

# Detection of hereditary hearing loss gene by DNA microarray

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**Abstract. – OBJECTIVE:** Screening genes in patients suffering clinically sporadic deafness, using DNA microarray, and evaluating the application value of the clinical detection.

**PATIENTS AND METHODS:** DNA extracted from patients' venous blood was amplified by PCR, and hybridization was carried out in a myriad class clean room. Nine mutation sites of four deaf genes commonly seen in Chinese people were tested.

**RESULTS:** Among 24 patients, 7 cases with mutations were detected, with a positive rate of 29.17%. These include 4 cases with GJB2 gene mutation (16.67%), of which 1 case with 176 del 16 site heterozygous mutation; 1 with 235 del C site homozygous mutation; 2 with 299 del AT site heterozygous mutation; 1 with SLC26A4 gene IVS7-2A>G site heterozygous mutation (4.17%), 2 with mitochondrion 12SrRNA gene1555A>G site homogeneous mutation (8.33%). No GJB3 gene mutation was detected.

**CONCLUSIONS:** Gene chip technology of hereditary hearing loss can detect related mutation sites of hearing loss rapidly and with high-throughput, which meets the demands of clinical deaf gene detection.

Key Words:

Deafness, DNA microarray, Gene mutation, Carrier.

## Introduction

Deafness, as the most common sensory nervous system defect, influences severely communication, exchange and life quality. About 60% of deafness is an inherited affection characterized by the mutation of a single gene or a combined mutation of different genes. Indeed, deafness is usually expressed as autosomal inheritance, X-linked chromosome inheritance and mitochondria inheritance, etc.<sup>1</sup>. It has been shown in domestic deafness molecular epidemiological study that common genes causing deafness are several mutation sites of a few genes like GJB2,

SLC26A4, mitochondrial DNA, 12s rRNA and GJB3<sup>2</sup>. The current study measures mutation sites of twenty-four sporadic patients in ENT Department of the hospital by microarray.

## Patients and Methods

### Patients

Informed consent and deafness gene testing application form were filled and obtained before starting the study. 24 cases of patients with sporadic deafness were treated in ENT Department of Central Hospital of Xuzhou, among which, 16 cases were male and 8 cases were female, aged from 2 months-35 years old with an average age of  $17.2 \pm 9.9$  years old. Gene chip technology was used for genetic testing of hereditary deafness. This study was approved by the Ethics Committee of Xuzhou Central Hospital. Signed written informed consents were obtained from the patients and/or guardians.

### Key Instruments and Reagents

BioMixerII hybridization instrument, Slide-Washer 8 chip wash and dry instrument, LuxScan-10K/B scanner and nine hereditary hearing loss gene detection kit of Geedom, were purchased from Beijing Boao Biology Group Co., Ltd, (Beijing, China). PCR instrument (DL9700 Touch) was produced by Beijing Donglin Changsheng Biological Science and Technology Co., Ltd, (Beijing, China) and microfuge 20 high-speed centrifuge was produced by Beckman Coulter (Brea, CA, USA). This gene chip could detect common nine hotspot mutations of four deafness genes in Chinese people, and these nine spots were: 35delG, 176del16, 235delC, 299delAT of GJB2 gene, 2168A>G, IVS7-2A>G of SLC26A4 gene, 1494C>T, 1555A>G of mitochondria 12S rRNA, and 538C>T of GJB3. The gene

extraction process was conducted in biosafety cabinet (Biobase) of myriad class clean room to avoid cross contamination.

### **Test Method**

#### *DNA Extraction*

1.5 ml Eppendorf tube was used to extract EDTA anti-freezing whole blood of 600  $\mu$ l, and the equal amount of cell lysis buffer was added. The tube was reversed to mix up the liquid, centrifuged at 12000 rpm for 1 min. The supernatant was left, then 900  $\mu$ l lysate were added, mixed up with vortex vibration, centrifuged, and the supernatant was left. 300  $\mu$ l mixed liquor of buffer solution and protease was added, mixed up with vortex immediately, and bathed in water at 65°C for 10 min. After centrifugation, the supernatant was taken and transferred into Eppendorf tube with 300  $\mu$ l isopropanol. The tube was reversed and mixed up until alcohol DNA could be seen. After centrifugation at 12000 rpm, the supernatant was left and 300  $\mu$ l of 70% ethyl alcohol were added. The new solution was mixed up with vortex and centrifuged. The supernatant was left. DNA sediment was dried with air. 60  $\mu$ l elution buffer solution were added and bathed in water at 65°C for 30-60 min to make DNA completely dilute. This was the preparative DNA extracting solution. The concentration was required to be 100-200 ng/ $\mu$ l, and degree of purity OD<sub>260/280</sub> = 1.7-2.0. If it could not be detected immediately, it was preserved at -20°C for inspection.

