ORIGINAL ARTICLE

Genetic mutations of deafness-related gene among pregnant women in Fujian province of South China

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Abstract: To analyze the mutations of deaf-related gene among pregnant women in Fujian province of South China and provide a prenatal diagnosis system for their families. 2000 peripheral blood of pregnant women in Fujian province of South China was collected, and the genetic mutations of four common deafness genes (GJB2, SLC26A4, mitochondrial 12SrRNA and GJB3) were detected by using hereditary deafness gene chip. In this study, 61 pregnant women were diagnosed with deafness genes mutation. The rate of deaf-related gene mutation was 3.05% (61/2000). Among them, 38 pregnant women have GJB2 mutation (1.9%, 38/2000), which contained 29 pregnant women with heterozygous 235 del C mutation and 9 pregnant women with heterozygous 299 del AT mutation. 16 pregnant women have SLC26A4 mutation (0.8%, 16/2000), which contained 13 pregnant women with heterozygous IVS7-2 A>G mutation and 3 cases with heterozygous 2168 A>G mutation. 3 pregnant women have heterozygous GJB3 538 C>T mutation (0.15%, 3/2000). 2 pregnant women have mitochondrial 12SrRNA homozygous 1555 A>G mutation (0.1%, 2/2000). In addition, one pregnant women have GJB2 heterozygous 235 del C and SLC26A4 heterozygous 2168 A>G mutations, and one have SLC26A4 heterozygous IVS7-2 A>G and 2168 A>G mutations. There are not same mutations of deaf-related genes in husband of 61 pregnant women, and follow-up with newborn hearing of 61 pregnant women was all normal. In conclusion, deaf-related gene mutations were found among the investigated areas of Fujian province in pregnant women. A diagnostic system for deafness-related gene mutations in pregnant women may provide a basis for prenatal diagnosis and genetic counseling with non-syndromic hearing loss.

Keywords: Deafness, pregnant, deaf-related gene, mutation

Introduction

Hearing loss is one of the most common losses of meaningful function in human, and poses a persistent threat to public health worldwide [1]. Approximately 10% of people have mild or moderate hearing loss [2]. Genetic factors are important for the pathogenesis of deafness including syndromic and nonsyndromic forms [3, 4]. Nonsyndromic deafness accounts for 60~70% of inherited hearing impairment cases with autosomal recessive being the most common type of inheritance [5]. For many populations, the most common cause of nonsyndromic autosomal recessive hearing loss have mutations in GJB2 (gap junction protein beta 2) [6]. To date, more than 150 mutations, unclassified variants and polymorphisms and have been found in the GJB2 gene [7, 8]. The c.235 del C, c.35 del G, and c.167 del T mutations are the most frequent mutations in Asian, Caucasian and Ashkenazi Jewish populations, respectively [3, 6, 9]. In China, many previous genetic screening studies have shown that four common deafness genes (GJB2, SLC26A4, mitochondrial 12SrRNA and GJB3) mutations are the most common causes of deafness [3, 10].

China is a large country with the highest population in the world. Chinese people from different areas may have different genetic backgrounds due to geographical and language separation. Fujian province is situated in the southeastern part of China facing Taiwan across the Taiwan Straits, and adjacent to provinces with high deafness morbidity [11]. Moreover, there have been only a few studies on nonsyn-
Genetic mutations of deafness-related gene in pregnant women

In the present study, a total of 2000 pregnant women from all over Fujian province was collected. We comprehensively analyzed four deafness-related genes (GJB2, SLC26A4, mitochondrial 12SrRNA and GJB3) in the 2000 pregnant women. We have attempted to identify and expand the mutational spectrum of nonsyndromic deafness in this region in order to facilitate its molecular diagnosis, genetic counseling and prenatal diagnosis.

Materials and methods

Subjects

2000 pregnant women from all over Fujian province were enrolled in this study between August 2018 to July 2015 at from Fujian Provincial Maternity and Children’s Hospital (Fujian, China). The pregnant women from 20 to 29 years old with an average age of 22.43 ± 3.68 years, gestational age from 10 to 16 weeks with an average gestational age of 14.22 ± 1.74 years. Hearing tests indicated all pregnant women were normal, all peoples denied family history. No family had a consanguineous marriage. The study was approved by the ethics committees of the Fujian Provincial Maternity and Children’s Hospital (Fujian, China). Each participant provided written informed consent in compliance with ethics of the World Medical Association (Declaration of Helsinki).

