High accuracy of quantitative fluorescence polymerase chain reaction combined with non-invasive pre-natal testing for mid-pregnancy diagnosis of common fetal aneuploidies: A single-center experience in China

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Abstract. Quantitative fluorescence polymerase chain reaction (QF-PCR) may be used as a mid-pregnancy test to confirm the diagnosis of common fetal aneuploidies, but its use is controversial. The present study aimed to determine the value of QF-PCR for diagnostic confirmation of karyotyping and the impact of parental origin and meiosis stage on the detected aneuploidy. The present prospective cohort study included pregnant women (age, 21-45 years; gestational age, 17-25 weeks) who consulted between May 2015 and December 2016. Women were screened and only consecutive high-risk individuals were included (n=428). QF-PCR analysis of amniocytes was performed. Karyotype analysis was considered the gold standard. Parental karyotyping was performed if the embryo exhibited any aneuploidy. GeneMapper 3.2 was used for data analysis. There were no false-negative or false-positive QF-PCR results, with 100% concordance with the karyotype. The aneuploidy distribution (n=105) was 68.6% for trisomy 21, 19.0% for trisomy 18, 7.6% for sex chromosome aneuploidy, 3.8% for trisomy 13 and 1.0% for 48,XXX,+18. Regarding trisomy 21, most cases (86.1%) were of maternal origin, 8.3% paternal and 6.5% undefined. Trisomy 18 was 88.2% maternal and 11.8% paternal. Maternal meiosis stage errors in trisomy 21 mainly occurred in meiosis I, while the origin of trisomy 18 exhibited similar proportions between meiosis I and II. The combination of non-invasive pre-natal testing and QF-PCR may become a rapid and effective method for fetal aneuploidy detection. QF-PCR may provide more genetic information for clinical diagnosis and treatment than karyotyping alone.

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Abbreviations: QF-PCR, quantitative fluorescence-polymerase chain reaction; CGH, comparative genomic hybridization; NIPT, non-invasive pre-natal testing; aCGH, array comparative genomic hybridization

Key words: aneuploidy, polymerase chain reaction, pre-natal diagnosis, karyotype analysis, meiosis

Introduction

Aneuploidy refers to the presence of an abnormal number of chromosomes in a cell and an extra or missing chromosome is a common genetic disorder (1). Aneuploidy frequently results in miscarriage, but is also the cause of a large number of birth defects. Among those defects, trisomy 21, 18 and 13 are most common (1). The prevalence of trisomy 21 (Down syndrome) is 10.3-13.6 per 10,000 live births in the USA and Europe (2), while the prevalence of trisomy 18 and trisomy 13 is 4.0 and 1.6 cases per 10,000 pregnancies, respectively (3). These syndromes are characterized by multiple malformations, concomitant medical conditions and cognitive impairment (4-6). These conditions cannot be cured and their impacts on the parents and society are important. Therefore, pre-natal diagnosis has a critical role in the management of aneuploidies.

Even if certain ultrasound features may hint toward the possibility of aneuploidy in a fetus, the only definitive and gold-standard diagnostic method remains karyotype analysis. Nevertheless, this method requires cell culture, time and viable cells, and has low sensitivity (7). Novel methods, including quantitative fluorescence polymerase chain reaction (QF-PCR), fluorescence *in situ* hybridization and comparative genomic hybridization (CGH) have certain advantages, including saving time and cost, the requirement of small numbers of cells and high accuracy. In general, the results for common aneuploidies may be obtained within 24-48 h (8). QF-PCR has high

sensitivity for aneuploidies involving chromosomes 21, 18, 13, X and Y, but not for rare aneuploidies. QF-PCR is increasingly considered as a complementary investigation (9-13) or as an alternative to conventional cytogenetic analysis.

In recent years, non-invasive pre-natal testing (NIPT) technologies have developed rapidly. As the associated conditions cannot be cured and their impact on the parents' and child's life and society are non-negligible, the parents desire result confirmation as soon as possible when the screening results indicate a high risk of aneuploidy. The value of QF-PCR as a screening method is controversial (11-14), but it may be used as a mid-pregnancy confirmation test if common fetal aneuploidies have been identified. Therefore, the aim of the present study was to examine the value of QF-PCR for diagnostic confirmation of aneuploidies and the impact of the parental origin and meiosis stage on the detected aneuploidy.

Materials and methods

Study design and subjects. The study was approved by the Ethics Committee of Hebei General Hospital (Shijiazhuang, China). All participants provided written informed consent.

The present study was a prospective cohort study of 428 consecutive high-risk pregnant women (age, 21-45 years; gestational age, 17-25 weeks) who consulted between May 2015 and December 2016 at Hebei General Hospital (Shijiazhuang, China). Patients with at least one of the following indications were included: i) High-risk NIPT (Z score >3); ii) mid-pregnancy screening high-risk value (>1/100); and iii) >2 fetal abnormalities on ultrasound (15). No other genetic testing was performed. All indicators prior to amniotic fluid examination were required to be normal. All female patients provided written informed consent for the pre-natal diagnosis.

The indications for NITP were: i) Serological screening or imaging examinations suggesting borderline risk of common chromosomal aneuploidy; ii) contraindications to interventional pre-natal diagnosis (including threatening abortion, fever, coagulation problems, communicable diseases to the fetus, including hepatitis B and C viruses, syphilis and human immunodeficiency virus, ongoing infection and incompatibility of maternal and fetal RH blood group); and iii) gestational age $>20^{+6}$ weeks, i.e., women who missed the optimal timing for serological screening. The indications for serological screening in the second trimester were: i) Singleton pregnancy of 16-20^{+6} weeks; ii) for female patients with irregular menstruation, the biparietal diameter of the fetus was required to be ≤48 mm; iii) the age of the mother at the expected due time was <35 years; iv) without history of abnormal pregnancy; and v) the ultrasound examinations of the fetus exhibited no abnormalities.

In the context of routine pre-natal examinations, color fetal ultrasound is first performed to search for morphological and developmental abnormalities. Subsequently, blood routine examinations were performed and blood coagulation indexes, blood type and viral indexes [including hepatitis B surface antigen or antibody, hepatitis B antigen or antibody, hepatitis B core antibody, hepatitis B virus, syphilis and HIV] were determined, and vaginal secretions (routinely examined for vaginal cleanliness, pus, vaginal *Lactobacillus*, *Chlamydia trachomatis*, Clue cells, *Candida albicans*, and *Trichomonas vaginalis*, body temperature and pulse of the pregnant subjects were measured to ensure that no surgical contraindications were present. The women with any contraindications or with multiple gestations were excluded.

