

Application of gene detection technique in the antenatal diagnosis of hereditary hearing loss

Y. FANG¹, M.-S. GU¹, F. SUO¹, C.-X. WANG¹, X.-H. LIU¹, F.-M. LIU²

¹The Affiliated Maternal and Child Health Care Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China

²Department of Obstetrics and Gynecology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China

Abstract. – **OBJECTIVE:** Gene chip and gene sequencing techniques were used to detect the main pathogenic genes in pregnant women with hereditary hearing loss.

PATIENTS AND METHODS: From May 2015 to May 2016, 1080 pregnant in Xuzhou Maternal and Child Health Hospital were enrolled in this study. Women age range was 18 to 40 years. 4 genes and 9 mutation sites, including 4 sites (35delG, 176, 235delC and 299) in GJB2 gene, 2 sites (2168A>G and IVS-7-2A>G) in SLC26A4 (PDS) gene, 2 sites (1494C>T and 1555A>G) in 12s rRNA gene and 1 site (538C>T) in GJB3 gene, were detected using the GeeDom® 9-item hereditary hearing loss gene detection kit. Deafness genes in the husband of the pregnant woman with GJB2 and SLC26A4 positive gene mutations were verified using Sanger sequencing. Fetuses with the same deafness genes as their parents were diagnosed before delivery using amniocentesis.

RESULTS: 48 patients (4.45 %) were detected positive for hereditary hearing loss. Most of them (28 cases) were identified with GJB2 gene mutation (1 case with 176 site mutation, 22 cases with 235delC site mutation and 5 cases with 299 site mutation). We had 15 cases of the SLC26A4 gene mutation (3 cases of 2168A>G site mutation and 12 cases of IVS-7-2A>G site mutation), 2 cases of 538C>T site mutation of GJB3 gene and 3 cases of 1555A>G site mutation of 12s rRNA gene.

CONCLUSIONS: The gene detection technique has a great health-economic significance in screening the main pathogenic genes involved in the hereditary hearing loss.

Key Words:

Gene chip, Gene sequencing, Hereditary hearing loss, GJB2 gene, SLC26A4(PDS) gene, 12s rRNA gene, GJB3 gene.

Introduction

Deafness is the most common sensorineural dysfunction, and 80% to 90% of deafness cases are congenital¹. More than 80 genes have been identified to be associated with the hereditary deafness, including 67 autosomal dominant/recessive genetic genes, 3 X-linked genes and 7 mitochondrial genes². The hot-spot mutation in deafness genes is mainly the single-gene biallelic mutation³. Currently, DNA sequencing is the “gold standard” for clinical molecular diagnosis⁴. The epidemiological survey in China found that the prevalence rate of congenital deafness is about 2% to 2.5‰ with an increasing trend year by year⁵. GJB2, SLC26A4 (PDS), 12s rRNA and GJB3 genes have the highest mutational load⁶. GJB2 gene is the most common gene causing the non-syndromic hereditary hearing loss⁷. In mitochondrial 12s rRNA genes, A1555G site mutation is the most common maternal deafness gene associated with the aminoglycoside drug-induced deafness and non-syndromic hereditary hearing loss⁸. In this study, the gene chip and gene sequencing technique were used to detect the main pathogenic genes in 1080 cases of pregnant women with hereditary hearing loss.

Patients and Methods

From May 2015 to May 2016, 1080 pregnant in Xuzhou Maternal and Child Health Hospital were enrolled in this study. Women age range was 18 to

40 years. This study was authorized by the Hospital Ethics Committee with the informed consent of patients and their families.

