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Specific short tandem repeat loci detection in prenatal diagnosis of fetal chromosomal diseases

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ABSTRACT

It was to investigate the value of quantitative fluorescence PCR (QF-PCR) for the selection of specific short tandem repeat (STR) in prenatal diagnosis of fetal chromosomal diseases. Amniotic fluid (AF) and villus samples were obtained from 80 pregnant women at 16-20 weeks of gestation, and venous blood samples were obtained from 60 normal individuals to extract and prepare peripheral blood chromosome, AF cell chromosome, and villus cell chromosome samples for specific STR locus detection. It showed that the area ratio of AMX peak to AMY peak in the Genescan typing map of peripheral blood DNA of normal males was close to 1:1, while the Genescan typing map of peripheral blood DNA of normal females had only AMX peak and no AMY peak. Normal heterozygous individuals had an area ratio between 1 and 1.45 for venous blood, 1.002 and 1.27 for villous samples, and 1 and 1.35 for AF samples. The karyotype of a male fetus was 46, XY, inv [9] (p11: q13), and the structure of fetal chromosome 9 was inverted (interarm), and the site of structural inversion was band 1 in the short breech 1 region and band 3 in the long arm 1 region of chromosome 9. It suggested that QF-PCR can effectively identify the normal human body and cases by selecting specific STR locus detection, which has a good application value for prenatal diagnosis of fetal chromosomal diseases.

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Introduction

Chromosomal diseases refer to diseases caused by chromosomal abnormalities, and more than 100 chromosomal diseases have been found, and they can often cause reproductive dysfunction such as multiple abortions, malformations and infertility, abnormal secondary sexual characteristics, external genital hermaphroditism, congenital multiple malformations and mental retardation, and cancer tumors in clinical practice (1-3). The most common chromosomal abnormalities in prenatal diagnosis are numerical chromosomal, structural chromosomal, and microstructural chromosomal abnormalities. Among them, numerical chromosomal abnormalities are the most important in clinical practice, of which 13, 21, and 18, X, and Y chromosomal abnormalities are the most common, accounting for more than 95% of chromosomal aneuploidy aberrations (4,5). These chromosomal abnormalities account for 80% to 90% of all chromosomal diseases, such as 21-trisomy, 18-trisomy, 13-trisomy, and sex chromosome abnormality syndromes. According to statistics, there are about 100,000 newborns born with chromosomal abnormalities each year in China, and 0.3% of live infants have chromosomal abnormalities (6). Common causes of chromosomal abnormalities are ionizing radiation, exposure to chemicals, microbial infection, and genetics. Fetal chromosome examination during pregnancy can detect fetal chromosomal abnormalities early and gain time for early diagnosis and early intervention, which is an important measure to implement secondary prevention of birth defects and implement prenatal and postnatal care (7). Therefore, it is very important to popularize prenatal screening and prenatal diagnosis of chromosomal diseases to reduce the occurrence of birth defects.

There are many methods for early screening of clinical fetal chromosomal diseases, such as Down syndrome screening, noninvasive DNA screening, and amniocentesis (8). Down syndrome screening refers to drawing the mother's peripheral blood in the first and second trimesters, measuring the corresponding biochemical markers, synthesizing various information such as gestational age, maternal age, and weight, and calculating whether the fetus has the risk of chromosomal abnormalities through professional screening software, which is suitable for all singleton and twin pregnant women (9-11). Amniocentesis is the acquisition of fetal cells for cell culture and karyotype analysis by amniocentesis or chorionic villus sampling or umbilical cord blood puncture, which is currently the gold standard for prenatal diagnosis of fetal chromosomal diseases, but 100% success can't be ensured and chromosomal micros-

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tructural changes, monogenic genetic diseases, polygenic genetic diseases, environmental and drug-induced intrauterine dysplasia, low proportion chimerism, and maternal contamination can't be completely excluded (12,13). At present, noninvasive prenatal testing (NIPT) is considered by many to be a means of prenatal diagnosis of chromosomal diseases that can replace amniocentesis (14). NIPT is performed by collecting peripheral blood from pregnant women, extracting free DNA from the fetus, and using next-generation high-throughput sequencing combined with bioinformatics analysis to derive the risk rate of chromosomal aneuploidy disease in the fetus. It has the advantages of non-invasiveness, a large detection range, and a high detection rate (15,16).