Sampling. The amniotic fluid was sampled using a 21-G sterile needle and syringe under real-time ultrasound guidance (ALOKA5500; ALOKA). The first 1 ml of amniotic fluid was discarded and 5 ml were sampled for QF-PCR and 20 ml for karyotype analysis. Furthermore, 2 ml of venous blood were collected from the antecubital vein of 74 couples (both parents) with confirmed fetal aneuploidy.

For NITP, 5 ml peripheral blood were obtained from the pregnant female subjects and used for fetal free-DNA screening of the plasma at the Boao Clinical Examination Center (Yizhuang Biomedical Park, Beijing, China). The free DNA was obtained for high-resolution sequencing and the actual number of nucleotide fragments distributed in each chromosome was measured. The number was compared with the result of the high-performance computing sequencing fragment count. Combined bioinformatics analysis was then performed to assess whether the fetus had any chromosomal aneuploidy. The assessment of the stage of abnormal chromosome segregation was performed using the genetic map. If the redundant short tandem repeat (STR) was of the parental double-STR type, the abnormal chromosome segregation had occurred in the first meiosis. If the redundant STR was of the parental single-STR type, the abnormal chromosome segregation had occurred in the second meiosis. If the two types were present, it was not possible to determine the time of abnormal chromosome segregation due to exchanges of parental chromosomes.

QF-PCR. Loci D21S11 (located at 21q21.1), D21S1435 (at 21q21) and PENTAD (at 21q22.3) were used to assess chromosome 21. The loci D18S51 (located at 18q21.33), D18S1002 (at 18q11.2), D18S535 (at 18q12.2) and D18S391 (at 18p11.22) were used to assess chromosome 18. The loci D13S317 (at 13q31.1) and D13S634 (at 13q14.3) were used to assess chromosome 13. The loci Amelogenin (located at Xp22.3), DXS8106 (at Xq27.3) and DXS7132 (at Xq11.2) were used to assess the sex chromosomes (16,17). Primer sequences are presented in Table I. All primers were synthesized by Shanghai YingweiJieji Trading Co., Ltd. Fluorescent labeling was performed at the 5' end using carboxyfluorescein (blue), carboxytetramethylrhodamine (yellow) or carboxy-4',5'-dichloro-2',7'-dimethylfluorescein (green).

Amniotic fluid and peripheral blood DNA were extracted using the Lab-Aid 820 DNA extraction kit (Xiamen Zeesan Biotech Co., Ltd.), according to the manufacturer's protocols. Primers were generated with fluorescent tags according to the sequences in the National Center for Biotechnology Information database (strbase.nist.gov/str_fact.htm; www.ncbi.nlm.nih.gov). The reaction mixture was prepared using 100 μl GoTaq hot start colorless master mix (Promega Corp.), primers (10 μl) and H_2O (70 μl). Amplification was performed using 9 μl of this reaction mixture with 1 μl DNA. The QF-PCR program was: i) 2 min at 95˚C, followed by 20 sec at 95˚C and 80 sec at 59˚C; ii) 30 cycles of 20 sec at 95˚C, 80 sec at 59˚C and 40 sec at 73˚C; and iii) final extension for 10 min at 73˚C. The QF-PCR products (1 μl) were added to 10 ml

Table I. Primers for chromosomes 21, 18, 13 and X.

STR	Primers
AMEL	F: 5'-(TAMRA)-CCCTGGGCTCTGTAAAGAA-3'
	R: 5'-ATCAGAGCTTAAACTGGGAAGCTG-3'
D13S317	F: 5'-ATTACAGAAGTCTGGGATGTGGAGGA-3'
	R: 5'-(JOE)-GGCAGCCCAAAAAGACAGA-3'
D13S634	F: 5'-GGCAGATTCAATAGGATAAATAGA-3'
	R: 5'-(TAMRA)-GTAACCCCTCAGGTTCTCAAGTCT-3'
D18S1002	F: 5'-(TAMRA)-CAAAGAGTGAATGCTGTACAAACAGC-3'
	R: 5'-CAAGATGTGAGTGTGCTTTTCAGGAG-3'
D18S391	F: 5'-GGACTTACCACAGGCAATGTGACT-3'
	R: 5'-(JOE)-TAGACTTCACTATTCCCATCTGAG-3'
D18S51	F: 5'-(FAM)-TTCTTGAGCCCAGAAGGTTA-3'
	R: 5'-ATTCTACCAGCAACAACACAAATAAAC-3'
D18S535	F: 5'-CAGCAAACTTCATGTGACAAAAGC-3'
	R: 5'-(JOE)-CAATGGTAACCTACTATTTACGTC-3'
D21S11	F: 5'-ATATGTGAGTCAATTCCCCAAG-3'
	R: 5'-(FAM)-TGTATTAGTCAATGTTCTCCAGAGAC-3'
D21S1435	F: 5'-CCCTCTCAATTGTTTGTCTACC-3'
	R: 5'-(TAMRA)-GCAAGAGATTTCAGTGCCAT-3'
DXS7132	F: 5'-AGCCCATTTTCATAATAAATCC-3'
	R: 5'-(FAM)-AATCAGTGCTTTCTGTACTATTGG-3'
DXS8106	F: 5'-(FAM)-CTTGCACTTGCTGTGG-3'
	R: 5'-AGCTGTAGAGTTGAGGAATG-3'
PENTAD	F: 5'-(JOE)-GAAGGTCGAAGCTGAAGTG-3'
	R: 5'-ATTAGAATTCTTTAATCTGGACACAAG-3'

STR, short-tandem repeat; R, reverse; F, forward; AMEL, amelogenin; FAM, carboxyfluorescein; TAMRA, carboxytetramethylrhodamine; JOE, carboxy-4',5'-dichloro-2',7'-dimethylfluorescein.

formamide (Applied Biosystems; Thermo Fisher Scientific, Inc.), containing 0.25 μl LIS 600 (Promega Corp.) as a standard. After denaturation at 95˚C for 5 min, the mixture was cooled quickly and capillary electrophoresis was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) using a POP4 polymer.

Karyotype analysis. Karyotyping was performed using a routine method (18). Amniocytes were centrifuged and precipitated, and cultured in the appropriate medium. G banding was performed and the chromosome smear was prepared. An automatic scanning microscope and image analysis system (GSL-120; Leica Microsystems) were used to perform chromosome karyotype analysis.