#### *PCR Amplification*

PCR amplification reagent was prepared according to the number of specimens. A and B, two kinds of reaction systems, were prepared for each sample and 12.5  $\mu$ l amplification primer mixture, as well as 4.5  $\mu$ l amplification reagent mixture and 3  $\mu$ l genome DNA extraction, were added, respectively, in a total of 20  $\mu$ l reaction system. After centrifugation and mixing up for a short time, PCR instrument was opened to set PCR amplification procedure: pre-degeneration at 37°C 10 min, 95°C 15 min, 96°C 1 min. A total of 32 circulation conducted DNA amplification at 4°C 30 s, 55°C 30 s, 70°C 45 s; the amplification was completed after 60°C 10 min. During the amplification process, parameters were set to make the temperature decrease from 94°C to 55°C at a speed of 0.4°C/s and increase from 55°C to 70°C at a speed of 0.2°C/s, and the total reaction time lasted about 3 h 20 min.

#### *Hybridization*

After PCR product was placed into PCR instrument to degenerate for 5 min at 95°C, it was immersed in ice water mixture for ice-bath for 3 min. 2.5  $\mu$ l PCR product with mixed components from A and B, two amplification systems of the same sample template, was extracted and added into the centrifuge tube with 10  $\mu$ l hybridized buffer solution, mixed up fully and centrifuged at an instant. 14  $\mu$ l hybridized reaction mixture was extracted, added vertically into the microarray area of the chip from wells of the chip. The hybridization box was sealed, horizontally placed into hybridization instrument of 60°C preheating, and hybridized for 60 min at a rotate speed of 5 rpm.

#### *Chip Scrubbing*

The hybridized chip was taken out and placed into chip wash and dry instrument; scrubbing parameters were taken out according to the following procedures: scrubbing solution 1, 42°C 2 min, scrubbing solution 2, 42°C 1 min, twice. It was centrifuged for 2 min at 1000 rpm and tumble-dried.

#### *Chip Scanning*

LuxScan 10K-B microarray chip scanner was opened, and DeafTest software was preheated for 10 min. The sample information was typed in, and the dried chip was placed into the chip card slot of scanner to conduct chip scanning.

#### *Results Interpretation and Explanation*

Testing result was interpreted automatically by discrimination system of hereditary hearing-loss gene testing chip. The interpretation was conducted for nine mutation sites of four mutation genes, respectively. If W detection probe was positive at the same site, the site was interpreted as wild type, which means both sites on two allelic genes do not mutate. If M detection probe was positive, the site was interpreted as homozygote mutation type, which means both sites on two allelic genes mutate. If both W and M detection probe were positive, the site was interpreted as heterozygous mutation type, which means the site on one of the two allelic genes mutates, as for the mutation site of mitochondria 12S rRNA gene. If M detection probe was positive, the site was interpreted as homogeneous mutation type, which means the site on all mitochondria 12S rRNA gene mutate. If both M and W detection probes were positive, the site was interpreted as heterogeneous mutation type, which means the site on some mitochondria 12S rRNA genes mutate.

**Table I.** Distribution of gene mutation in 24 cases of patients with hereditary deafness.

Mutation gene	Mutation site	Positive detection number	Mutation type	Detection rate (%)
GJB2	176 del 16	1	Heterozygous mutation type	4.17
	235 del C	1	Homozygous mutation type	4.17
SLC26A4	299 del AT	2	Heterozygous mutation type	8.33
Mitochondria 12SrRNA	IVS7-2 A>G	1	Heterozygous mutation type	4.17
	1555 A>G	2	Homogeneous mutation type	8.33

### Quality Control

Each gene chip had its own quality control probe, including surface chemistry quality control probe (QC), hybridization positive quality control probe (PC), blank control probe (BC) and negative control probe (NC).

### Results

7 cases of hereditary hearing loss gene mutation carriers were detected in 24 samples, the positive detection rate was 29.17% (there was no case detected with 2 mutation sites) (Table I).

### Discussion

The morbidity of deafness in newborns is about 1-3%, and is increasing with more than 30,000 deaf newborns every year. The disabled people caused by hearing and voice disorder account for about 30% of total disabled people of the country, ranking top among all disabled types, and it is a common disease threatening human health<sup>3</sup>. About 60% deafness is caused by hereditary factors, usually shows up as non-syndromic (NSHI) hearing loss, which only has deafness symptom without accompanied other systematic diseases. Its modes of inheritance are mainly autosomal dominant, recessive, sex linkage and mitochondria matrilineal inheritance. At present, located by NSHI are more than 170, involving ninety genes and more than 1,000 mutation sites<sup>4</sup>. Deaf people in China are mainly related to nine mutation sites of four genes, GJB2, SLC26A4, mtDNA 12s rRNA and GJB3. High-frequency mutation of GJB2, closely related to NSHI, is called susceptibility gene of deafness. It can show up as autosomal dominant and recessive inheritance, and most are recessive inheritance, causing many sporadic affected individuals without family

