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Original Article

Analysis and prenatal diagnosis of deafness-related gene mutations in patients with fourteen Chinese families

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Abstract: To analyze deaf-related genes in patients with fourteen Chinese families and provide a prenatal diagnosis system for such patients and their families. Peripheral blood of fourteen family members and the fetal amniotic fluids or fine hairs was collected. Four common deafness genes (GJB2, SLC26A4, mitochondrial 12SrRNA and GJB3) were detected by using hereditary deafness gene chip. Here, 14 probands were found in the fourteen Chinese families. Among them, 9 cases was homozygous GJB2 235 del C mutations, 4 cases was homozygous SLC26A4 IVS7-2 A>G mutations and 1 case was heterozygous GJB2 235 del C and 299 del AT mutation. There were heterozygous GJB2 235 del C mutations on both sides of 9 couples, heterozygous SLC26A4 IVS7-2 A>G mutations of 4 couples and heterozygous GJB2 235 del C and 299 del AT of 1 couple. In these patients, 10 cases had GJB2 mutations and 4 cases had SLC26A4 gene mutations. In 14 cases of prenatal diagnosis, heterozygous GJB2 235 del C mutations was presented in 6 cases, homozygous GJB2 235 del C mutations in 2 cases, heterozygous SLC26A4 IVS7-2 A>G mutations in 2 cases, homozygous SLC26A4 IVS7-2 A>G mutation in 1 cases, heterozygous GJB2 299 del AT mutation in 1 cases and wild type in 2 cases. The overall detection rate of deaf-related gene mutations was 96.43% (54/56). The prenatal diagnosis of deaf-related gene mutations was 85.71% (12/14) and the rate of homozygous mutations was 21.43% (3/14). In conclusion, deaf-related gene mutations was common in families with hearing loss. A diagnostic system for deafness-related gene mutations may provide a basis for prenatal diagnosis and genetic counseling to families with hearing loss.

Keywords: Deaf-related genes, mutations, prenatal diagnosis, heterozygous, homozygous

Introduction

Hearing loss is the most common sensory impairment that affects normal communication and life quality of the patients [1]. Approximately half of patients with hearing impairment are caused by genetic factors [2-4]. 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 [5-7]. Of these mutations, GJB2 235 del C mutation is the most common in the Chinese deaf population [8]. The most common mutation of SLC26A4 seen in the Chinese deaf population is IVS7-2 A>G and its detection rate can be as high as 12.5% [9, 10]. Ethnic differences exist in the genetic pathogenesis of deafness. In Caucasian, Asian, Ashkenazi Jew, and African populations, the most common mutations in the GJB2 gene are 35 del G, 167 del T, 235 del C and 427 C>T, respectively [11].

Prenatal diagnosis is testing for diseases or conditions in fetuses before born, which gives parents the chance to prepare psychologically and medically for the probable health and educational needs of the affected neonates [12]. Meanwhile, appropriate genetic counseling could be of necessity and importance in providing information concerning the nature of the disease, the implications of being mutation carriers, the inheritance modes and other reproductive choices. It is also important to empower participating families to help them avoid over-

dependence on professionals and to involve them in decision making courses. Genetic counseling and prenatal diagnosis are very necessary and accurate to detect hereditary hearing loss, especially in high-risk families [13].

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. Until now, no systematic genetic analysis and prenatal diagnosis of deaf patients in Chinese families has been reported previously. To provide accurate genetic testing and counseling, we evaluated the molecular etiology of deafness in a deaf population from fourteen Chinese families. In total, patients with fourteen Chinese families were recruited for this investigation. In the present study, we comprehensively analyzed four deafness-related genes (GJB2, SLC26A4, mitochondrial 12SrRNA and GJB3) in fourteen Chinese families. Detailed genotype and phenotype analyses were performed to provide effective risk assessment and genetic counseling for hearing loss patients and their families.