Data analysis. GeneMapper 3.2 (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to analyze the results and calculate the peak areas. Allele ratios (shorter allele/longer allele) between 0.8 and 1.4 were considered as normal, and ratios of >1.8 or <0.65, or the presence of three alleles of equal peak area were considered as trisomy. The presence of a single peak was considered as uninformative and a minimum of two concordant informative markers for each chromosome (21, 18, 13 and X) were required for a confident result (19). Parental origin was obtained from the genotypes of STR markers in the same locus of the fetus. If parental heterozygosity was retained in the trisomic offspring, non-disjunction error from meiosis I was considered. If parental heterozygosity was reduced to homozygosity, non-disjunction error from meiosis II was considered (20). When the two types existed at the same time, non-disjunction error from the parental chromosome exchange was considered.

Results

Patient and specimen characteristics. The amniotic fluid from the 428 pregnant women was analyzed by karyotype and QF-PCR. The mean age of the pregnant women was 30.4 years (range, 21-45 years) and the mean gestational age was 20.0 weeks (range, 17-25 weeks). The QF-PCR results for representative cases of aneuploidy by are provided in Figs. 1-7. Technically, the two methods were 100% successful, a readable result was obtained from each test and no retesting was required.

Aneuploidy distribution by karyotype. The tests identified 105 cases of aneuploid karyotype, including 71 cases of common trisomy 21 and one case of ectopic type trisomy 21. Trisomy 21 accounted for 68.6% of all trisomy cases. There were 20 cases of trisomy 18 (19.0%), 8 cases of sex chromosome

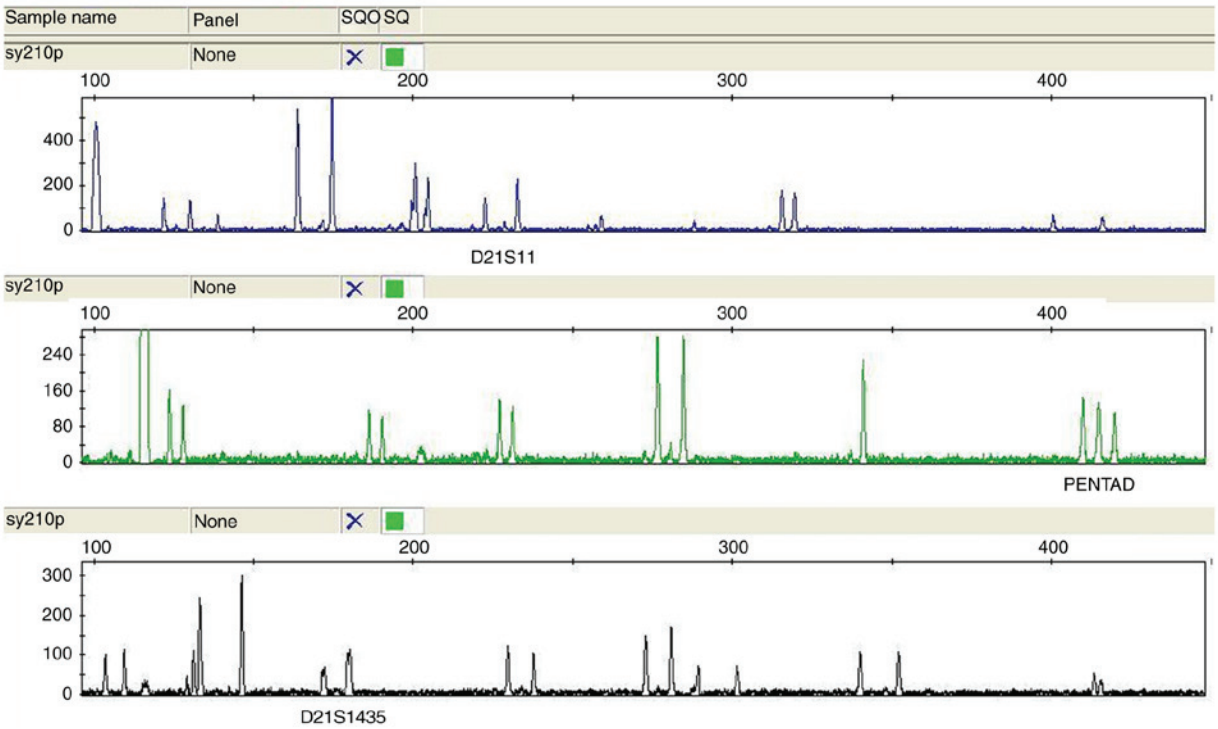

Figure 1. Electrophoregrams of a fetus diagnosed with trisomy 21. All chromosome 21-specific short-tandem repeats exhibited three copies. Loci D21S11 and D21S1435 have 2:1 peaks. Locus Penta D has 1:1:1 peaks.