Methods

Gene micro-array chip inspection

Fasting peripheral venous blood samples (2 ml) were collected and stored at 4°C. 300 µl whole blood was transferred into 1.5 ml EP tube. DNA extraction kit (Jiangsu Biyuntian Science and Technology Co., Ltd) was used to extract DNA. DNA was dissolved in 50 µl of TB and DNA concentration and purity was determined by ultraviolet spectrophotometry (Europe BV, Venlo, Netherlands). PCR amplification system was prepared according to the description of hereditary hearing loss gene detection kits (Beijing Boao Biotechnology Co., Ltd). Samples were divided in two and transferred into two 200 µl centrifuge tubes (tubes A and B). Primers A1 and B1 were naturally thawed at room temperature, and the amplification reagents A2 and B2 were shaken evenly. A1 (12.5 µl)+A2 (4.5 µl)+DNA (3 µl) was added into Tube A and B1 (12.5 µl)+B2 (4.5 µl)+DNA (3 µl) was added into Tube B1 (total volume=20 µl). One negative reference group was prepared using the same method. The reaction condition was as follows: 37°C for 10 min, 95°C for 15 min, 96°C for 1 min, 94°C for 30 s, 55°C for 30 s, 70°C for 45 s (32 cycles) and 60°C for 10 min. PCR products were left on ice for 3 min for hybridization. The hybridization instrument, kit and hybridization solution were preheated to 50°C. Samples were added into 200 µl centrifuge tube and 15 µl of hybridization solution was added. 10 µl into the tube and 2.5 µl into the amplification system A and B. 14.5 µl of solution were transferred to the chip and sealed. It was placed in the hybridization instrument for 60 min under 50°C and 5 r/min. Then, it was washed, dried and scanned. Deaf Test software was used to set up the QC (quality control probe), PC (positive probe), BC (blank control probe), NC (negative control probe) and W (wild type) and M (mutant type) probes for 9 detection sites. The specification of chip: (76.20 ± 0.50) mm × (25.40 ± 0.25) mm × (1.00 ± 0.10) mm, scanning area: 22 mm × 72 mm, detection sensitivity: ≤ 0.1 fluorescent molecules/µm².

Sanger Sequencing

The deafness gene sequencing was conducted on husbands with pregnant women with GJB2 and

SLC26A4 gene positive mutant. PCR amplification was conducted on hot-spot mutant gene locations, and the one-generation sequencer 3130X1 and Sanger sequencing were used for analysis.

Antenatal Diagnosis of Amniocentesis

Pregnant women's amniotic fluid samples were extracted, and the cast-off cells from fetus were collected and cultured under specified conditions. DNA in the amniotic cell was directly extracted for the gene diagnosis of hereditary hearing loss. Fetuses with parents with the same genotypes were diagnosed using amniocentesis.

Statistical Analysis

We used SPSS19.0 software (SPSS Inc., Chicago, IL, USA) for our statistical analysis. The measurement data was presented as mean ± standard deviation, and the enumeration data was presented as the case number (%).

Results

48 patients (4.45%) were detected positive for hereditary hearing loss. Most of them (28 cases) were identified with GJB2 gene mutation (1 case with 176 site mutation, 22 cases with 235delC site mutation and 5 cases with 299 site mutation). We had 15 cases of the SLC26A4 gene mutation (3 cases of 2168A>G site mutation and 12 cases of IVS-7-2A>G site mutation), 2 cases of 538C>T site mutation of GJB3 gene and 3 cases of 1555A>G site mutation of 12s rRNA gene. There were three couples who were diagnosed with the positive mutation in the same gene, including 2 pairs of GJB2 gene (mutation site in 235delC) and 1 pair of SLC26A4 gene (mutation site in IVS-7-2A>G). Amniocentesis results revealed that in 1 case GJB2 gene homozygous was mutated, in 1 case GJB2 gene heterozygous was mutated and in 1 case SLC26A4 gene heterozygous was mutated (Figure 1).

