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Establishment of Preimplantation Genetic Diagnostic Technique for Hereditary Hearing Impairment

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Abstract

Objective*: The aim of this study is to establish a method by single nucleotide polymorphism detection from a single cell using the whole genomic amplification and restriction fragment length polymorphism-PCR (RFLP-PCR).*

Methods*: Genomic DNA was first prepared and wholly amplified from 80 biopsied blastomeres using the Sure Plex DNA Amplification System. Then, PCR was carried out on a single blastomere for the most frequent mutations, 235delC in GJB2 gene and 1555A>G in 12SrRNA gene.*

Results*: For GJB2, 62 samples were successfully diagnosed, and all samples were carried out for 12S rRNA. , The PCR products were digested with special restriction enzymes to further determine genetic mutation. Finally, the CYP2B6 genotype was determined by the RFLP-PCR.*

Conclusion*: This study provides the first attempt of RFLP-PCR technology applications in determining genetic deafness, and shows the reliability of the technology in IVF clinical settings.*

Keywords: *Deafness gene, Single cell; the polymerase chain reaction-restriction fragment length polymorphisms ; Single nucleotide polymorphism(SNP); Vertical transmission; whole genome amplification (WGA)*

I. Background

Congenital hearing loss (HL) is one of the most common sensory impairments in humans, occurring in 1 per 1,000 infants [1]. It is also the most common sensory disability in humans [2]. Hearing disability seriously affects the quality of life of human beings since humans obtain 30% of their sensory information from hearing. The onset of HL in early childhood can have serious detrimental effects on language acquisition [3]. The second sampling survey for the disabled in our country in 2006 indicates that there are 27.8 million people who live with hearing impairment, accounting for 33.52% of the total number of persons with disabilities, with an increase at an annual rate of 30,000 deaf children. It is important to note that more than 50% of these hearing loss cases are attributable to genetic factors [4]. Genetic deafness is divided into two categories: syndrome and non-syndrome. Syndrome deafness accounts for 70% of genetic hearing loss and is the most common sensorineural deafness. At the present time, the best provision for congenital HL is early diagnosis and continuous management. Due to the high prevalence and clinical impact of this condition, the CDC have advocated for early detection through genetic diagnosis as the most effective supportive therapy in the prevention and treatment of hereditary HL. By common genetic screening, 40% of these cases can be diagnosed. It is also found that GJB2, SLC26A4 and mitochondrial gene mutations are the main genetic risk factors in deaf disease of Chinese. GJB2 encodes the gap junction connexin 26 protein, one of the most important members of the connexin family, and is expressed in epithelial and connective tissue cells in the inner ear. This gap junction protein serves as the structural basis for recycling potassium ions back to the endolymph of the cochlear duct after sensory hair cell stimulation [5]. The loss of connexin 26 protein is expected to lead to disrupted potassium ion flow and may potentially lead to hearing loss [6].The most frequent pathogenic mutations in the gene GJB2 are known to be a major cause of hereditary pre-lingual non-syndrome hearing loss [7,8] . SLC26A4 (PDS) gene mutation, an autosomal recessive inheritance patterns, is a high-risk mutation that lead to China's National People's Congress vestibular conduit syndrome. Mitochondrial(MT)-RNR1 gene mutations is the main cause of

drug-induced non matriarchal hereditary deafness syndrome type in which deafness is bilateral in varying degrees and is associated with the use of aminoglycoside drugs. Bilateral sensorineural deafness that is caused by mild collisions such as falling in bed, or children's play and sports activities, is an important cause of late-onset deafness. As we know, preimplantation genetic diagnosis (PGD) can help new families with known heritable syndromes to avoid the birth of diseased infant.

The 235delC mutation of GJB2 causes a loss of targeting activity to the cell membrane and severe deterioration of gap junction activity [9]. GJB2 gene defects is the main causes of congenital deafness, an autosomal recessive inheritance patterns, leading to 50% of autosomal recessive hereditary deafness with non-syndrome type. Heterozygous mutations in GJB2 can also cause autosomal dominant deafness, or also known as DFNA3A [10]. 235delC is the most important deletion mutation in GJB2, since its allele frequency is 24.1%, which accounts for 63.3% of all mutations. Furthermore, in some groups, the m.1555A>G mutation in the MT-RNR1 gene has been identified as a relatively common determinant in deafness in Spanish and far eastern populations [11, 12]. This mutation has also been shown to cause aminoglycoside ototoxicity [13].