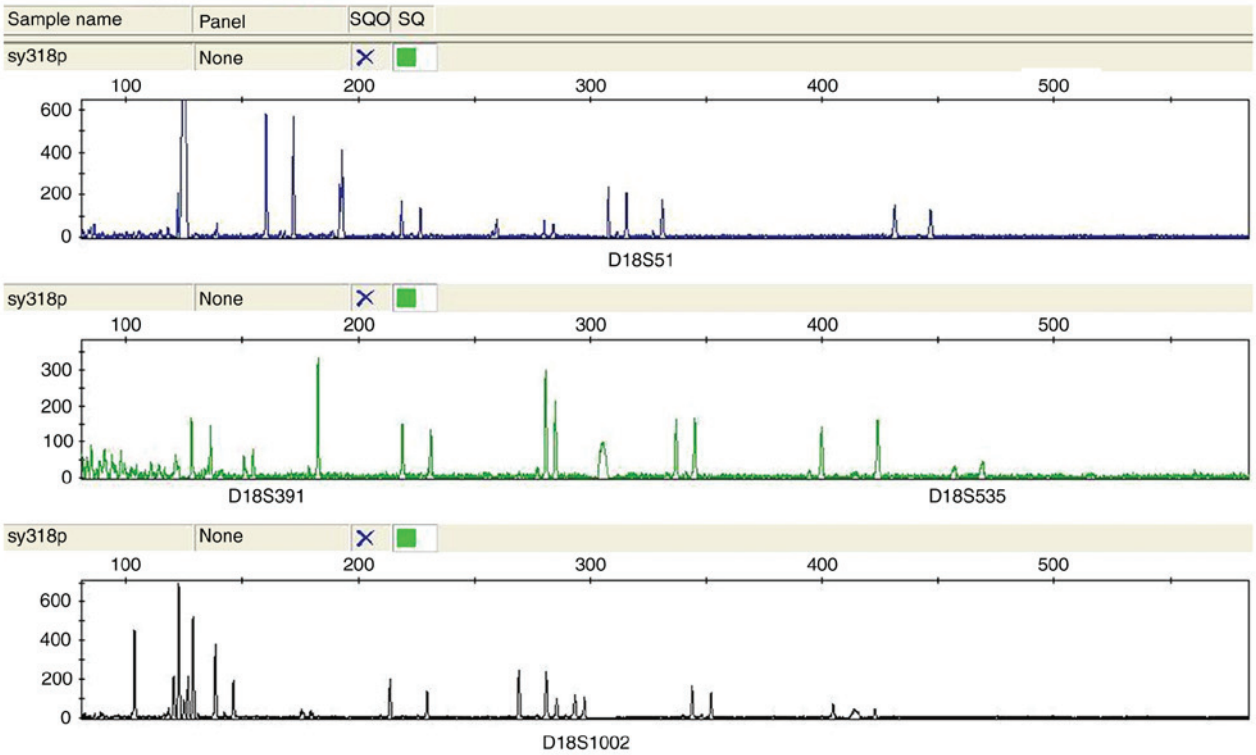

Figure 2. Electrophoregrams of a fetus diagnosed with trisomy 18. All chromosome 18-specific short-tandem repeats have three copies. Loci D18S51 and D18S1002 have 1:1:1 peaks. D18S391 and D18S535 have 2:1 peaks.

aneuploidy (7.6%; 5 cases of 47,XXX; 2 cases of 47,XXY; and 1 case of 45,XO), 4 cases of trisomy 13 (3.8%; including one case of ectopic type trisomy 13) and 1 case of 48,XXX,+18 (1.0%) (Table II).

Concordance between karyotype and QF-PCR. By using karyotyping and QF-PCR analysis, 105 cases of aneuploidy were identified. Using karyotype as the gold standard, QF-PCR indicated no false-positive or false-negative results, due to a

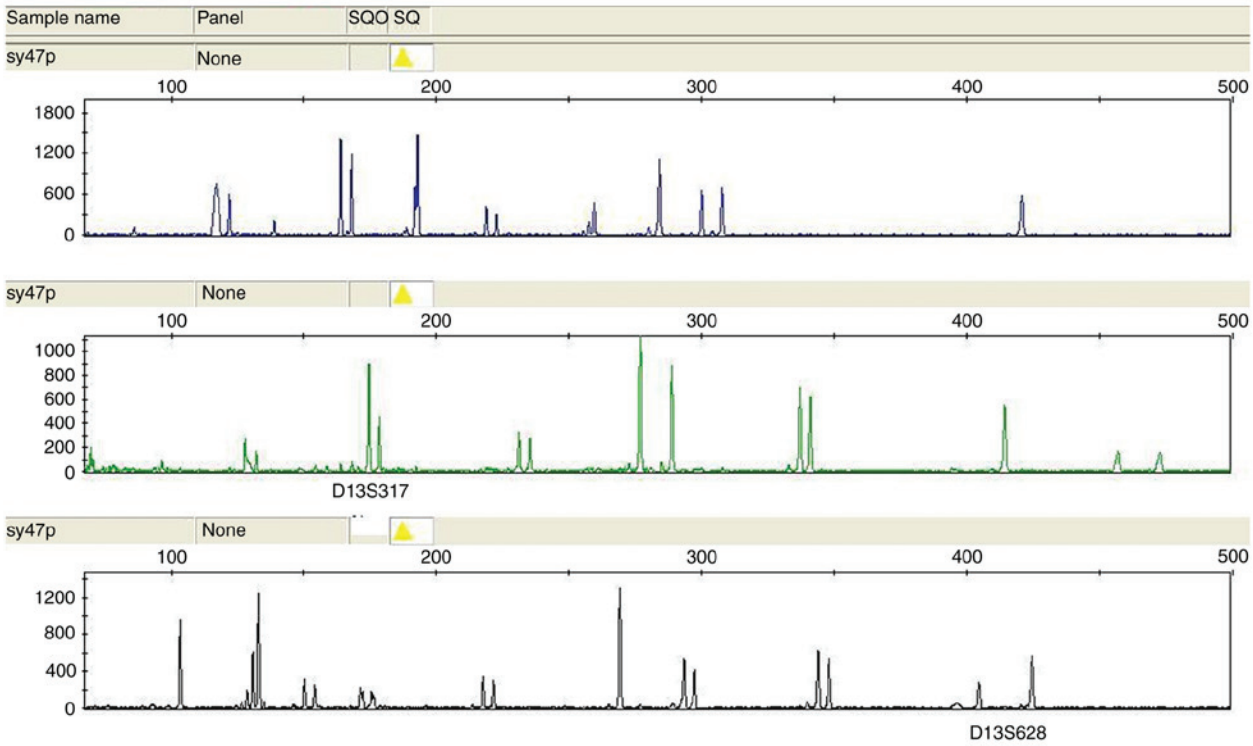

Figure 3. Electrophoregrams of a fetus diagnosed with trisomy 13. All chromosome 13-specific short-tandem repeats have three copies. Loci D13S317 and D13S628 have 2:1 peaks.

