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CASE REPORT

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Detection of partial deletion and mosaicism using quantitative fluorescent polymerase chain reaction: Case reports and a review of the literature

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Abstract

Background: Aneuploidy of chromosomes 13, 18, 21, X, and Y can be detected by the quantitative fluorescence polymerase chain reaction (QF-PCR) performed with short tandem repeat (STR) markers. Although QF-PCR is designed to detect whole chromosome trisomy, the partial deletion or mosaic of chromosomes may also be detected.

Methods: Partial deletion or mosaic of chromosomes in three cases was detected by QF-PCR. Karyotyping and chromosome microarray analysis(CMA) were performed. We further reviewed the clinical utility of QF-PCR in detecting mosaicisms and deletions/duplications.

Results: QF-PCR demonstrated structurally abnormal 21, X, and Y chromosomes in primary amniotic cells. QF-PCR results in these three cases showed abnormal peak height/peak area, which could not be interpreted according to the kit instructions. QF-PCR results suggested that there were partial deletions or mosaicism, which were confirmed by karyotyping and CMA.

Conclusion: In addition to detecting trisomies of whole chromosomes, QF-PCR can also detect deletion and mosaicism of chromosomes 13, 18, 21, X, and Y, which could suggest the presence of copy number variants (CNVs). Additional testing with genetic technologies, such as karyotyping or microarrays, is recommended when an uninformative pattern is suspected.

KEYWORDS
chromosomal microarray analysis, karyotyping, mosaic, partial deletion, QF-PCR

1 | INTRODUCTION

The most common type of chromosome abnormality is known as aneuploidy, which is also the most common fetal genetic disorder detected by prenatal diagnosis. The most common chromosome abnormalities include trisomy 13, 18, 21 and sex chromosomal abnormalities, such as Klinefelter syndrome (47,XXY), Jacobs syndrome (47,XXX), XYY syndrome (47,XYY), and Turner syndrome (45,X), which account for more than 80% of the clinically relevant chromosomal abnormalities. Karyotyping can detect chromosomal

abnormalities with unclear or mild clinical relevance, which can cause patient anxiety and emotional dilemmas concerning the continuation of pregnancy. Therefore, high accuracy and rapid detection methods are needed to reduce maternal anxiety and speed up intervention.[1] In invasive prenatal diagnosis, karyotyping on amniocytes is considered the gold standard. Karyotype analysis takes a long time and quantitative fluorescence polymerase chain reaction (QF-PCR) can be completed within 24–48 h at low cost. In this study, QF-PCR was used to detect prenatal specimens, and the results of chromosome karyotypes and chromosome microarray analysis (CMA) were compared to explore the application value of QF-PCR in the prenatal diagnosis of fetal chromosomal abnormalities. We further reviewed the clinical utility of QF-PCR in detecting mosaicism and deletions/duplications.

2 | CASE INTRODUCTION

2.1 | Case 1

A 33-year-old woman underwent amniocentesis at 17 weeks of gestation because her first-trimester screening revealed that the fetus had a high risk for Down syndrome. QF-PCR results indicated the presence of mosaic trisomy 21. The karyotype was identified as mos 47,XY,+21,der(21;21)(q10;q10)[13]/46,XY[103], as shown in Figure 1. The pregnant woman chose to terminate the pregnancy.

2.2 | Case 2

A 25-year-old woman had a failed noninvasive prenatal testing at 17+ weeks of gestation, so amniocentesis was performed. The QF-PCR assay detected a decreased chromosome Y number and fragment deletion. The karyotype analysis result was mos 45,X[19]/46,X,Yqh-[96]. CMA identified a 10.1 Mb deletion at Yq11.221–q11.23 and a 16 Mb mosaic deletion at Yp11.32–q11.221, as shown in Figure 2. Yq11.221–q11.23 contains the azoospermia factor b + c region, which is a critical region for spermatogenesis, and its deletion can lead to azoospermia. The pregnant woman delivered a male infant with a normal external genital appearance at 39 weeks.