II. Materials And Methods

Discarded embryos were collected from the Reproductive Center of Yangzhou Subei Hospital in Jiangsu Province, including 3PN and other abnormal fertilized embryos. An informed consent with patients agreeing to disposed embryos used for scientific research was signed. On fertilization Day 3, holes were punched into discarded embryos using a biopsy needle with an internal diameter of 30-35 μm and a single blastomere was drawn under the laser rupture of membranes instrument. Each biopsied blastomere was gently put into a 200 μl PCR tube containing 2.5 μl of lysis buffer. The genomic DNA of a single blastomere was amplified using the SurePlex DNA Amplification System. After single cell genomes were amplified, PCR was carried out for 235delC in GJB2 and 1555A>G in 12S rRNA. (The sequences of the primers used in the PCR reaction are presented in Table 1.) Amplification for the two genes was as follows: denaturation step of 95°C for 5 mins, followed by 34 cycles of denaturation at 94°C for 45 seconds, annealing at 62°C for 1 min, extension at 72°C for 1 min, and then a final extension step of 72°C for 10 min. RFLP was carried out for GJB2 and 12S rRNA. The PCR products were fragmented by the restriction enzyme Apa I (Biolabs Inc., R0114S) for GJB2 and Bsma I (Biolabs Inc., R0529S) for 12S rRNA. The total PCR reaction mixture of 20 μl contained: 2 μl buffer, 10 μl (for GJB2) or 3 μl (for 12S rRNA) PCR production, 1 μl restriction enzyme, and 7 μl or 14 μl distilled water and was incubated at a temperature of 25°C (for GJB2) or 55°C (for 12S rRNA) for 30 min. Lastly, 10 μl production was observed by 2 % agarose gel electrophoresis. The amplification primers of the two kinds of deafness gene sequences are shown in Table 1.

III. Results

WGA was carried out using 80 discarded embryos. All single blastomeres were successfully amplified. The 12S rRNA gene was well amplified in all samples and 62 samples were amplified for GJB2 shows in Figure 1. All PCR products were successfully fragmented by special restriction enzymes as shown in Figure 2. At the same time, sequencing results show that the base sequences of sites matching the restriction enzyme sites are consistent to the results of RFLP-PCR in 12Sr RNA or GJB2 as shown in Figure 3.

IV. Discussion

The onset of HL in early childhood can have serious adverse effects on language acquisition. At the present time, the best management for hereditary HL is prevention through early diagnosis and continuous monitoring. During the past years, major progress has been made in the identification of genes associated with deafness. Molecular genetic testing offers a new technique to determine the genetic causes of HL in a neonate, and the availability of such tests is increasing rapidly. Early prenatal diagnosis technology, namely human PGD, is the regular prenatal diagnosis. Early embryo in the uterus before biopsy and genetic analysis, selecting embryos implanted into the uterus cavity without genetic disease, to obtain normal fetal diagnostic methods, and effectively avoiding regular prenatal diagnosis may face the choice of the dangers of therapeutic abortion and physical and mental damage that accompany the ethical conflict. Most of the genetic deafness belongs to the strong heterogeneity of single gene disease, which is in accordance with PGD medical indications. To date, conventional gel-based and direct sequencing techniques have been used to screen for known deafness-related genes.

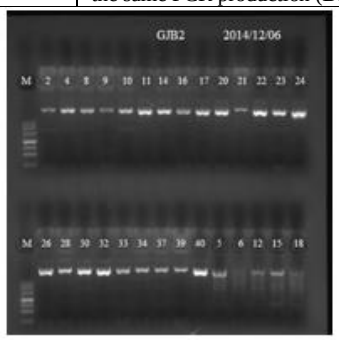
GJB2 mutation (235delC) and 1555A>G mutation are important causes of sensorineural non-syndromic hearing loss in China. MT-RNR1 gene mutations cause the syndrome type of matrilineal hereditary deafness. Improving prevention interventions and diagnostic measures, as well as effectively avoiding births of infants with these heriditary syndromes is the aurists' objective by now. In this study, we explored a simple diagnostic technique for some mutations, which are known to be responsible for the major etiologies of hereditary HL in Chinese neonates. In this study, not all the samples were diagnosed for GJB2. We believe that the main reason for this is the quality of these discarded embryos. Importantly, this technology offers many

advantages, such as simple operation and low time requirement. The entire procedure can be completed in six hours, in which time, embryos could be transplanted in the fresh cycle and get ready for the IVF..