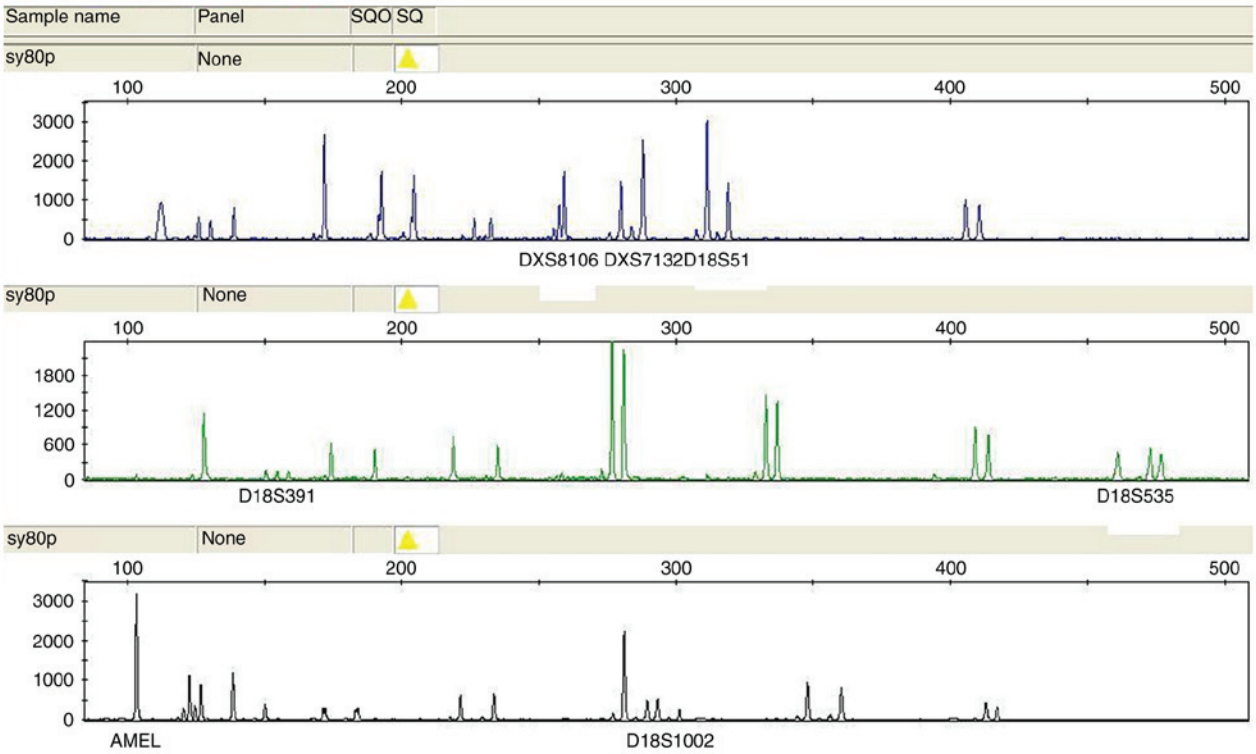

Figure 4. Electrophoregrams of a fetus diagnosed with 48,XXX,+18. All chromosome 18-specific and X-specific short-tandem repeats have three copies. Loci DXS8106, DXS7132 and D18S51 have 2:1 peaks. Loci D18S391, D18S535 and D18S1002 have 1:1:1 peaks. AMEL, amelogenin locus.

concordance of 100%. Among the 62 cases with a high risk of trisomy 21 according to NIPT, 58 cases had positive results in the karyotype and STR analyses, while 4 cases exhibited no obvious abnormality, leading to a true-positive rate of 93.5%. Among the 18 cases with a high risk of trisomy 18 according to NIPT, 15 had positive results in the karyotype and STR analyses, while 3 cases exhibited no obvious abnormality, leading to a true-positive rate of 83.3% (Table III).

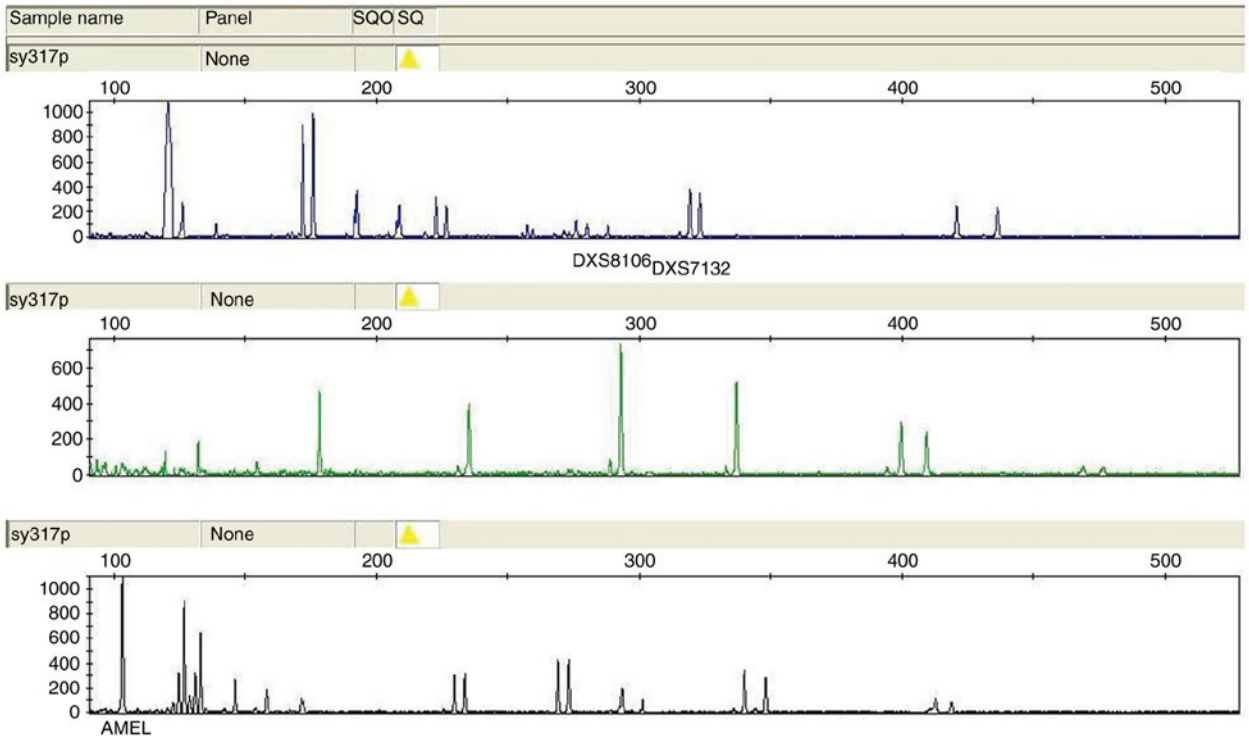

Figure 5. Electrophoregrams of a fetus diagnosed with 47,XXX. All chromosome X-specific short-tandem repeats have three copies. Locus DXS8106 has 2:1 peaks. Locus DXS7132 has 1:1:1 peaks. AMEL, amelogenin locus.

