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Research paper

Rapid prenatal diagnosis of aneuploidy for chromosomes 21, 18, 13, X, and Y using segmental duplication quantitative fluorescent PCR (SD-QF-PCR)

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ABSTRACT

Background: In our previous studies, the rapid diagnosis of aneuploidy has been achieved using the segmental duplication molecular markers-based SD-QF-PCR technique. However, it is also insufficient due to the drawbacks including less detection loci and incompetence in single-tube detection.

Methods: In this paper, we developed 13 new segmental duplications as molecular markers, as well as designed 13 pairs of primers and 1 fluorescence-labeled universal primer, which could detect chromosome aneuploidies in one PCR tube.

Results: Two hundred and thirty samples were detected using SD-QF-PCR, the samples were collected from individuals with trisomy 21 (n = 16); trisomy 18 (n = 4); trisomy 13 (n = 3); 45,X (n = 3); 47,XXY (n = 2); 47,XYY (n = 2); suspected mosaic 46,XX/46,XY (n = 2); and unaffected controls (n = 198).

Conclusions: The detection results of SD-QF-PCR were consistent with those of conventional karyotype analysis. SD-QF-PCR based on the newly developed segmental duplications enables the single-tube and multi-locus simultaneous detection on the number of chromosomes 13, 18, 21, X and Y. Therefore, this technique offers a new alternative for the diagnosis of chromosome aneuploidies.

1. Introduction

Genetic diseases induced by chromosomal abnormalities are responsible for over 50% of spontaneous abortion, stillbirth and premature deaths and exhibit an incidence rate of about 1% in newborns (Yusuf and Naeem, 2004; Driscoll and Gross, 2009). Being important causes for sexual abnormality as well as male and female infertility and sterility, they also act as one of significant origins leading to congenital heart disease, intelligence hypoplasia and other disorders. Chromosomal abnormalities consist of numerical abnormalities and structural abnormalities, where chromosomal aneuploidy abnormalities prove to be one of the most common human chromosomal aberrations, which seriously threats human health considering the severe mental retardation and malformation of tissues and organs generally developed in the sufferers. The most common chromosomal numerical abnormalities include trisomy 21 (Down's syndrome), trisomy 18 (Edward's syndrome), trisomy 13 (Patau syndrome), 45,X (Turner's syndrome), 47,XXY (Klinefelter syndrome), triploids, mosaicism, among others, which together account for > 80% of prenatally diagnosed chromosomal abnormalities (Hochstenbach et al., 2005). Existing heavy mental and financial burden to the society and the family, these diseases cannot be effectively cured at present, and the only feasible approach is to reduce or avoid the birth of such children via prenatal screening or diagnosis. Consequently, it is particularly vital to perform prenatal diagnosis against chromosomal disorders.

Currently, as the main diagnostic methods for chromosomal disorders, cell culture and karyotype analysis method is accompanied by shortcomings like complicated operation and long diagnostic reporting cycle (Caine et al., 2005). With the development of molecular biology, a number of rapid diagnostic techniques and methods have been developed and reported in succession, such as fluorescence in situ hybridization (FISH) (Ho et al., 2012), quantitative fluorescent PCR (QF-

Abbreviations: FISH, fluorescence in situ hybridization; QF-PCR, quantitative fluorescent PCR; qPCR, real-time quantitative PCR; MLPA, multiplex ligation-dependent probe amplification; HGQ-PCR, homologous gene quantitative PCR; PSQ, paralogous sequence quantification; HRM, high-resolution melting curve; aCGH, array comparative genomic hybridization; NGS, next generation sequencing; dPCR, digital PCR; SD-QF-PCR, segmental duplication quantitative fluorescent PCR

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PCR) (Mann et al., 2004), SNP melting curve analysis (Pont-Kingdon and Lyon, 2003; Nagy et al., 2005), real-time quantitative PCR (qPCR) (Zimmermann et al., 2002), multiplex ligation-dependent probe amplification (MLPA) (Schouten et al., 2002),homologous gene quantitative PCR (HGQ-PCR) (Lee et al., 1997), paralogous sequence quantification (PSQ) (Deutsch et al., 2004), MLPA/qPCR (Guo et al., 2010), high-resolution melting curve analysis (HRM) of segmental duplication (Guo et al., 2012), array comparative genomic hybridization (aCGH) (Hung et al., 2012), next generation sequencing (NGS) (Norton et al., 2015), and digital PCR (dPCR) (Fan and Quake, 2007). All these methods have the ability to diagnose aneuploidies, and exhibit respective disadvantages or shortcomings, such as complicated operation, poor locus specificity, high requirements for technical conditions, and expensive costs.