Materials and methods

Patients

Fourteen Chinese families from Fujian Provincial Matenity and Children's Hospital (Fujian, China) were enrolled in this study between August 2013 to September 2015. This cohort of patients consisted of 14 males and 14 females from 19 to 27 years old, with an average age of 21.32 ± 4.17 years. Hearing tests demonstrated that the level of hearing loss was severe to profound in all cases. No family had a consanguineous marriage. The protocol for this investigation was performed with the approval of the ethics committees of the Fujian Provincial Matenity and Children's Hospital (Fujian, China). Each participant provided written informed consent in compliance with ethics of the World Medical Association (Declaration of Helsinki). Questionnaires included basic information, including name, age, address, family history, health records of the mother during pregnancy, and a clinical history of the patient, such as infections, possible head or brain injury, and the use of aminoglycoside antibiotics. All subjects underwent hearing tests and medical examinations.

DNA samples and variant analysis

DNA was extracted from peripheral blood leukocytes by using a commercially available DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. The coding exons plus approximately 50~100 bp of the flanking intron regions of GJB2, SLC26A4, mitochondrial 12SrRNA and GJB3 were amplified by polymerase chain reaction (PCR). The hereditary deafness gene chip (CapitalBio, Beijing, China) for simultaneously screening the 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 IVS7-2 A>G; mitochondrial 12SrRNA: 1494 C>T and 1555 A>G) was used to determine the sequences in all patients. The data was analyzed by a LuxScan™ 3.0 software (CapitalBio, Beijing, China).

Genetic counseling

Elaborate genetic counseling was provided to each participating families that might play a role in a specific family according to the genetic test results. Moreover, detailed information was offered to assist families with deaf children in making informed decisions concerning medical managements and appropriate educational interventions. In addition, as for those carrier couples anticipating another children, recurrence information and reproductive choices were provided. Carrier couples identified prepregnancy could have the options of taking their chances, avoiding pregnancy, preimplantation diagnosis, or gamete donation. Those identified during pregnancy could have the opportunity of prenatal diagnosis and early preparation for the health and educational needs of neonates.

Prenatal diagnosis

For those couples at risk and might need prenatal diagnosis, detailed information was offered, regarding to the fetal DNA sampling procedures, the risk of fetal mortality, and the limitations of the testing. With ultrasound-guid-

Table 1. Analysis of deafness-related gene mutations by using hereditary deafness gene chip

Gene	Sequence variants	Amino acid changes
GJB2	35 del G	Yes
	176 del 16	Yes
	235 del C	Yes
	299 del AT	Yes
GJB3	538 C>T	Yes
SLC26A4	2168 A>G	Yes
	IVS7-2 A>G	Yes
Mitochondrial 12SrRNA	1494 C>T	Yes
	1555 A>G	Yes

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

ed procedures, fetal amniotic fluids or fine hairs samples were obtained for fetal DNA analysis. Since the potential presence of maternal cells in fetal amniotic fluids or fine hairs samples may pose a significant preanalytical risk for prenatal misdiagnosis, maternal cell contamination testing was performed to determine the pure fetal origin of all prenatals pecimens undergoing genetic analysis.

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. A value of $P<0.05$ was considered to be a statistically significant difference.

Results

GJB2 mutations

14 probands were found in the fourteen Chinese families. As shown in **Table 1**, seven variants were identified in this cohort. The overall detection rate of deaf-related gene mutations was 96.43% (54/56). The mutant alleles of GJB2 accounted for 71.4% (10/14) of the total alleles in the fourteen Chinese families. Among them, 9 cases was homozygous GJB2 235 del C mutations and 1 case was heterozygous GJB2 235 del C and 299 del AT mutation (**Table 2**). There were heterozygous GJB2 235 del C mutations on both sides of 9 couples and heterozygous GJB2 235 del C and 299 del AT of 1 couple (**Table 2**).

SLC26A4 mutations

The mutant alleles of SLC26A4 accounted for 28.57% (4/14) of the total alleles in the fourteen Chinese families. 4 cases of the probands was homozygous SLC26A4 IVS7-2 A>G mutations (**Table 2**). There were heterozygous SLC26A4 IVS7-2 A>G mutations of 4 couples.

Mutation analysis

GJB2 235 del C and 299 del AT mutations were common found in patients from previous study. We predicted the pathogenicity of this mutation by SIFT (http://sift.jcvi.org/) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and the results suggested "damaging". SLC26A4 IVS7-2 A>G mutation has been proposed to exert a likely pathogenic effect according to the reported data (http://deafnessvariationdatabase.org/). These data suggest that these mutations are responsible for hearing loss in the fourteen Chinese families.