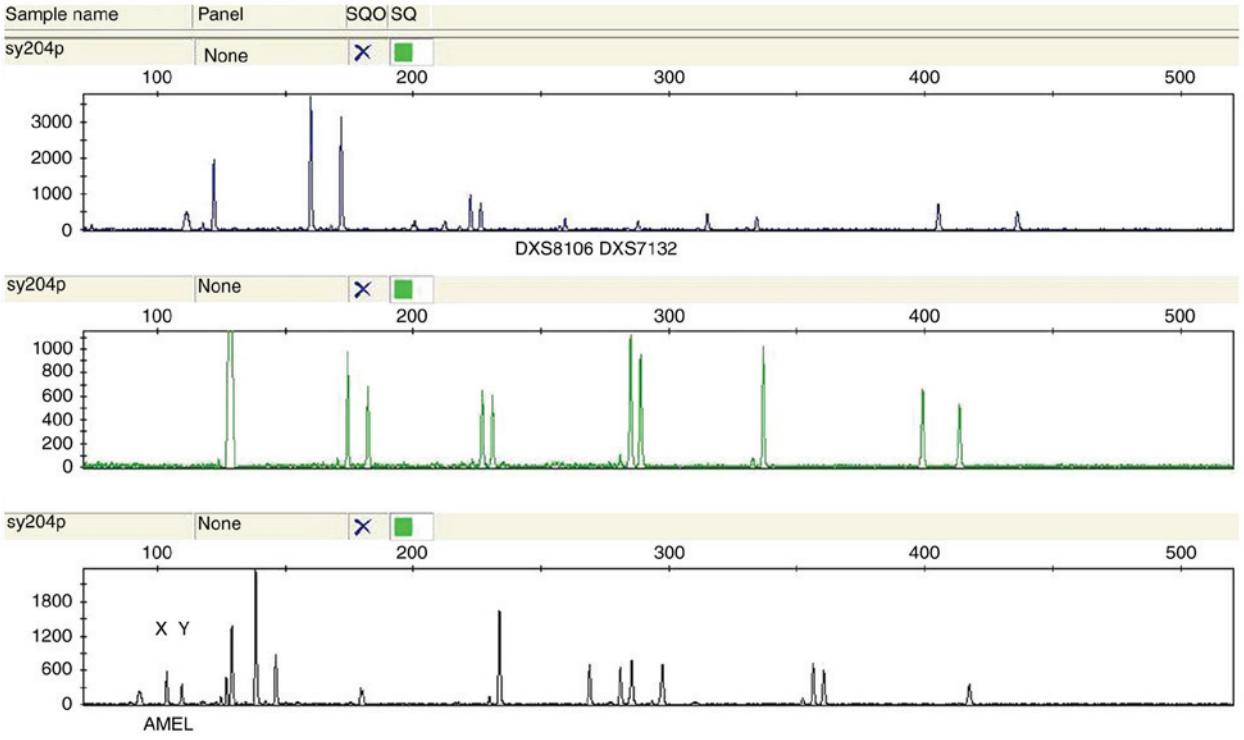

Figure 6. Electrophoregrams of a fetus diagnosed with 47,XXY. The AMEL has 2:1 peaks (X:Y). AMEL, amelogenin locus.

Parental origin of non-disjunction errors of chromosomal aneuploidy. Blood samples from 81 couples (out of 105 cases of fetal chromosomal aneuploidy; 77.1%) were collected and tested for parental origin of non-disjunction error. The results indicated that 47 cases of trisomy 21 were of maternal origin (85.4%) and five were of paternal origin (9.1%), while the source was unknown in three cases (5.5%). Among the 17 cases of trisomy 18, 15 cases were of maternal (88.2%) and two of paternal origin (11.8%). Among the 4 cases of trisomy 13, 3 cases were of maternal (75.0%) and 1 of paternal

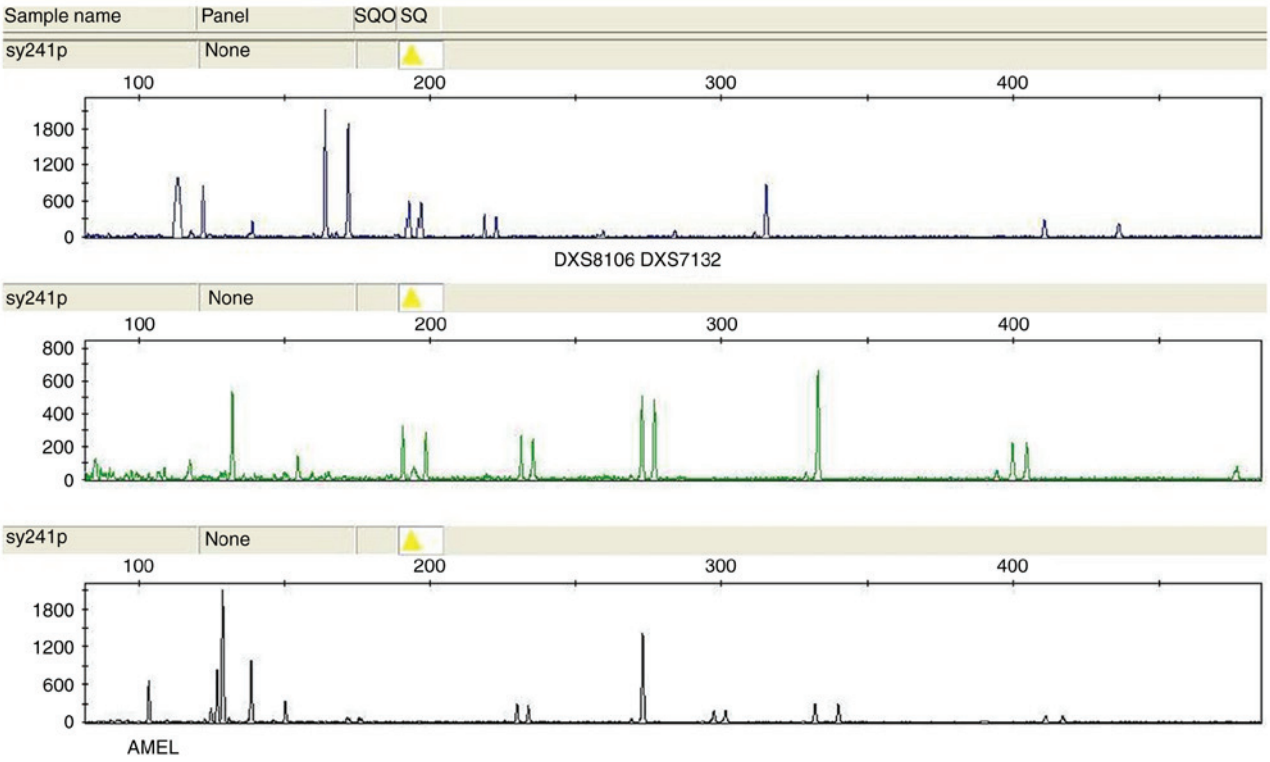

Figure 7. Electrophoregrams of a fetus diagnosed with 45,XO. Loci DXS8106 and DXS7132 have only single peaks. AMEL, amelogenin locus.

origin (25.0%). Among the 5 cases of sex chromosome abnormality, 2 cases were of maternal (40.0%) and 3 of paternal origin (60.0%) (Table IV).