Short tandem repeat (STR), also known as microsatellite DNA, refers to DNA sequences that are repeated in series in a few nucleotides (1 to 6, or more) (sequences are usually repeated 5 to 50 times). Compared with other DNA sequences, these sequences have a higher mutation rate, thus bringing a high degree of genetic diversity (17). STR is species-specific and suitable for multiplex amplification, degradation, and detection of trace samples, which has been widely used in the fields of genetic mapping of the human genome, cancer diagnosis, kinship analysis, and forensic individual identification (18). Therefore, amniotic fluid (AF) and villus samples were obtained from 80 pregnant women at 16-20 weeks of gestation who visited the Prenatal Diagnosis Center of Maternal and Child Health Hospital Affiliated with Nantong University from October 2020 to November 2022, and venous blood samples were obtained from 60 normal unrelated individuals. Peripheral blood chromosome samples, AF cell chromosome samples, and villus cell chromosome samples were extracted and prepared for the detection of specific STR loci by quantitative fluorescence PCR (QF-PCR). The value of QF-PCR to select specific STR detection for the prenatal diagnosis of fetal chromosomal diseases was deeply explored by analyzing the PCR results.

Materials and Methods

Study subjects

AF and villus samples were collected from 80 pregnant women at 16-20 weeks of gestation in the Prenatal Diagnosis Center of Maternity and Child Health Care Hospital from October 2020 to November 2022. Among them, pregnant women were 21-40 years old, with a mean age of 26.85 ± 7.37, a mean height of 155.68 ± 12.42, and an average gestational age of 18.55 ± 4.39. Venous blood samples were obtained from 60 people.

Rapid DNA extraction method from whole blood

The extraction steps were as follows: (I) 1 mL of ethylenediaminetetraacetic acid anticoagulated blood samples were placed in four centrifuge tubes (1.5 mL capacity), 200 μL in each tube. 300 μL dodecyl trimethyl ammonium bromide solution was put in each centrifuge tube, mixed well, and placed in a water bath at 60℃ for 5 min; (II) 500 μL chloroform was added into each tube, inverted, and mixed well. Centrifugation at 4℃ for 10 min, the supernatant was transferred to a new centrifuge tube, the addition of cetyltrimethylammonium bromide solution to each centrifuge tube. It was mixed well, letting it stand at 4℃ for 20 min, and centrifuged for another 10 min. Then, 1,000 μL of a mixed solution of 1.2 mmol/L NaCl and absolute ethanol was added into each tube. It was mixed well and centrifuged at 4℃ for 10 min; (III) The precipitate was extracted. It was centrifuged twice again, and the finally obtained precipitate was dissolved by adding TE buffer solution (Tris-EDTA buffer solution), a water bath at 37℃ for 40 min, and stored in a -20℃ refrigerator.

Routine DNA extraction method from whole blood

The extraction steps: (I) 5 mL venous blood samples were drawn, mixed with 1 mL of glucose citrate solution, and centrifuged at 3,000 rpm for 10 min. The supernatant was removed and it was washed with 10 mL of normal saline and centrifuged at 3,000 rpm for 10 min; (II) the final obtained pellet was dissolved with a 5-fold volume of hemolytic reagent and mixed well to stand for 10 min, and it was centrifuged at 3,000 rpm for 20 min after the solution became transparent; (III) 5 mL of DNA extraction buffer and 5 μL of 20 μg/μL RNase were added after the supernatant was removed to react at 37℃ for 60 min. 15 mg/mL protease was put and reacted in a water bath for 1 h. On the second day, the same volume of Tris-balanced phenol solution was put. It was mixed well and centrifuged at 5,000 rpm for 20 min. The extraction was repeated, followed by centrifugation; (IV) 7.5 mmol/L ammonium acetate and 3-fold volume of absolute ethanol were put in the obtained supernatant, and white floccules appeared after mixing, which were transferred to a 1.5 mL centrifuge tube, washed with 70% ethanol, and centrifuged at 5,000 rpm for 20 min. The supernatant was removed and it was dried in the air, and then dissolved with 100 μL TE buffer solution.