Prenatal diagnosis of deafness-related gene mutations in patients with fourteen Chinese families

The hereditary deafness gene chip of prenatal diagnosis was shown in **Figure 1**. In 14 cases of prenatal diagnosis, heterozygous GJB2 235 del C mutations was presented in 6 cases, homozygous GJB2 235 del C mutations in 2 cases, heterozygous SLC26A4 IVS7-2 A>G mutations in 2 cases, homozygous SLC26A4 IVS7-2 A>G mutation in 1 cases, heterozygous GJB2 299 del AT mutation in 1 cases and wild type in 2 cases. In these patients, 10 cases had GJB2 mutations and 4 cases had SLC26A4 gene mutations. The prenatal diagnosis of deaf-related gene mutations was 85.71% (12/14) and the rate of homozygous mutations was 21.43% (3/14).

Discussion

It is generally recognized that genetic testing and prenatal diagnosis should not reflect a eugenic policy, so the genetic counseling should be provided without a self-censoring filter, because attitudes and biases can influence both content and presentation of information relevant to decision-making [14, 15]. Generally,

Table 2. Analysis and prenatal diagnosis of deafness-related gene mutations in patients with fourteen Chinese families

Families	Probands	wives	Husbands	Fetuses	Follow up
1	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Heterozygous 235 del C	Normal hearing
2	Homozygous IVS7-2 A>G (deafness)	Heterozygous IVS7-2 A>G	Heterozygous IVS7-2 A>G	Heterozygous IVS7-2 A>G	Normal hearing
3	Homozygous 235 del C (deafness)	Heterozygous 235 del AT	Heterozygous 235 del C	Heterozygous 235 del C	Normal hearing
4	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Heterozygous 235 del C	Normal hearing
5	Homozygous IVS7-2 A>G (deafness)	Heterozygous IVS7-2 A>G	Heterozygous IVS7-2 A>G	Heterozygous IVS7-2 A>G	Normal hearing
6	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Heterozygous 235 del C	Normal hearing
7	Homozygous IVS7-2 A>G (deafness)	heterozygous IVS7-2 A>G	heterozygous IVS7-2 A>G	Wild type	Normal hearing
8	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Heterozygous 235 del C	Normal hearing
9	Heterozygous 235 del C and 299 del AT (deafness)	Heterozygous 235 del C	Heterozygous 299 del C	Heterozygous 299 del AT	Normal hearing
10	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Heterozygous 235 del C	Normal hearing
11	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Wild type	Normal hearing
12	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Homozygous 235 del C	Induced labour
13	Homozygous 235 del C (deafness)	Heterozygous 235 del C	Heterozygous 235 del C	Homozygous 235 del C	Induced labour
14	Homozygous IVS7-2 A>G (deafness)	Heterozygous IVS7-2 A>G	Heterozygous IVS7-2 A>G	Homozygous IVS7-2 A>G	Induced labour