Frequency of maternal meiosis stage errors. The frequency of maternal meiosis stage errors was significantly different (P=0.041) between trisomy 21 [76.6% meiosis I (n=36), 12.8% meiosis II (n=6), 8.5% maternal transition (n=4) and 2.1% maternal reproductive cell chimeras (n=1)] and trisomy 18 [46.7% meiosis I (n=7), 40.0% meiosis II (n=6) and 13.3% maternal transition (n=2)] (Table V).

Discussion

The value of QF-PCR as a screening test is controversial, but it may be used as a mid-pregnancy test to confirm the diagnosis of common fetal aneuploidies (11-14). In this light, the present study aimed to examine the value of QF-PCR in diagnostic karyotype confirmation and the impact of the parental origin and meiosis stage on the aneuploidy. The results suggest that the combination of NIPT and QF-PCR may become a rapid and effective method for fetal aneuploidy detection. Testing of the parental origin and meiosis stage of non-disjunction errors by QF-PCR provides additional genetic information for the diagnosis and management of aneuploidies, as opposed to karyotyping alone.

QF-PCR has numerous advantages. Without a doubt, the major advantages of QF-PCR are that the results are obtained rapidly and only require a small amount of amniotic fluid and no cell culture. Hence, QF-PCR is more cost-effective than karyotyping (21). Furthermore, QF-PCR is able to detect >90% of clinically significant chromosomal abnormalities (11,22-25), but this is controversial and certain studies suggest that QF-PCR may fail to detect 15-30% of the abnormalities identified by karyotyping (26,27). On the other hand, one limitation of QF-PCR is that it fails to detect structural abnormalities and mosaicism of <30% (28). It has been suggested that restricting the use of QF-PCR for low-risk pregnancies or combining QF-PCR with other modalities, e.g. nuchal translucency, may be sufficient for screening, but there is a concern regarding the lack of high-quality data from karyotyping, and this issue remains controversial (12).

In the present study, QF-PCR had a concordance rate of 100% with the karyotype, without any false-positive or -negative results. This observation is particularly good and is supported by previous studies that also obtained accuracies of almost 100%. Indeed, Badenas *et al* (11) reported a concordance rate of 98.8% among 7,679 pre-natal samples. In a study by De la Paz-Gallardo *et al* (12), the results of 99% of the 928 samples included were concordant; in addition, if QF-PCR had been used as the major diagnostic method, with confirmation by karyotyping only in high-risk individuals identified based on imaging, only 12.5% of the samples would have required karyotype confirmation. Rostami *et al* (13) reported that among 4,058 pre-natal samples, 98.6% were successfully diagnosed by QF-PCR, but karyotyping detected additional cases. On the other hand, Papoulidis *et al* (14) reported that karyotyping detected 320 aneuploidies among 13,500 cases, but QF-PCR did not detect 70 (21.9%) of these 320 cases. In a Chinese study, only two cases out of 210 were discordant between karyotype and QF-PCR (16). However, there are certain variations among studies, populations and methods (10). A recent meta-analysis indicated that the sensitivity/specificity of QF-PCR compared with karyotyping vary depending on the

Table II. Fetal chromosome aneuploidy distribution in mid-pregnancy.

Chromosome aneuploidy type	n (%)
Trisomy 21 (47,XX,+21) (including one ectopic type)	72 (68.6)
Trisomy 18 (47,XX,+18)	20 (19.0)
Sex chromosome aneuploidy	8 (7.6)
Trisomy 13 (47,XX,+13)	4 (3.8)
48,XXX,+18 (including one ectopic type)	1 (1.0)
Total	105 (100.0)

Table III. Concordance between karyotype and QF-PCR.

Abnormality	n (%)
Chromosome 21 (according to NIPT)	62 (100)
Positive on karyotype and STR (true positive)	58 (93.5)
No obvious abnormality	4 (6.5)
Chromosome 18	18 (100)
Positive on karyotype and STR (true positive)	15 (83.3)
No obvious abnormality	3 (16.7)

Table IV. Parental origin of non-disjunction errors.

Parental origin of non-disjunction error	n (%)
Chromosome 21	
Maternal origin	47 (85.4)
Paternal origin	5 (9.1)
Parental origin not determined	3 (5.5)
Total	55 (100)
Chromosome 18	
Maternal origin	15 (88.2)
Paternal origin	2 (11.8)
Total	17 (100)
Sex chromosome	
Maternal origin	2 (40.0)
Paternal origin	3 (60.0)
Total	5 (100)

Table V. Maternal meiosis stages of non-disjunction errors.

Maternal meiosis stage of non-disjunction errors	n (%)
Chromosome 21	
Meiosis I	36 (76.6)
Meiosis II	6 (12.8)
Maternal transition	4 (8.5)
Maternal reproductive cell chimeras	1 (2.1)
Total	47 (100)
Chromosome 18	
Meiosis I	7 (46.7)
Meiosis II	6 (40.0)
Maternal transition	2 (13.3)
Total	15 (100)

tested chromosome, with respective values of 99.4/99.9% for trisomy 21, 97.7/99.9% for trisomy 18, 92.9/99.9% for monosomy X and 90.6/100% for trisomy 13 (29).

Early studies suggested that the detection of sex chromosome aneuploidies by QF-PCR was poor (30,31), but more recent studies indicated a good performance (32-34). The difficulties are due to the low polymorphic level of sex chromosome STRs, but the discovery of appropriate markers improved the detection of sex chromosome aneuploidies (33,34). Hence, the choice of the primers for QF-PCR influences the results and diagnostic performance. Additional studies are required to address these issues.