2.3 | Case 3

Amniocentesis was performed at 17 weeks of gestation due to sex chromosome abnormalities indicated by non-invasive prenatal testing. QF-PCR results indicated partial deletion of the X chromosome. The karyotype analysis result was 46,X,?psu dic(X;22)(p11.23;q11.21). Two chromosomal abnormalities were detected by CMA, as show in Figure 3. A 1.7 Mb duplication occurred on chromosome 22q11.1–q11.21, including 11 genes annotated in the Online Mendelian Inheritance in Man (OMIM) database. This copy number variant (CNV) overlaps with cat-eye syndrome (OMIM #115470), which is characterized by mental retardation, coloboma iris, genitourinary abnormalities, and anal atresia. A deletion of 56.0 Mb at Xp11.23–pter covered almost the entire short arm of the X chromosome, including 440 genes. Turner syndrome has been associated with this deletion and is characterized by short stature, abnormal skeletal development, and gonadal dysplasia. The pregnant woman chose to terminate the pregnancy.

3 | DISCUSSION

QF-PCR in detecting mosaicism and deletions/duplications has been used previously, as shown in the review of the literature displayed in Table 1. QF-PCR can detect mosaicism when the abnormal cell line contributes at least 10% of the whole sample.[2–10] In this study, we observed bimodal area ratios of STR markers on chromosome 21 that were around 0.7 and 1.4, which did not reach the standard for trisomy (bimodal area ratios <0.65 or >1.8), suggesting that there was mosaicism for trisomy 21 (Figure 1A,B). The result of karyotype analysis was mos 47,XY,+21,der(21;21)(q10;q10)[13]/46,XY[103] (cultured cells). In the present case, all the markers were diallelic and this may be indicative of a post-zygotic non-disjunction event. As described in the literature, when the bimodal area ratio is in the middle range (1.4 ~ 1.8)/(0.65 ~ 0.8), follow-up studies using either fluorescent in situ hybridization (FISH) or karyotype analysis are recommended.[2–5]

QF-PCR cannot detect inversions or translocations or when the deletion/duplication regions are not within the STR marker range.[11–13] Four cases in Table 1 had deletion or duplication regions containing the STR sites designed in the QF-PCR kit, which can be detected.[10,14–17] This result is consistent with the conclusion of our study. For example, Figure 2A,B of case 2 showed that AMXY (Xp22.3/Yp11.2) with a ratio of 2.0 and SRY (Yp11.2) presented a low peak, which indicates a copy number loss in the Yp11.2 region; the peak area ratio of TAF9b (3P24.2/Xq21.1) was 2:1, indicating that the presence of monosomy X, and the DXYS267 (Xq21.31/Yp11.31) peak height ratio was 0.66, which indicated that there was a deletion at the Yp11.2 region. DYS448 (Yq11.2) showed no peak height/area signal, indicating the loss of the Yq11.2 region. The result of karyotype analysis was mos 45,X[19]/46,X,Yqh-[96]; CMA detected a 10.1 Mb deletion at Yq11.221–q11.23 (chr Y:18,674,015–28,799,654) and a 16 Mb mosaic deletion at Yp11.32–q11.221 (chr Y: 2,650,424–18,641,290) (representing a proportion of mosaicism of 20%). The pregnant woman delivered a male infant with normal external genital appearance at 39 weeks. Sex chromosome abnormalities may manifest during puberty. Here, we demonstrated that case 2 involved both mosaicism and a deletion, which has not been seen in the literature previously. With the development of molecular biology, a series of diagnostic techniques have been developed, such as FISH, array comparative genomic hybridization, and next-generation sequencing (NGS). All these methods can be used to diagnose aneuploidies, but exhibit respective shortcomings, such as poor locus specificity,