In order to pursue a more fast, simple and stable method, an investigation was carried out regarding the existing techniques and methods. We found that the HGQ-PCR developed by Lee HH et al. provided an excellent detection method for the rapid diagnosis of Down's syndrome (Lee et al., 1997). However, in-depth exploration on the study found that there was an incompletely matched nucleotide in both upstream and downstream of the used primer, so it is difficult to maintain the original ratio in PCR amplification providing incomplete binding of the primer to the target sequence. At the same time, with single agarose gel electrophoresis as the quantitative method, the quantitative accuracy and stability could not be guaranteed in different laboratory tests. Nevertheless, the study did exhibit a highly promising prospect in the homologous gene-based detection of gene copy number. In 2012, Guo Q et al. developed the segmental duplication molecular markers based on the same principle with homologous genes, whose integration with the high-resolution melting analysis of segmental duplication (SD-HRM) permitted the rapid diagnosis of aneuploidy (Guo et al., 2012), thus markedly simplifying the assays and reducing costs. Regrettably, this method required a multi-tube operation, and 6 tubes would be involved in order to detect 3 chromosomes. At all events, it once again highlighted the advantage of segmental duplication-based detection methodology. We started to develop segmental duplication molecular markers considering their potential application prospects. In the early development and application, we combined segmental duplication molecular markers with STR molecular markers, which were used to simultaneously detect aneuploidies and showed the advantage of mutual diagnosis and mutual verification (Long et al., 2013). Unfortunately, STR required several fluorescent labels, so the labeling costs were higher; besides, STR's requirement for multi-locus detection could easily cause the reduction in reaction system stability. In addition, we further combined the developed segmental duplications molecular markers with QF-PCR technique. The detection system consisted of two tubes, which could enable the aneuploidy screening of 5 chromosomes (Kong et al., 2014). However, the less detection loci, excessive fluorescent labels and incompetence in single tube detection, may lead to reduced stability and accuracy of the experiment.

In view of the shortcomings in the previous detection system, we rescreened and verified a series of new segmental duplication molecular markers. The new molecular markers can realize the single-tube and multi-locus simultaneous detection of the abnormalities in chromosomes 13, 18, 21, X and Y, thus providing a new theoretical basis and diagnostic alternative for the diagnosis of prenatal common chromosome aneuploidies. In this paper, 230 cases of routine clinical amniotic fluid samples were further detected using SD-QF-PCR, whose clinical application value in the diagnosis of aneuploidy abnormalities was explored via a more in-depth comparison with the conventional karyotype analysis.

2. Materials and methods

2.1. Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. The protocol for this study was approved through the Research Ethics Committee of Qinzhou Maternal and Child Health Hospital. Written informed consent was obtained from each participant for the collection of samples and subsequent analyses.

2.2. Samples

A total of 230 samples were collected in this study, of which 16 samples were obtained from individuals with trisomy 21, 4 samples from individuals with trisomy 18, 3 samples from individuals with trisomy 13, 3 samples with 45,X, 2 samples from individuals with 47,XXY and with 47,XYY respectively, 2 samples of suspected mosaic 46,XX/46,XY, and 198 samples from unaffected individuals. All samples have been verified through amniotic fluid karyotype analysis.

2.3. DNA extraction

DNA was extracted from uncultured amniotic fluid samples and umbilical cord blood samples using the DNA extraction kit (Tiangen) according to the manufacturer's instructions. The concentration and purity of the extracted genomic DNA were determined via spectrophotometry (Quawell); with the concentration adjusted to 30 ng/μL, the DNA samples were stored at − 80 °C for subsequent molecular detection.

2.4. Development of segmental duplication molecular markers

The segmental duplications used in this study referred to the interspersed repeats with two copies in their genome rather than the tandem repeats in the form of unique sequences, such as satellite DNA, minisatellite DNA and microsatellite DNA. Such segmental duplication included two sequences with highly similar bases (similar sequences) and were blasted against the human chromosomal segmental duplications in NCBI; we selected two copies: one copy must be located on the target chromosome, and the other must be located on a chromosome other than the target chromosome (Fig. 1A). For the screened similar segmental duplications, both the high base similarity and the differences in the number of some bases were required, since the identical bases could be used for subsequent universal primer design while those with numerical or structural differences for subsequent capillary electrophoresis analysis (Fig. 1B). Using capillary electrophoresis to acquire the fluorescence ratio between the PCR products of two similar sequences of segmental duplication, their ratio on the initial template could be determined, thereby allowing to judging the number of target chromosomes (Fig. 1C).

Primers were designed according to the selected segmental duplications respectively, followed by the single-primer and multi-primer verification. After that, the segmental duplication molecular markers and their amplification primers were finally developed.

2.5. SD-QF-PCR and capillary electrophoresis

The PCR amplification was performed using PCR System 9700 (Applied Biosystems) in a total reaction volume of 25 μL containing 1 × Reaction Master Mix (CWBio Biological Technology Co., Ltd.), 30 ng genomic DNA and 0.5 m mol/L of each forward and reverse primer. The reaction mixture was preheated at 95 °C for 3 min, followed by 28 cycles of 30 s at 95 °C and 30 s at 60 °C, an extension step at 72 °C for 10 min, and the final preservation at 15 °C for subsequent use.

1 μL of the PCR product was mixed with 23 μL of formamide and