Table 1. Nine mutations in the deafness-related gene

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Position</th>
<th>Sequence changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJB2</td>
<td>35</td>
<td>del G</td>
</tr>
<tr>
<td></td>
<td>176</td>
<td>del 16</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td>del C</td>
</tr>
<tr>
<td></td>
<td>299</td>
<td>del AT</td>
</tr>
<tr>
<td>GJB3</td>
<td>538</td>
<td>C&gt;T</td>
</tr>
<tr>
<td>SLC26A4</td>
<td>2168</td>
<td>A&gt;G</td>
</tr>
<tr>
<td>IVS 7-2</td>
<td></td>
<td>A&gt;G</td>
</tr>
<tr>
<td>mitochondrial 12SrRNA</td>
<td>1494</td>
<td>C&gt;T</td>
</tr>
<tr>
<td></td>
<td>1555</td>
<td>A&gt;G</td>
</tr>
</tbody>
</table>

GJB2: gap junction protein beta 2; GJB3: gap junction protein beta 3; SLC26A4: solute carrier family 26 member 4; del: delete.

DNA isolation

4 ml peripheral blood in each pregnant women was collected in our hospital. DNA was extracted from peripheral blood leukocytes by using a commercially available DNA extraction kit (Qia-gen, Hilden, Germany), according to the manufacturer’s instructions. The extracted genomic DNA was quantified by a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, MA, USA) to ensure that the DNA concentration at >100 ng/μl and the optical densities at 260/280 nm of 1.8~2.0. The genomic DNA was then stored at -80°C for further experiments.

Polymerase chain reaction (PCR) amplification

PCR was carried out with Nine Deafness Gene Mutations Detection Kit (CP.300065; CapitalBio Technology Inc., Beijing, China). The PCR was conducted with the primers provided in the kit with the following cycling conditions: 95°C for 10 min and 95°C for 10 sec, followed by 55°C for 30 sec and 72°C for 30 sec for 32 cycles, then 72°C for 10 min. An CFX96 Quantitative PCR Instrument (Bio-Rad Laboratories, Inc., CA, USA) was used to analyze the data.

Hybridization, chip washing and drying

The PCR products were placed in a hot bath (98°C) for 10 min in order to denature the nucleotides, and then immediately placed into an ice-water mixture for 5 min. Subsequently, 2.5 µl PCR products of each amplification system were added. Following adequate mixing, samples were centrifuged at 160×g and added into the chip-array area. The hybrid box was then closed and horizontally placed into preheated LuxScan™ 3.0 chips hybridization instrument (CapitalBio, Beijing, China) for 1 h. Finally, the chips were removed and washed with the SlideWasher™ 8-chip washing instrument (CapitalBio, Beijing, China). Washing liquid I was used to wash the chips once for 120 sec with a cleaning force of 5, and then the chips were washed twice with washing liquid II (42°C) for 60 sec with a cleaning force of 5. The chips were then put into a drying chamber for centrifugal drying at 240×g for 5 min.
Scanning and variant analysis

The hereditary deafness gene chip (CapitalBio, Beijing, China) for simultaneously screening nine mutations leading to hearing impairment (GJB2: 35 del G, 176 del 16, 235 del C and 299 del AT; GJB3: 538 C>T; SLC26A4: 2168 A>G and IVS 7-2 A>G; mitochondrial 12SrRNA: 1494 C>T and 1555 A>G) was used to determine the mutations in all pregnant women. The mutations was shown in Table 1. Data was analyzed by using a LuxScan™ 3.0 software (CapitalBio, Beijing, China).

Statistical analysis

The statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Data was presented as mean ± SD (standard deviation) from three independent experiments with each measured in triplicate. The differences among groups were analyzed using One-way ANOVA. A value of $P<0.05$ was considered to be a statistically significant difference.

Results

Clinical description

The pregnant women were from 20 to 29 years old with an average age at 22.43 ± 3.68 years. The gestational age was from 10 to 16 weeks with an average gestational age at 14.22 ± 1.74 years. Hearing tests indicated all pregnant women and theirs husband were normal, all peoples denied family history.

Microarray analysis of deafness gene mutations

As was shown in Figure 1. Nine mutation was found by hereditary deafness gene chip. There are GJB2 mutations (heterozygous 235 del C and heterozygous del AT), SLC26A4 mutations (heterozygous IVS7-2 A>G and heterozygous 2168 A>G), GJB3 mutation (heterozygous 538 C>T), mitochondrial 12SrRNA mutation (homozygous 1555 A>G), GJB2 heterozygous 235 del C and SLC26A4 heterozygous 2168 A>G muta-
Genetic mutations of deafness-related gene in pregnant women

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Cases</th>
<th>Mutational rate (%)</th>
<th>Constituent ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous 235 del C</td>
<td>29</td>
<td>1.45% (29/2000)</td>
<td>47.54% (29/61)</td>
</tr>
<tr>
<td>Heterozygous 299 del AT</td>
<td>9</td>
<td>0.45% (9/2000)</td>
<td>14.75% (9/61)</td>
</tr>
<tr>
<td>Heterozygous IVS7-2 A&gt;G</td>
<td>13</td>
<td>0.65% (13/2000)</td>
<td>21.31% (13/61)</td>
</tr>
<tr>
<td>Heterozygous 2168 A&gt;G</td>
<td>3</td>
<td>0.15% (3/2000)</td>
<td>4.92% (3/61)</td>
</tr>
<tr>
<td>Homozygous 1555 A&gt;G</td>
<td>2</td>
<td>0.1% (2/2000)</td>
<td>3.28% (2/61)</td>
</tr>
<tr>
<td>Heterozygous 538 C&gt;T</td>
<td>3</td>
<td>0.15% (3/2000)</td>
<td>4.92% (3/61)</td>
</tr>
<tr>
<td>Heterozygous 235 del C and 2168 A&gt;G</td>
<td>1</td>
<td>0.05% (1/2000)</td>
<td>1.64% (1/61)</td>
</tr>
<tr>
<td>Heterozygous IVS7-2 A&gt;G and 2168 A&gt;G</td>
<td>1</td>
<td>0.05% (1/2000)</td>
<td>1.64% (1/61)</td>
</tr>
</tbody>
</table>