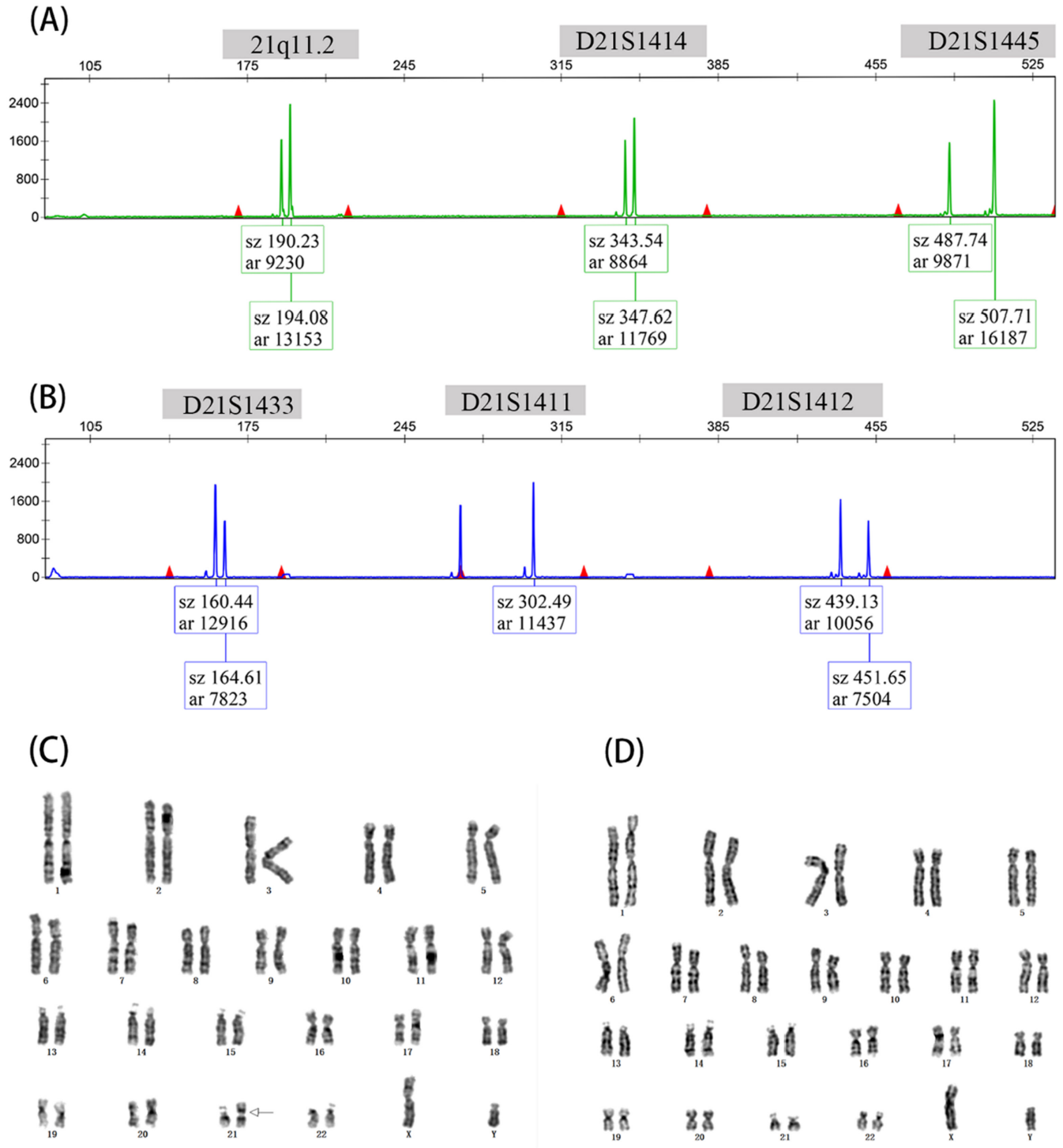

FIGURE 1 A, B: Among the six STR markers on chromosome 21, the peak height ratios of allele dosage was about 0.7 or 1.4, which did not meet the standard of trisomy (a ratio of ≤0.65 or ≥1.8), suggesting a fetus with low-level mosaicism for trisomy 21; C, D: Karyotype analysis result: mos 47,XY,+21,der(21;21)(q10;q10)[13]/46,XY[103]

complicated operation, high requirements for technical conditions, and expensive cost. Compared with FISH/CMA/CNV-seq, QF-PCR is markedly less labor-intensive, has a shorter turnaround time, and is inexpensive. QF-PCR can also be used to identify prenatal diagnostic samples for maternal cell contamination. QF-PCR could detect some cases of partial deletion/duplication based on the suggest rather than diagnose due to other possible factors which may affect the QF-PCR results, such as PCR efficiency and SNPs at the primer binding site. As stated in the literature, QF-PCR cannot determine the starting location and fragment length of the deletion/duplication region.[10,14–17] As shown in Figure 4, when two STR loci are designed to be far apart on chromosomes,