NIPT for aneuploidy using cell-free DNA in maternal plasma is a novel direction for pre-natal screening and diagnosis. Clinical trials have demonstrated the efficacy of NIPT for trisomy 21, 18 and 13 in high-risk females, but positive NIPT results must be confirmed using invasive techniques (35). Of note, the NIPT data for the three abovementioned aneuploidies had 100% (or close to) diagnostic sensitivity and specificity; the test also correctly identified the fetal sex in all cases (36-38). However, it must be emphasized that for aneuploidies, the diagnostic performance of cell-free fetal DNA methods, including QF-PCR, may be affected by disease prevalence and placental mosaicism, and QF-PCR should be considered, for now, as a screening test (29). In the present study, chromosome analysis and QF-PCR detection indicated that the true-positive rates of NIPT for trisomy 21 and 18 were 93.5 and 83.3%, respectively. These discrepancies among previously published studies may be due to differences in populations regarding factors including genetics or polymorphisms. Additional studies are required to examine this issue.

Determining from which parent the aneuploidy originates may be useful in certain situations, e.g. in egg or sperm donation, or to determine which parent is at higher risk of yielding aneuploid gametes (39). Aneuploid embryos mostly occur due to maternal aneuploid gametes, but 1-2% of aneuploid embryos are due to aneuploid gametes from the sperm. The aneuploidy incidence of oocytes is higher than that of sperm due to more effective checkpoints in the processes of spermatogenesis compared to oogenesis (40). This strong maternal bias occurs mainly in autosomal chromosomes, and sex chromosome abnormalities (e.g. those associated with Klinefelter's syndrome) are usually from the father (41). The present study indicated that 85.4% of the cases of trisomy 21 had a maternal and 9.1% a paternal source, while the source was unknown for 5.5% (the selected markers were present in the mother and father, and the exact source remained undetermined), as supported by previous studies (20,42). Studies suggested that 5-9% of trisomy 21 cases result from paternal meiosis errors (43,44). The development of complete human

gametes involves two meiotic divisions. The first meiotic division is the separation of homologous chromosomes and the second separates sister chromatids. Previous studies suggested that in trisomy 21, more errors occur in meiosis I than in meiosis II (45). In the present study, for trisomy 21, 76.6% meiosis I and 12.8% meiosis II errors, 8.5% maternal transition and 2.1% maternal reproductive cell chimeras were detected. This result was in disagreement with a previous study from Europe (42). Differences in ethnicity and genetics may explain, at least in part, the discrepancies.

Trisomy 18 is the second most common trisomy syndrome after trisomy 21 (5). It is also important in pre-natal diagnosis due to being associated with a high risk of fetal loss and still-birth (46-48). The present study indicated that in the context of mid-pregnancy diagnosis, trisomy 18 accounted for 19.0% of the cases, second to trisomy 21. The extra chromosome of trisomy 18 cases was usually of maternal origin. Indeed, 88.2% of cases of trisomy 18 were maternal and 11.8% were paternal. This is different from other autosomal abnormalities, which more frequently arise in meiosis I. About half of the non-disjunction errors occur in meiosis II of oocytes (49,50). The results of the present study were consistent with these results and indicated that the stages of meiotic separation affected in trisomy 18 were 46.7% for meiosis I, 40.0% for meiosis II and 13.3% for maternal transition.

The present study included certain cases of trisomy 13 and sex chromosome aneuploidy. In the two cases in which the parents' blood was provided, the aneuploidies were of paternal origin. Paternal sex chromosome non-disjunction is associated with reduced recombination between X and Y (51,52). It has been indicated that G-group and sex chromosomes are more likely to exhibit aneuploidy than other chromosomes (53). Most individual autosomes have a disomic frequency of about 0.1%, but sex chromosomes have a disomic frequency of about 0.43% (54), likely due to these chromosomes normally having only one crossover; if recombination fails and this single chiasma is not present, the homologous chromosomes do not properly move to opposite poles (55).

QF-PCR is not a perfect technique and the results may be negative even in the presence of fetal abnormalities on ultrasound. Array comparative genomic hybridization (aCGH) is able to detect copy number variations with high resolution (56). In cases of fetal abnormalities identified on ultrasound but with negative QF-PCR results, aCGH may indeed detect a gain or deletion in a portion of a chromosome. In the present study, no cases of negative QF-PCR were encountered due to the strict selection criteria applied to the population (pregnancies at high risk of aneuploidy). aCGH will be evaluated in a future large-scale screening study performed by our group. In addition, QF-PCR cannot fully replace the traditional karyotype analysis, but it may be used to screen for common chromosomal aberrations, including trisomy 21, 18 and 13, and sex chromosome aneuploidy (9-13,29). The advantage of the technique is that the results may be quickly obtained. In the presence of normal QF-PCR screening test results but fetal abnormalities on ultrasound, more invasive, time-consuming and costly karyotyping may be performed.

In the present study, 62 subjects had ≥2 fetal ultrasound abnormalities; among them, karyotyping indicated that 18 subjects had aneuploidies. QF-PCR confirmed the karyotyping results. In addition, karyotyping also identified three cases of chromosomal translocation, which were not detected by QF-PCR. However, the concordance between QF-PCR and karyotyping in examining aneuploidies in chromosomes 21, 18 and 13, and sex chromosomes in the second trimester was 100%. The frequency of aneuploidies was 29.0% (18/62) for the cases with ≥2 fetal ultrasound abnormalities in the second trimester. Therefore, for those cases with fetal ultrasound abnormalities in the second trimester, particularly those with ≥2 abnormalities, pre-natal diagnosis should be performed, even if pre-natal screening was not performed or suggested a low risk, in order to rule out any chromosomal abnormalities (57).

Of note, the present study had certain limitations. The patients were from a single center and their number was relatively small. However, as the study population, all females with a high risk of aneuploidies were selected from all consecutive and consenting females encountered during the recruitment period according to the criteria. In addition, due to limited funding, the recruitment period was restricted to 18 months. Of note, increasing the sample size would increase the likelihood of observing false-negative and false-positive results. A multi-center study may further address this issue. In the present study, only the most common aneuploidies were examined, which is a limitation of QF-PCR itself. Additional studies are required to improve the generalizability of these results. It is important to highlight that only a limited number of chromosomes were tested using QF-PCR in the present study. Testing for additional chromosomes should be developed, examined for cost-benefits and implemented if required (11,58).

In conclusion, the combination of NIPT and QF-PCR may become a rapid and effective method for the detection of fetal aneuploidy. Assessment of parental origin and meiosis stage of non-disjunction errors by QF-PCR may provide additional genetic information for the diagnosis and management of aneuploidy compared to karyotyping alone.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XW conceived and supervised the study; PH, BJ and LR performed the experiments; BJ and JZ analysed the data; PH and JL wrote the manuscript; XW and QL revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hebei General Hospital (Shijiazhuang, China). All participants provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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