DNA extraction method from AF

Extraction steps: (I) 2 mL AF was centrifuged at 3,500 rpm for 10 min, and the supernatant was removed. It was washed 3 times with 1 mL normal saline centrifuged at 300 rpm for 10 min each time; (II) the obtained precipitate was transferred to a new centrifuge tube, the addition of 350 μL STE buffer and 10 μL 20% sodium dodecyl sulfate to mix well and let it stand at 37℃ for 180 min; (III) addition of the same volume of Tris balanced phenol solution, it was mixed well and centrifuged at 10,000 rpm at 4℃ for 10 min to extract the supernatant, the 0.5-fold volume of 7 mmol/L ammonium acetate and 3-fold volume of absolute ethanol was put. It was mixed well and should stand at -20℃ for half an hour, centrifuged at 10,000 rpm at 4℃ for 10 min; (IV) the final precipitate was dissolved with 30 μL TE buffer solution, water bath at 37℃ for 40 min, and the absorbance (OD) at 250 nm was determined using UV-1900i double beam UV spectrophotometer (Shimadzu Company).

Chorionic villi DNA extraction method

After 0.5g villous tissue was washed, 1.5 mL phosphate buffer was added and mixed well and a series of steps including digestion, extraction, and precipitation were performed. After it was dried in the air, 45μL deionized water was used to dissolve at 65℃, and 2μL of the solution was adopted for the PCR template.

PCR product detection method

The obtained DNA was subjected to a PCR reaction to obtain PCR products. First, 25 μL of deionized formamide

and 0.5 μL of ROX-500 were mixed, and 15 μL of the mixed solution and 2 μL of the PCR product were mixed, denatured using a ProFlex™ PCR instrument (Beijing Zhonglilan Biotechnology Co., Ltd., China) for 5 min, ice bath for 5 min. Finally, Genotypev@2.1 genotyping and Genescan@3.1 were applied for quantitative detection.

Preparation method for peripheral blood chromosome samples

(I) 3 mL venous blood was drawn from the patient with a sterilized syringe, a small amount of heparin was mixed well, 0.5 mL of the sample was inoculated into RPMI 1640 medium, and placed in a 37°C incubator to wait for 3 days. 0.2 mL of 10 μg/mL colchicine was added 3 h before the end, and it was gently shaken well and continued to cultivate in the incubator; (II) After the end of the reaction, the flask wall was washed with a pipette, and the cells were transferred to a new centrifuge tube and centrifuged at 1,500 rpm for 20 min to remove most of the supernatant to obtain the cell suspension, it was fixed with 0.75 mmol/L KCl solution, centrifuged at 1,500 rpm for 15 min, and the supernatant was discarded; (III) 10 mL of the fixative was adopted, waiting for half an hour, it was centrifuged at 1,500 rpm for 15 min, and the supernatant was discarded. 8 mL of fixative was put in, waiting for half an hour, and centrifugation was performed at 1,500 rpm for 15 min, with the supernatant discarded, and the operation was repeated 3 times to prepare the cell suspension; (IV) A drop of cell suspension on a glass slide, the cells were blown with a mouth to obtain the chromosome specimen for microscopic observation and karyotype analysis.

Preparation of chromosome specimens from AF cells

(I) 20 mL AF was extracted from pregnant women and centrifuged at 1,500 rpm for 5 min. The supernatant was removed. It was blown evenly and inoculated in a culture flask containing 5 mL culture medium and cultured in a 37°C incubator for 6 days. The culture was observed under a high-definition microscope. If many transparent small round cells appeared, the culture could be terminated. 0.2 mL of 10 μg/mL colchicine was added 3 h before the end. (II) The culture medium was removed from the culture flask, 1.5 mL trypsin-EDTA solution was put to culture in a 37°C incubator for 10 min. After the end, it was blown 3 times with a dropper, and the digestive juice and residual cells were transferred to a centrifuge tube and centrifuged at 15,00 rpm for 10 min. (III) The supernatant was removed and the hypotonic solution was added for precipitation, with a dropper to blow evenly, waiting for 5 min. 5 mL of fixative was put in, standing half an hour, and centrifugation was carried out at 1,200 rpm for 10 min, with most of the supernatant discarded, and the operation was repeated 3 times to prepare the cell suspension. A drop of cell suspension on a glass slide, the cells were blown with a mouth to obtain the chromosome specimen for microscopic observation and karyotype analysis.