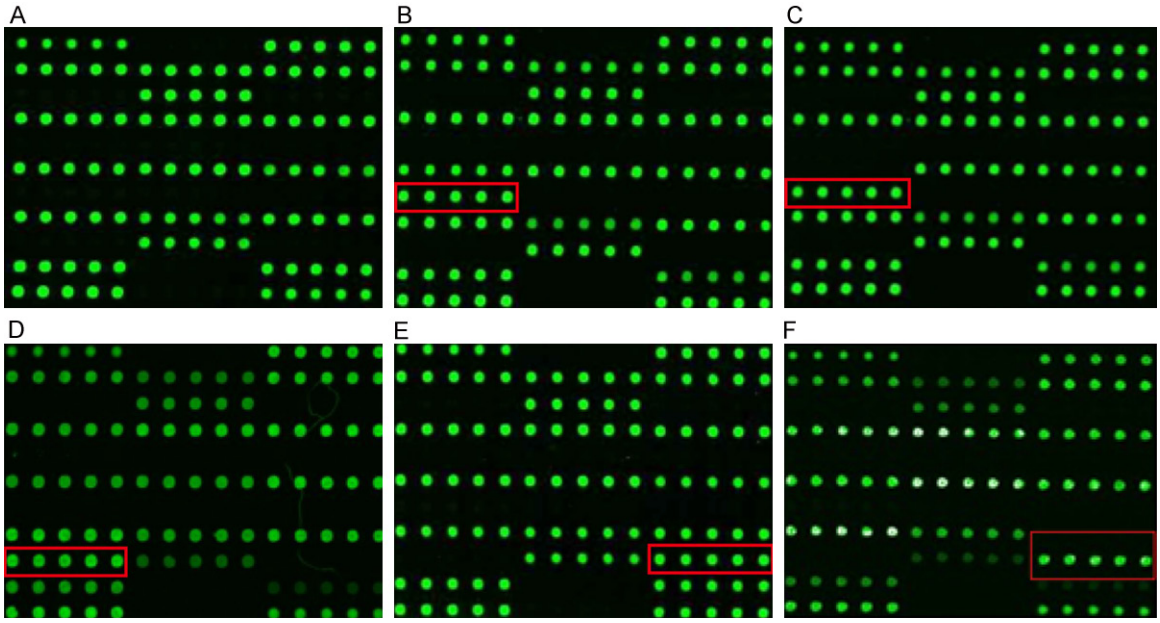

Figure 1. The hereditary deafness gene chip of prenatal diagnosis was shown. A: Wild type; B: Heterozygous 235 del C; C: Homozygous 235 del C; D: Heterozygous 299 del AT; E: Heterozygous IVS7-2 A>G; F: Homozygous IVS7-2 A>G. Red box: mutation position.

the genetic counseling should contain both pre-test and post-test sections. The pre-test counseling should include the nature of hearing loss, different causes of deafness, modes of inheritance, as well as genetic testing options, concerning their risks, benefits, and limitations. The post-test counseling should include explanations of test results in the context of the pre-test session and an assessment of the psychosocial impact that the results may have on the family. It is also important for counselors to know about appropriate referral networks specific to hearing loss for emotional and decision-making support [12, 13].

Many hearing loss-related gene mutations have been described to date, most of which are isolated mutations in some populations. In this study, mutation analysis was performed in fourteen Chinese families with hearing loss. A total of 96.43% deaf patients showed evidence of genetic involvement based on either genetic screening or family history, 96.43% and 28.57% of the patients were determined to have inherited hearing impairment caused by GJB2 and SLC26A4. These results will facilitate effective risk assessment and genetic counseling for hearing loss patients and their families.

Screening of the GJB2 gene coding region revealed that GJB2 235 del C and 299 del AT mutations are common in Chinese people with hearing loss [16]. The 235 del C mutation was markedly associated with the risk of hearing loss in East Asian and Southeast Asian populations, but not significantly in European or Oceanian populations [11]. In our study, the most common mutation was 235 del C, and followed by 299 del AT. The common Caucasian mutation 35 del G was not found. The 235 del C and 299 del AT mutations accounted for 90.00% and 10.00% of the GJB2 mutations in our patients. The IVS7-2 A>G is the hotspot mutation in SLC26A4. The mutant alleles of GJB2 accounted for 28.57% of the total alleles in the fourteen Chinese families. This mutation spectrum was different from that in Japanese, Korean, northern European and Danish populations. In Japan, H723R is the most prevalent mutation [17, 18]. In Korea, IVS7-2 A>G and H723R are the two most prevalent mutations [19].

In the present study, the prenatal diagnosis of deaf-related gene mutations was 85.71% and the rate of homozygous mutations was 21.43%, which indicated a positive attitude of normal hearing couples with hearing impaired children held towards genetic testing and prenatal diagnosis of hereditary hearing loss. They valued prenatal testing for the information provided for future family planning and probably the preparation for the health and educational needs of the affected neonate [20]. As indicated in the

study, hearing parents' attitudes towards prenatal testing and their decisions about affected pregnancy were influenced by a number of factors, including ethical and moral values, religious beliefs, economic considerations, as well as the laws and rules regulating the field.

In conclusion, our results revealed special hotspots and spectra of mutations in fourteen Chinese families, and this information will be helpful in designing the protocol for genetic testing for deafness and achieving an accurate molecular diagnosis in patients with hearing loss. A diagnostic system for deafness-related gene mutations may provide a basis for prenatal diagnosis and genetic counseling to families with hearing loss.

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Disclosure of conflict of interest

None.

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