FIGURE 2 A: The presence of AMXY (Xp22.3/Yp11.2) with a ratio of 2.0 and SRY (Yp11.2) showed a low peak, which indicates that copy number loss in the Yp11.2 region; B: The peak area ratio of TAF9b (3P24.2/Xq21.1) was 2:1, indicating monosomy X. The DXYS267 (Xq21.31/Yp11.31) peak height ratio was 0.66, which indicates that there is a deletion at the Yp11.2 region. DYS448 (Yq11.2) showed no signal of peak height/area, indicating the loss of Yq11.2 region; C, D: Karyotype analysis result: mos 45,X[19]/46,X,Yqh-[96]; E: CMA: a 10.1 Mb deletion at Yq11.221–q11.23 (chrY: 18674015–28,799,654) and a 16 Mb mosaic deletion at Yp11.32–q11.221 (chrY: 2650424–18,641,290) (mosaicism proportion 20%)

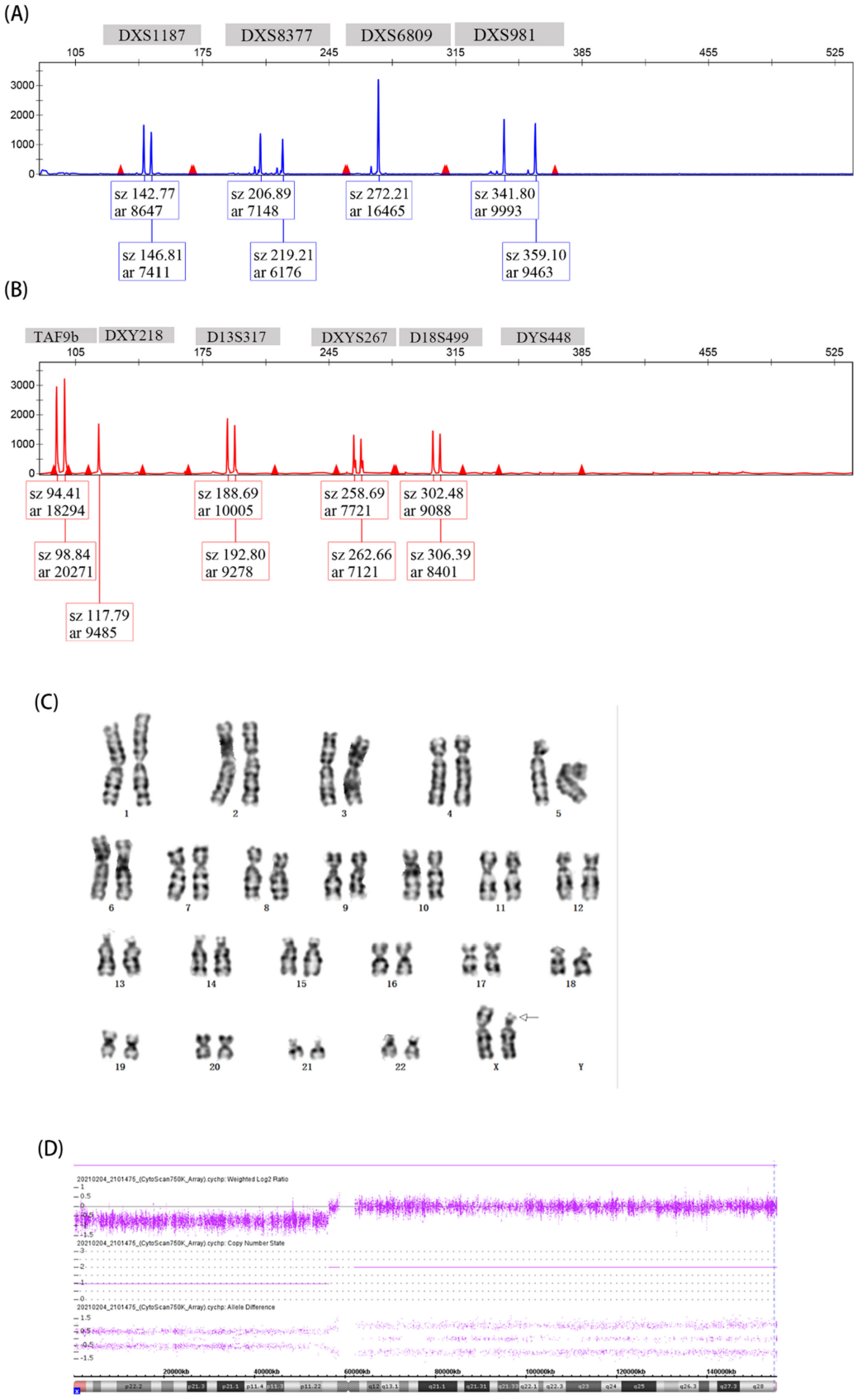

FIGURE 3 A: The four STR markers on X chromosome were bimodal, indicating the existence of two X chromosomes. B: The peak height ratios of DXY218 (Xp22.3/Yp11.2) are almost half that of TAF9b (3P24.2 /Xq21.1), indicating that the DNA copy number of Xp22.3 is half that of 3P24.2/Xq21.1; C: Karyotype analysis result: 46,X,?psu dic(X;22)(p11.23;q11.21); D: CMA: a duplication 1.7 Mb 22q11.1–q11.21 (16888900–18,630,593) and a 56 Mb deletion at Xp11.23-pter Xp22.33p11.23 (168552–48,183,279)

TABLE 1 QF-PCR detected mosaicism and deletions/duplications in our cases and published literature

Reference	Abnormal Type	STR	Karyotype analysis (uncultured cells)	CMA/NGS	Outcome and phenotype
Vicic A, et al.[2]	Sex chromosome mosaicism	Indicated abnormal	mos 45,X (90%)/46,XX (10%)		
Quaife R, et al.[3]	Mosaic trisomy 18	D18S386; D18S535; D18S380	mos 47,XN,+18 (40%)/46,XN (60%)		
Donaghue C, et al.[4]	Mosaic trisomy 13, 18, 21	All STR markers	Abnormal cell line contributes at least 15%		
