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APPLICATION OF GENETIC DEAFNESS GENE CHIP FOR DETECTION OF GENE MUTATION OF DEAFNESS IN PREGNANT WOMEN

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Abstract

Objective The study is to identify the carrier rate of common deafness mutation in Chinese pregnant women via detecting deafness gene mutations with gene chip. **Methods** The pregnant women in obstetric clinic without hearing impairment and hearing disorders family history were selected. The informed consent was signed. Peripheral blood was taken to extract genomic DNA. Application of genetic deafness gene chip for detecting 9 mutational hot spot of the most common 4 Chinese deafness genes, namely GJB2 (35delG，176del16bp, 235delC, 299delAT), GJB3 (C538T) ,SLC26A4 (IVS72A>G, A2168G) and mitochondrial DNA 12S rRNA (A1555G, C1494T) . Further genetic testing were provided to the spouses and newborns of the screened carriers. **Results** Peripheral blood of 430 pregnant women were detected，detection of deafness gene mutation carriers in 24 cases(4.2%), including 13 cases of the GJB2 heterozygous mutation, 3 cases of SLC26A4 heterozygous mutation, 1 cases of GJB3 heterozygous mutation, and 1 case of mitochondrial 12S rRNA mutation. 18 spouses and 17 newborns took further genetic tests, and 6 newborns inherited the mutation from their mother. **Conclusion** The common deafness genes mutation has a high carrier rate in pregnant women group，235delC and IVS7-2A>G heterozygous mutations are common.

Key words: Gene chip, Hereditary deafness, Carrier rate, Mutation detection

Deafness, one of the most common sensory dysfunction, is the biggest obstacle to language learning and communication. In China, it is estimated that 30,000 babies are born with congenital hearing loss every year[1]. 50% - 70%of deafness may be related to genetic factors. According to the phenotype of hereditary，deafness can be divided into Syndromic hearing loss (SHL; about 30%) and Nonsyndromic hearing loss (NSHL; about 70%)[2]. By the end of April 30, 2014, we have found the following results through the data query（http://hereditaryhearingloss.org）, including 53 autosomal recessive cloned, 34 autosomal dominant, 4 X-linked, and 2 mitochondrial NSHL. A large number of studies show that hereditary deafness has strong genetic heterogeneity and multiple gene mutations, in addition, the morbidity and the rate of pathogenic gene are high. Therefore, the establishment of a rapid, high-throughput gene diagnosis method is very necessary in the clinical examination. Studies on molecular epidemiology of deafness in China shows that most NSHL caused only by a small number of mutations, such as GJB2, SLC26A4, mitochondrial DNA (mtDNA) 12S rRNA and GJB3 etc[3] [4]. In this study, gene diagnosis chip of hereditary deafness (Beijing CapitalBio Corporation) [5] were used to detect deafness gene mutations among pregnant women, and the statistics of the carrying rate of common deafness mutation

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was performed. Application prospect of deafness gene chip technology was discussed in the research.

Materials and methods

Materials

The peripheral blood of 430 pregnant women, husband of 18 pregnant women and 17 newborns from January to December, 2013 was collected in the department of Obstetrics and Gynecology of the Third Hospital of Peking University. The age of the pregnant women ranged from 22 to 43, and the average age was 30.1 ± 3.2. Pregnant women whose family has hearing impairment were not included in the study. The informed consent was signed by all participating pregnant women.

Methods

DNA extraction: 3ml venous blood was collected and transferred to EDTA anticoagulant tube. DNA was extracted by Blood genomic DNA Extraction System Kit (Beijing Tiangen biotech companies, DP319-02). The concentration of DNA was 100-200ng/μl, the purity of DNA (OD 260/280) was 1.7-2.0. The specimens were kept under -20℃.

Chip detection: 9 of the mutation sites in 4 common gene associated with Chinese deafness, including GJB2(13q11-12), SLC26A4(7q22-31.1), GJB3 (1p33-p35) and mtDNA 12S rRNA, were detected using Jingxin nine genetic deafness gene detection kit (Beijing Capital Bio corporation, 300065). PCR amplification, microarray hybridization, scanning and chips washing were performed. The data was finally processed and analyzed. The details are as follows.

PCR reaction: Based on 9 sets of primers of the 9 mutation sites, the PCR was divided into 2 reaction systems, including A and B. Respectively by multiplex PCR was carried out respectively. 3 μ l template DNA, 12.5 μ l mixture of amplification primer and 4.5 μ l mixture of amplification reagent were added to the 20 μ l reaction system individually. PCR reaction was performed according to the thermal cycling program in table 1; temperature rate was set by using RAMP function of PCR instrument. The total reaction time was about 3 hours and 20 minutes.

Hybridization: 3 μ L of PCR products obtained from two different amplification (A, B) of the same sample template was added to the 10 μ l hybridization buffer tube. Mixed liquid of hybridization was added to the microarray zone of the chip. The slide was incubated at 60℃ for 1 hour.

Chips washing: The hybrid chips were collected and rinsed in cleaning solution under 42℃ for 2 min, followed by washing and drying in the air.

Scanning and interpretation of the results: he chip was imaged with a Jingxin LuxScan 10K-B chip microarray scanner and the corresponding judging system. The statistical analysis was performed using SPSS 17.0.

Table 1. PCR amplification program of gene detection of hereditary deafness

temperature (℃)	37	95	96	94	RAMP	55	RAMP	70	60	4
time (S) Cycle	600	600	60	30	0.4℃/s	30	0.2℃/s	45	600	-
	1	1	1	32					1	1

Result

Among 430 pregnant women with no obvious abnormal hearing, 18 carriers of heterozygous mutation of deafness gene were detected, and the detection rate was 4.2%. Homozygous mutant was not found. As for gene GJB2, 8 cases of 235delC and 4 cases of 299delAT were detected, no 235delG or 176del16bp were found. About gene SLC26A4, 3 cases of IVS7-2A>G were detected, no A21 68G was found. 1 case takes 299del AT and IVS7-2A>G simultaneously.1 case of 538 C>T in gene GJB3 was detected; 1 case of A1555G in mtDNA 12S rRNA was detected, no C1494T was detected in the gene. The carrier rate of GJB2, SLC26A4, GJB3, mtDNA 12S rRNA were 3.1%, 0.7%, 0.2%, 0.2% respectively, and the total carrier rate was 4.2%. The details are listed in table 2.

Deafness gene chip detection was also performed on spouse of 18 pregnant women carrying mutations and their newborns. The results of all 18 cases of male spouses were normal. 17 cases of newborn have passed the newborn hearing screening. 1 case of pregnancy was terminated due to fetal polymeria during midtrimester. 6 cases of the newborns inherited the at present known gene mutation. See Table 3 for details.

Table 2. Mutation detection and carrier rate

mutant gene	mutant site (heterozygosis)	cases	Proportion	Carrying rate
GJB2	235delC	8	44.4%	1.9%
	299delAT	5 #	27.8%	1.2%
SLC26A4	IVS7-2A>G	3	16.6%	0.7%
GJB3	538 C>T	1	5.6%	0.2%
mtDNA 12S rRNA	1555	1	5.6%	0.2%
total		18	100.0%	4.2%

* Indicate a pregnant woman carries 299delAT and IVS7-2A>G simultaneously, statistical analysis is included in the 299delAT group