Statistical methods

Data were processed adopting SPSS 19.0, with measurement data expressed as mean ± standard deviation ($\bar{x}\pm s$), and enumeration data expressed as a percentage (%). Repeated measures analysis of variance was adopted for comparisons between groups and two-way analysis of variance for comparisons within groups. Two-sided tests were statistically significant at $P<0.05$.

Results

PCR images of normal males

Figure 1A shows a photograph of a normal male staining with a 46, XX karyotype. Figure 1B shows the Genescan typing map of peripheral blood DNA from normal men, with four sites in the first row showing blue fluorescence peaks, D21S11, D21S1270, D13S317, and D5S818, all of which were heterozygous. The three sites in the second row indicated green fluorescence peaks, D18S391, D3S1358, and D21S1414, all of which were heterozygous. The two sites in the third row were black, AMXY and D13S631, in which D13S631 was heterozygous, and the area ratio of AMX peak to AMY peak was close to 1:1.

PCR images of a normal female

Figure 2A illustrates a photograph of a normal female staining with a 46, XX karyotype. Figure 2B shows a Genescan typing plot of peripheral blood DNA from normal women, with the same sites in rows 1 and 2 as in normal men. The two sites in the third row showed black, AMXY and D13S631, of which D13S631 was heterozygous, only

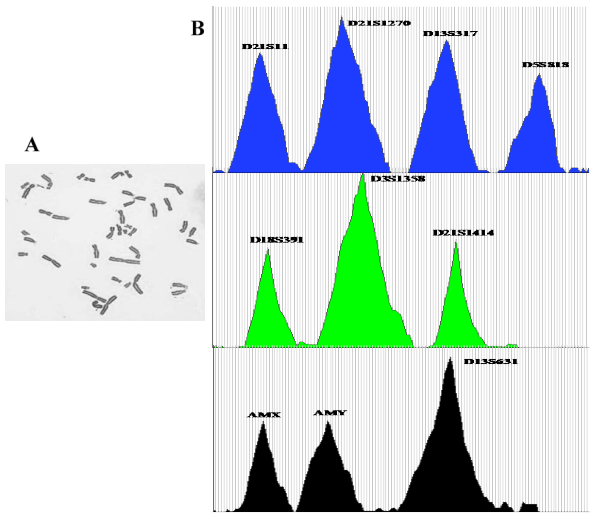

Figure 1. PCR results of normal males. (A: photograph of normal male staining; B: Genescan typing map of peripheral blood DNA).

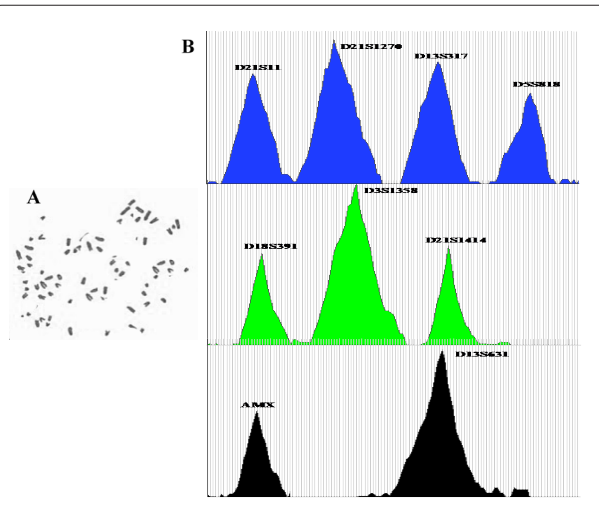

Figure 2. PCR results of a normal woman. (A: photograph of normal female staining; B: Genescan typing map of peripheral blood DNA).

