA haplotype may also be significant factors causing hearing loss.

**CONCLUSION**

The GJB2 and SLC26A4 genes were much more prevalent than MT-RNR1 and GJB3 in South China according to this gene chip. Minuscule differences in the mutation spectrum or prevalence of GJB2 c.235delC and SLC26A4 c.919-2A>G were found in our study, and there was a relatively high incidence of variations among these individuals with NSHI.

The gene chip technology is a biological technology that was developed along with the human genome project. It is high-throughput, highly integrated, microminiaturized, automated, and highly sensitive and specific. It enables rapid diagnosis and precise identification of the cause of hearing loss in large-scale screenings and provides a theoretical basis for the screening, intervention, and prevention of hearing loss. Screening at the special schools in Guangdong using gene chip technology helped infer the hot-spot genes leading to deafness in the Guangdong area as a preliminary step. This technology will enable early treatment with hearing aids or cochlear implantation. It will also facilitate the education of high-risk individuals and their family members who are not yet symptomatic as well as facilitate the establishment of instructions regarding the use of drugs, consultation on hereditary hearing loss, marriage counseling, and advice on reproduction to eventually reduce the occurrence of hearing loss and to lower the birth rate of hearing-impaired babies in the Guangdong province.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Guangzhou Otorhinolaryngology Head and Neck Surgery Hospital.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.


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**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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