Discussion

The sensitivity and accuracy of the postnatal hearing test are low, and the late-onset, progressive and drug-sensitivity deafness cannot be screened early. But the gene detection technique can sequence and screen the deafness genes, and the single nucleotide polymorphism and monoclonal amplification method can be used to define the mutation sites. This can explain the mechanism

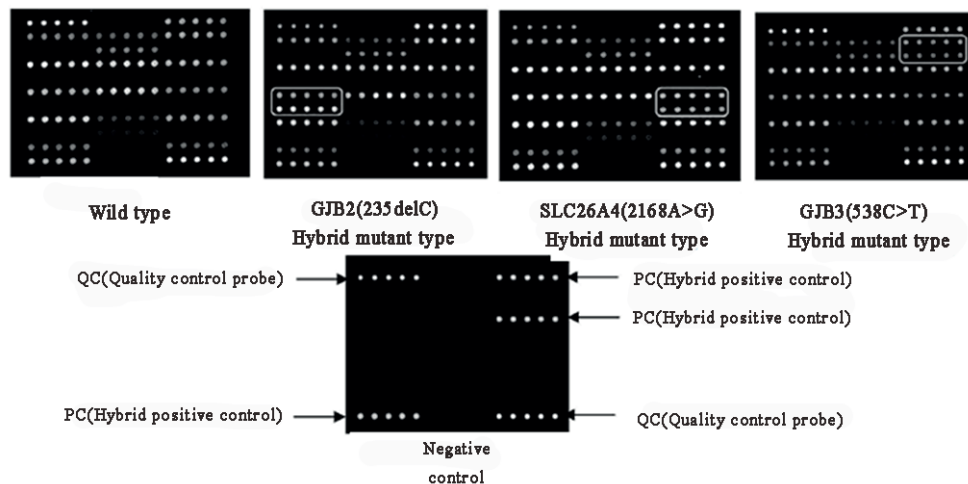


Figure 1. Gene chip scan.

of hereditary hearing loss from the perspective of genes and molecules, screen the high-risk pregnant women and fetuses before the delivery, and avoid the birth of children with congenital deafness⁹. In this study we used a large sample population-based screening and discovered that the positive mutation rate of hereditary hearing loss was about 4.45%. Our findings were consistent with the results obtained from previous reports. GJB2 and SLC26A4 (PDS) genes had higher mutation rates (58.33% and 31.25 % respectively), and they were two kinds of mutant genes with the higher genetic load. Aminoglycoside drug should be avoided for the pregnant women and fetuses carrying 12s rRNA for tertiary prevention.

Sanger sequencing is characterized by the low sequencing efficiency and high cost¹⁰. The conventional digestion, hybridization and TaqMan probe only analyze the mutation hot spots, but the detection throughput does not increase¹¹. Micro-array chips can detect 9 genes associated with the deafness simultaneously, characterized by the simple method, high accuracy and good repeatability¹². Boao deafness gene detection chip approved by CFDA plays an important role in the gene screening of hereditary hearing loss¹³. In addition, APEX, Invader and other chip technologies can detect hundreds of mutation sites simultaneously with high throughput¹⁴, but these methods have a long cycle and a high cost with limited detection ability for other types of mutations except the point mutation¹⁵. We discovered that the mutation type of deafness gene includes the point mutation, copy number variation, small segment insertion deletion, and structure variation¹⁶. The

single detection technique has a large limitation, and the pathogenesis of a great number of deafness genetic variation is still unclear¹⁷.

The latest high-throughput sequencing technique can sequence millions of DNA molecules in parallel simultaneously, looking for mutation sites and identifying the pathogenic genes. This has become the most effective method for monogenic disease study¹⁸. Rehman et al¹⁹ used NimbleGen chip trapping technique, combined with 454 sequencing platforms, and discovered that TPRN homozygous nonsense mutation in 2.9 Mb of DFNB79 site was the new pathogenic gene of deafness. Sirmaci et al²⁰ showed that MASP1 gene was another pathogenic mutation.

Conclusions

The gene detection technique has a great health-economic significance in screening the main pathogenic genes involved in the hereditary hearing loss.