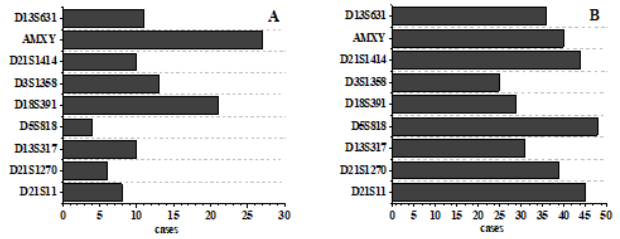

Figure 3. PCR quantitative data results of normal unrelated individuals (A is homozygous; B is heterozygous).

AMX peak, no AMY peak.

Quantitative PCR results in normal unrelated individuals

Figure 3 illustrates the number of homozygotes and heterozygotes for the nine sites of venous blood DNA in normal unrelated individuals, and the analysis suggested that the peaks area ratio of normal heterozygous individuals was between 1 and 1.45, and there was no obvious difference between the expected value and the observed value.

Quantitative PCR results of villous specimens

Figure 4 indicates the number of homozygotes and heterozygotes at 9 sites of DNA in villus samples, and the analysis revealed that the heterozygous peaks area ratio was between 1.002 and 1.27, which belonged to the normal range.

AF specimen PCR quantitation results

Figure 5 shows the number of homozygotes and heterozygotes for the nine sites of DNA in AF specimens, and the heterozygous peaks area ratio was 1-1.35, which was the normal range.

PCR results of a case

Figure 6 illustrates the karyotype analysis of a fetus AF, male, 46, XY, inv [9] (p11: q13). The structure of chromosome 9 was inverted (interarm), and the location of structural inversion was band 1 in the short buttock 1 region and band 3 in the long arm 1 region of chromosome 9.

Figure 7 indicates the karyotype analysis of AF in a female fetus, female, with three chromosomes 21, and no abnormalities in the number and structure of other chromosomes, which was identified as a 21-trisomy syndrome.

Figure 8 shows the karyotype analysis of AF in a male fetus, 46, XY, t (12; 13) (p13; q21), balanced translocations on chromosomes 12 and 13, and breakage translocations occurred on band 3 in the short arm 1 region of chromosome 12 and band 1 in the short arm 2 region of chromosome 13.

Discussion

Chromosomal diseases are diseases caused by numerical or (and) structural chromosomal abnormalities due to various causes, also known as chromosomal aberration syndrome, which are divided into two categories: autosomal diseases and sex chromosome diseases. The overall incidence in neonates is approximately 0.6%. There are many causes of chromosomal lesions in patients, including physical factors, chemical factors, biological factors, maternal age, and genetic factors (19,20). Prenatal screening can be performed in the first or second trimester of pregnancy in pregnant women, and prenatal diagnosis can be performed by measuring maternal serum β-human cho-

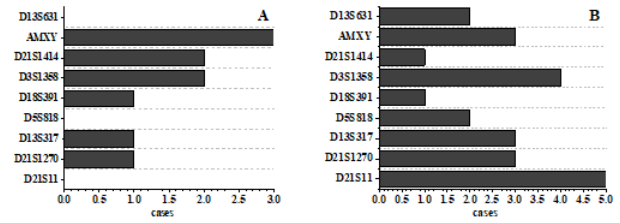

Figure 4. PCR quantitative data of villous samples.

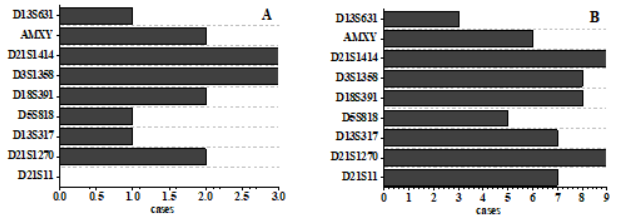

Figure 5. PCR quantification data of AF specimens.

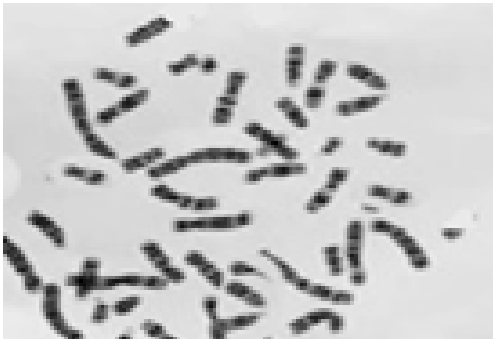

Figure 6. Chromosome photograph of a male fetus.