Del: delete.

Genetic mutation analysis

As shown in Table 2, among the 2000 pregnant women, 61 pregnant women have existed deafness gene mutation. The rate of deaf-related gene mutation was 3.05% (61/2000). The mutation rate of GJB2 was 1.9% (38/2000) accounting for 62.30% of all positive mutations (38/61), including 29 cases with heterozygous 235 del C mutation and 9 cases with heterozygous 299 del AT mutation. 16 pregnant women have SLC26A4 mutation (0.8%, 16/2000) accounting for 26.22% of all positive mutations (16/61), which contained 13 pregnant women with heterozygous IVS7-2 A>G mutation and 3 cases with heterozygous 2168 A>G mutation. 3 pregnant women have heterozygous GJB3 538 C>T mutation (0.15%, 3/2000) accounting for 4.91% of all positive mutation (3/61). 2 pregnant women have mitochondrial homozygous 12SrRNA 1555 A>G mutation (0.1%, 2/2000) accounting for 3.28% of all positive mutations (2/61). One pregnant women one have GJB2 heterozygous 235 del C and SLC26A4 heterozygous 2168 A>G mutations, and one have SLC26A4 heterozygous IVS7-2 A>G and heterozygous 2168 A>G mutations. There are not mutations of deaf-related genes in husband of 61 pregnant women, and follow-up with newborn hearing of 61 pregnant women was of all normal.

Discussion

Genetic factors have been shown to influence the development of deafness. The new-onset and aggravation of deafness may be prevented to a certain extent through identifying genetic factors of deaf patients and high-risk groups and giving appropriate guidance and interventional measures [12, 13]. To date numerous deafness genes have been reported and the mutation spectrum is known to be widespread. In this study, mutation analysis was performed among 2000 pregnant women in Fujian province of South China. A total of 3.05% pregnant women showed evidence of genetic mutations caused by GJB2, SLC26A4, GJB3 and mitochondrial 12SrRNA. These results will facilitate effective risk assessment and genetic counseling for pregnant women and their families.

GJB2 was the first disease-causing gene identified for Chinese non-syndromic hearing loss [10]. Previous study showed that GJB2 mutation was observed to be the most common deafness-associated mutation in Yongchuan district accounting for 73.68% (14/19) of all gene mutation loci detected [14]. In this study, the detection rate of the GJB2 mutation was 1.9% accounting for 62.30% of all positive mutations (38/61). The results were similar with previous study. These mutations contained 29 cases with heterozygous 235 del C mutation and 9 cases with heterozygous 299 del AT mutation. The present study detected 29 case of GJB2 heterozygous 235 del C, and this pregnant woman had lived in the local area for numerous years, with no descent from any ethnic minority and no family history of deafness; thus, it was suggested that a rare recessive genetic cause of deafness may present in those families within the Fujian province.

The SLC26A4 gene mutation has been previously reported to result in non-syndromic hearing loss and syndromic deafness [15, 16]. The present study detected 16 pregnant women with SLC26A4 mutation (0.8%) accounting for...
26.22% of all positive mutations (16/61), which contained 13 pregnant women with heterozygous IVS7-2 A>G mutation and 3 cases with heterozygous 2168 A>G mutation. The detection rate of the SLC26A4 mutation was lower than that reported in previous studies, and the possible causes were differences may exist in the deafness gene mutation spectrum in Fujian province. Previous literatures observed that in one patient, the mitochondrial 12S rRNA gene mutation was associated with maternal inheritance, and the application of aminoglycosides resulted in irreversible hearing loss [17, 18]. Here, the detection rate of mitochondrial 12S rRNA homozygous 1555 A>G mutation was 0.1%. In addition, 3 pregnant women have heterozygous GJB3 538 C>T mutation (0.15%).

In conclusion, the present study revealed spectra of deafness-related gene mutations among pregnant women in Fujian province of South China, suggesting that the government and medical institutions should intensify genetic counseling and pre-natal diagnosis of genetic diseases, in order to screen pregnant women for deafness gene mutations. The combination of guidance on medication, pre-natal diagnosis and clinical intervention may reduce deafness in the population.

Acknowledgements

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Disclosure of conflict of interest

None.

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