Conflict of interest

The authors declare no conflicts of interest.

References

- 1) COHEN M, PHILLIPS JA 3rd. Genetic approach to evaluation of hearing loss. *Otolaryngol Clin North Am* 2012; 45: 25-39.
- 2) HILGERT N, SMITH RJ, VAN CAMP G. Forty-six genes causing non-syndromic hearing impairment: whi-

- ch ones should be analyzed in DNA diagnostics. *Mutat Res* 2009; 681: 189-196.
- 3) ZHANG J, WANG P, HAN B, DING Y, PAN L, ZOU J, LIU H, PANG X, LIU E, WANG H, LIU H, ZHANG X, CHENG X, FENG D, LI Q, WANG D, ZONG L, YI Y, TIAN N, MU F, TIAN G, CHEN Y, LIU G, ZHANG F, YI X, YANG L, WANG Q. Newborn hearing concurrent genetic screening for hearing impairment-a clinical practice in 58397 neonates in Tianjin, China. *Int J Pediatr Otorhinolaryngol* 2013; 77: 1929-1935.
- 4) HÄKLI S, LUOTONEN M, SORRI M, MAJAMAA K. Audio-logical follow-up of children with the m.1555A>G mutation in mitochondrial DNA. *Audiol Neurotol* 2013; 18: 23-30.
- 5) HILDEBRAND MS, DeLUCA AP, TAYLOR KR, HOSKINSON DP, HUR IA, TACK D, McMORDIE SJ, HUYGEN PL, CASAVANT TL, SMITH RJ. A contemporary review of AudioGene audioprofiling: a machine-based candidate gene prediction tool for autosomal dominant nonsyndromic hearing loss. *Laryngoscope* 2009; 119: 2211-2215.
- 6) RODRIGUEZ-PARIS J, PIQUE L, COLEN T, ROBERSON J, GARDNER P, SCHRIJVER I. Genotyping with a 198 mutation arrayed primer extension array for hereditary hearing loss: assessment of its diagnostic value for medical practice. *PLoS One* 2010; 5: e11804.
- 7) WANG HY, ZHAO YL, LIU Q, YUAN H, GAO Y, LAN L, YU L, WANG DY, GUAN J, WANG QJ Identification of two disease-causing genes *tjp2* and *gjb2* in a chinese family with unconditional autosomal dominant nonsyndromic hereditaryhearing impairment. *Chin Med J* 2015; 128: 3345-3351.
- 8) ZHU Y, HUANG S, KANG D, HAN M, WANG G, YUAN Y, SU Y, YUAN H, ZHAI S, DAI P. Analysis of the heteroplasmy level and transmitted features in hearing-loss pedigrees with mitochondrial 12S rRNA A1555G mutation. *BMC Genet* 2014; 15: 26.
- 9) GIROTTI G, ABDULHADI K, BUNIELLO A, VOZZI D, LICASTRO D, D'EUSTACCHIO A, VUCKOVIC D, ALKOWARI MK, STEL KP, BADII R, GASPARINI P. Linkage study and exome sequencing identify a BDP1 mutation associated with hereditary hearing loss. *PLoS One* 2013; 8: e80323.
- 10) YING Y, XIE JJ, WANG HP, MAO P, ZHOU W, YANG Z, CHEN XY. Whole genome sequencing identified new somatic mutations for chronic myelomonocytic leukemia. *Eur Rev Med Pharmacol Sci* 2016; 20: 278.
- 11) CLARK MJ, CHEN R, LAM HY, KARCZEWSKI KJ, CHEN R, EUSKIRCHEN G, BUTTE AJ, SNYDER M. Performance comparison of exome DNA sequencing technologies. *Nat Biotechnol* 2011; 29: 908-914.