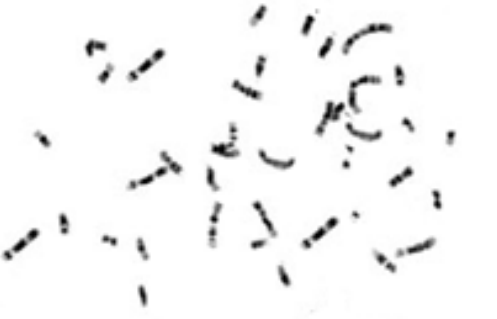

Figure 7. Chromosome photograph of a female fetus. (21-trisomy syndrome).

Figure 8. Chromosome photograph of a male fetus.

rionic gonadotropin (β-HCG), alpha-fetoprotein (AFP), and free estriol (FE3). Measurement of fetal nuchal skin thickness by B-mode ultrasound is also an important indicator of chromosomal disease (21). Karyotype analysis of AF cells is a diagnostic method for prenatal diagnosis of this disease. In recent years, non-invasive DNA prenatal detection technology has developed rapidly. Next-generation DNA sequencing technology is used to sequence free DNA fragments (including fetal free DNA) in maternal peripheral blood, obtain fetal genetic information through bioinformatics analysis, and detect whether the fetus has chromosomal diseases (22-24). AF and villus samples from 80 pregnant women at 16-20 weeks of gestation and 60 venous blood samples from normal unrelated individuals were obtained, and peripheral blood chromosome, AF cell chromosome, and villus cell chromosome samples were extracted and prepared. First, it was found that the AMX peak to AMY peak area ratio of the Genescan typing map of peripheral blood DNA in normal males was close to 1:1, while the Genescan typing map of peripheral blood DNA in normal females had only AMX peak and no AMY peak, which suggested chromosomal differences between male and female genders.

Clinically, chromosome-specific STR are selected as markers for amplification by QF-PCR. STR is repeated that are widespread in prokaryotic and eukaryotic genomes with core sequences of 2 to 7 nucleotides, with high polymorphism, low recombination rate, high information content, high heterozygosity, and easy and quick detection (25,26). Moreover, the simultaneous use of multiple STR specific for the same chromosome as markers can further improve the information content and diagnostic accuracy (27). Quantitative data analysis showed that the ratio of peaks area of venous blood was between 1 and 1.45 in normal heterozygous individuals, between 1.002 and 1.27 in heterozygous villous samples, and between 1 and 1.35 in heterozygous AF samples, which revealed the chromosomal status of normal individuals. In addition, from the PCR results of the cases, the fetus with the 21-trisomy syndrome had three chromosomes 21, and the number and structure of other chromosomes did not show abnormalities. The karyotype of a male fetus was 46, XY, inv [9] (p11: q13), showing that the structure of fetal chromosome 9 was inverted (interarm), and the site of structural inversion was band 1 in the short buttock 1 region and band 3 in the long arm 1 region of chromosome 9. A male fetus with AF indicated a 46, XY, t (12; 13) (p13; q21) karyotype, suggesting a balanced translocation between chromosomes 12 and 13, with breakage translocation sites occurring at band 3 in the short arm 1 region of chromosome 12 and band 1 in the short arm 2 region of chromosome 13. In summary, the use of specific STR locus detection can effectively identify normal humans and cases and has a good application value for prenatal diagnosis of fetal chromosomal diseases.

AF and villus samples of 80 pregnant women and 60 normal venous blood samples were collected from October 2020 to November 2022 at the Prenatal Diagnosis Center of Maternal and Child Health Hospital Affiliated with Nantong University, and peripheral blood chromosome, AF cell chromosome, and villus cell chromosome samples were extracted and prepared for specific STR locus detection. The results showed that QF-PCR can effectively identify normal human bodies and cases by selecting specific STR locus detection, which has a good application value and has important strategic significance in improving the quality of newborns in China. However, the inclusion of chromosomal cases is not comprehensive enough, many diseases have not been detected, and chromosomal case data need to be re-included for more perfect analysis in the subsequent. In conclusion, this result provides a reference basis for prenatal and postnatal care in China.

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