history. The mutation-carrying rate of GJB2 of normal people is 2.55%. Consequently, even if two people without family relation are married, there is possibility of deafness in their offspring. Therefore, it is necessary to screen deafness genes for young couple so as to better guide marriage and childbirth and reduce the birth of deaf children. Auditory nerve in cochlea of deaf patients with GJB2 gene mutation is normal with good hearing aid effect. Accordingly, transplanting electrical cochlea can greatly improve hearing of these patients<sup>5</sup>. In the current study, four GJB2 gene mutation was detected in 24 patients with a positive rate of 16.67%, including one case of heterozygous mutation type on 176 del 16 site and 299 del AT site, respectively, two cases of 235 del C homozygous mutation type. The detected GJB2 gene mutation constituent ratio was 57.14%. SLC26A4 gene, also called PDS gene, accounting for 14.5% of hereditary hearing loss, which is the second common mutation gene of deaf patients in our country. SLC26A4 gene is closely related to vestibular aqueduct syndrome (EVAS) and Pendred syndrome (PDS). SLC26A4 gene mutation can cause abnormal enlargement of vestibular aqueduct which is a gallery linking cranial cavity and inner ear thereby the change of intracranial pressure may lead to hearing decrease of EVAS patients. Therefore, for children carrying SLC26A4 gene mutation, parents should well supervise them to avoid aggravation caused by external factors such as strenuous exercise, head collision and cold<sup>6,7</sup>. In our study, one case of heterozygous mutation type on IVS7-2 A>G site was detected, the positive rate was 4.17%, the constituent ratio 14.29%, parent of the patient should make necessary prevention and protection. Mitochondria 12S rRNA gene has strict matrilineal inheritance characteristics. The female can pass the mutation gene down to her son and daughter, but only the daughter can pass it down to the next generation. The main

mutation site of this gene is 1555A>G, carriers of this site mutation are abnormally sensitive to aminoglycoside antibiotics. Injected aminoglycoside antibiotics will attack inner ear tissue, even a low dose can also cause tinnitus even severe hearing loss, and usually one injection will lead to deafness. For hereditary susceptible individuals carrying mitochondrial DNA 1555 A>G mutation site, one carrier discovered on average will provide warning and medication guide for more than ten members of maternal line free of disease. In our study, two cases of mitochondria 12S rRNA 1555 A>G homogeneous mutation type were detected, which show that patients and their mothers should be banned from using aminoglycoside antibiotics for their whole life, and such kind of deafness can be prevented. Two cases of homogeneous mutation type were detected with a positive detected rate of 8.33% and a constituent ratio of 28.57%. GJB3 gene is the first Chinese native deafness-related gene discovered by Xia et al<sup>8</sup> and is one of the commonly seen mutation genes of patients with acquired high-frequency phonosensitive nerve deafness. It mainly shows up as progressive high-frequency hearing phonosensitive nerve deafness after speaking. The mutation rate of GJB3 is lower than that of other common virulence genes of hereditary hearing loss<sup>9</sup>, a conclusion consistent with our study. No mutation of sites of this gene in 24 samples was detected. However, whether there is mutation impact of other genes involved in the influence of GJB3 gene on occurrence of hereditary hearing loss, is a feature that needs further study.

### Conclusions

Gene chip technology is a biological technology developing with the genome project with nucleic acid hybridization as its basic principle. Specific primer of gene sites with Tag label sequence was adopted with human genome DNA as template. Amplification and fluorescence labeling were conducted on the gene segments of relevant gene sites. These were hybridized with general gene chip that can identify the corresponding label sequence. The information of gene expression or mutation was got by scanning the chip and analyzing the data. Aimed at wild type and mutation type of nine mutation sites of four genes of common hereditary hearing loss among Chinese people to design primer and probe, microarray

chip method can detect the result of wild type and mutation type of nine sites simultaneously. Each chip can detect four samples at the same time, and several chips can be detected each time. With the advantages such as rapidness, high-accuracy, high-throughput, simultaneousness and high-efficiency, it can meet the detection requirements of clinical deafness gene. However, due to the complexity of human genome and high-heterogeneity of deafness gene, gene chip technology can only detect known mutation, and cannot discover unknown mutation. At present, mutation sites of only a few genes, GJB2, SLC26A4, mitochondria 12S rRNA and GJB3, can be detected, but other non hotspot mutation with low carrying rate or unknown spectrum of mutation cannot be detected. This causes misinterpretation of data or missed diagnosis. Therefore, the inspection results should be cautiously explained for the wild type patients<sup>9-11</sup>.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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