- 12) LIM BG, CLARK RH, KELLEHER AS, LIN Z, SPITZER AR; Pediatrix SoundGene® Study Group Principal Investigators and Contributors. Utility of genetic testing for the detection of late-onset hearing loss in neonates. *Am J Audiol* 2013; 22: 209-215.
- 13) SANTOS-CORTEZ RL, LEE K, GIESE AP, ANSAR M, AMIN-UD-DIN M, REHN K, WANG X, AZIZ A, CHIU I, HUSSAIN ALI R, SMITH JD; University of Washington Center for Mendelian Genomics., Shendure J, Bamshad M, Nickerson DA, Ahmed ZM, Ahmad W, Riazuddin S, Leal SM. Adenylate cyclase 1 (ADCY1) mutations cause recessive hearing impairment in humans and defects in hair cell function and hearing in zebrafish. *Hum Mol Genet* 2014; 23: 3289-3298.
- 13) BEHLOULI A, BONNET C, ABDI S, BOUAITA A, LELLI A, HARDELIN JP, SCHIETROMA C, ROUS Y, LOUHA M, CHEKNANE A, LEBDI H, BOUDJELIDA K, MAKRELOUF M, ZENATI A, Petit C EPS8, encoding an actin-binding protein of cochlear hair cell stereocilia, is a new causal gene for autosomal recessive profound deafness. *Orphanet J Rare Dis* 2014; 9: 55.
- 14) ZHENG J, MILLER KK, YANG T, HILDEBRAND MS, SHEARER AE, DeLUCA AP, SCHEETZ TE, DRUMMOND J, SCHERER SE, LEGAN PK, GOODYEAR RJ, RICHARDSON GP, CHEATHAM MA, SMITH RJ, DALLOS P. Carcinoembryonic antigen-related cell adhesion molecule 16 interacts with α -tectorin and is mutated in autosomal dominant hearing loss (DFNA4). *Proc Natl Acad Sci USA* 2011; 108: 4218-4223.
- 16) GNIRKE A, MELNIKOV A, MAGUIRE J, ROGOV P, LePROUST EM, BROCKMAN W, FENNELT T, GIANNOUKOS G, FISHER S, RUSS C, GABRIEL S, JAFFE DB, LANDER ES, NUSBAUM C. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nat Biotechnol* 2009; 27: 182-189.
- 17) HUEBNER AK, GANDIA M, FROMMOLT P, MAAK A, WICKLEIN EM, THIELE H, ALTMÜLLER J, WAGNER F, VIÑUELA A, AGUIRRE LA, MORENO F, MAIER H, RAU I, GIESSELMANN S, NÜRNBERG G, GAL A, NÜRNBERG P, HÜBNER CA, DEL CASTILLO I, KURTH I. Nonsense mutations in SMPX, encoding a protein responsive to physical force, result in X-chromosomal hearing loss. *Am J Hum Genet* 2011; 88: 621-627.
- 18) KOTHIAL P, COX S, EBERT J, HUSAMI A, KENNA MA, GREINWALD JH, ARONOW BJ, REHM HL. High-throughput detection of mutations responsible for childhood hearing loss using resequencing microarrays. *BMC Biotechnol* 2010; 10: 10.
- 19) REHMAN AU, MORELL RJ, BELYANTSEVA IA, KHAN SY, BOGER ET, SHAHZAD M, AHMED ZM, RIAZUDDIN S, KHAN SN, RIAZUDDIN S, FRIEDMAN TB. Targeted capture and next-generation sequencing identifies C9orf75, encoding taperin, as the mutated gene in non-syndromic deafness DFNB79. *Am J Hum Genet* 2010; 86: 378-388.
- 20) SIRMACI A, WALSH T, AKAY H, SPILIOPOULOS M, SAKALAR YB, HASANEFENDIOGLU-BAYRAK A, DUMAN D, FAROOQ A, KING MC, TEKIN M. MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of Carnevale, Malpuech, OSA, and Michels syndromes. *Am J Hum Genet* 2010; 87: 679-686.