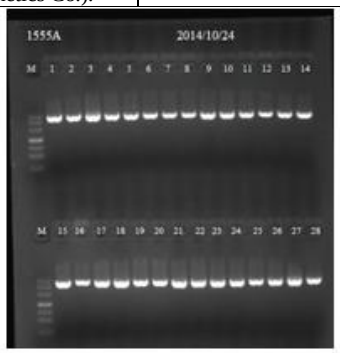
V. Figures And Tables

Table 1

Gene	Primers	Product length
GJB2[1]:	F: 5'-TGAGCACGGGTTGCCTCATC-3' R:5'-TGCTTACCCAGACTCAGAGAA-3'	844bp
12SrRNA	F: 5'-GCAGTAAACTAAGAGTAGAGT-3' R: 5'-GGCTCTCCTTGCAAAGTTAT-3'	463bp
	Sequencing Sequencing was performed to verify the RFLP method using the same PCR production (Beijing Microread Genetics Co.).	



The amplified specific product of GJB2.(844bp)



The amplified specific product of 12S rRNA.(463bp)

Figure 1 The results of PCR： M: marker; 1, 2 and so on was the number of sample.

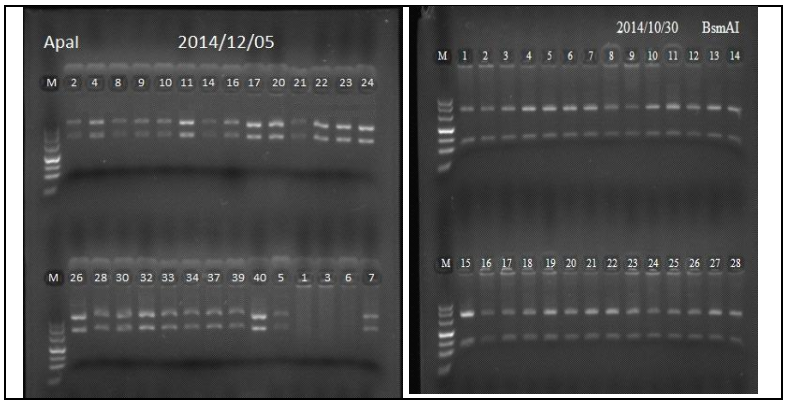


Figure 2: The product of PCR was digested by enzymes respectively: GJB2(844bp) becomes the bands of 511bp and 333bp； 12S rRNA(463bp) becomes the bands of 339bp and 124bp. M: marker, 1, 2 and so on was the number of sample.

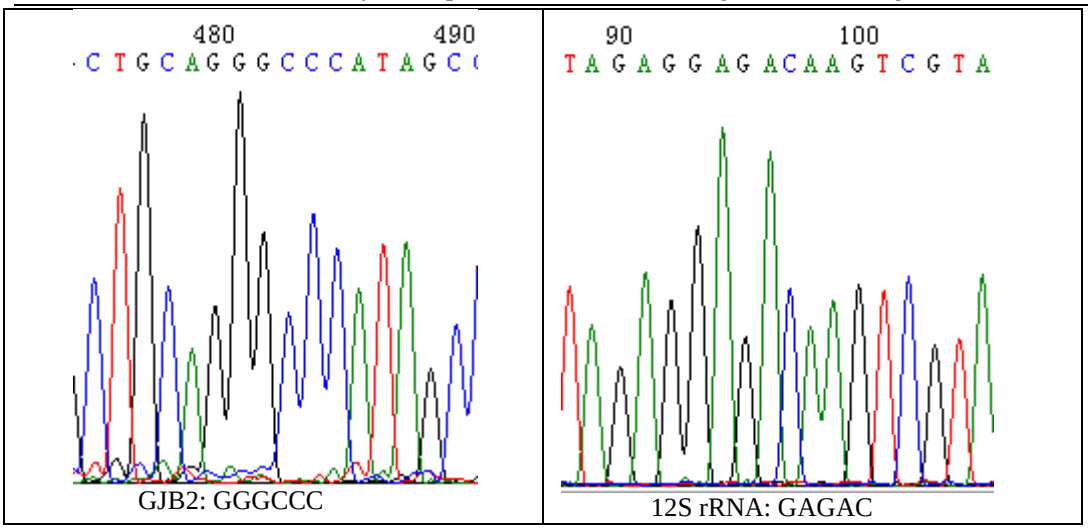


Figure 3: The results of sequencing:GJB2 has the bases of "GGGCCC" which was the restriction enzyme site of Apa I; 12S rRNA has the bases of "GAGAC"which was the restriction enzyme site.

VI. Conclusion

This study provides the first attempt of RFLP-PCR technology applications in determining genetic deafness, and shows the reliability of the technology in IVF clinical settings. Combining RFLP-PCR technology with WGA provides a rapid, sensitive and accurate approach to detect embryonic congenital hearing loss to block hereditary hearing impairment.

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