Fan Z, et al[5]	Sex chromosome mosaicism	Segmental duplication of chromosomes X and Y	Mosaicism is completely detectable for proportions above 10%		
de Moraes RW, et al.[6]	Mosaic trisomy 13, 18	D18S390 (18q22.3–18q23); D18S391 (18p11.31); D18S535 (18q12.3); D18S819 (18q11.2); D18S976 (18p11.31); D13S305 (13q13.3); D13S628 (13q31.1); D13S634 (13q21.33); D13S742 (13q12.13); D13S797 (13q33.2)	mos 46, XY/47, XY, +18; mos 47, XX, +13/48, XX, +13 mar; mos 47, XX, +mar/47, XX, +13 (abnormal cell line contributes at least 15%)		
Cirigliano V, et al.[7]	Sex chromosome mosaicism	HPRT(Xq26.1); DXS6803 (Xq12–Xq21.33); DXS6809 (Xq); DXS8377 (Xq28); SBMA (Xq11.2–Xq12)	Mosaicism 45,X/46,XX; the aneuploid cell line was present in at least 20% of the cells.		
Cirigliano V, et al.[8]	Sex chromosome mosaicism	X22 (Xq28 Yq) XHPRT (Xq26.1)	45,X (10%)/46,XX (90%)		
Sun L, et al.[9]	Sex chromosome mosaicism	SD11QFYX X:Y = 1008:710 = 1.42	mos 46, XY (17%)/47, XXY (83%)		
Liu Y, et al[10]	Sex chromosome mosaicism	AMEL SRY	mos 45,X (5%)/46,X, idic(Y) (95%) mos 45,X (10%)/46,X, idic (Y)(90%)	A 10.1 Mb deletion at Yq11.221–q11.23 (chr Y: 17,073,540–27,176,992)	The pregnancy was terminated at 30 weeks of gestation
Le TNU, et al.[14]	A 9.9 Mb deletion at 18p11.32–11.22	D18S391 (18p11.31); D18S976 (18p11.2)	18p deletion	A 9.9 Mb deletion at 18p11.32–18p11.22 (chr 18: 618,247–10,597,239)	A 19-month-old female with short stature and motor skill and speech delays
Bhola SL, et al[15]	Partial trisomy 21q22.2–q22.3	D21S1411 (21q22.3); D21S1412 (21q22.2)	46, XY.ish der(16)ins(16;21)(q22;q22.2q22.3)	A 4.98 Mb duplication at 21q22.2–q22.3 (39,119,758–44,102,267)×3	The pregnancy was terminated before 20 weeks of gestation
Inkster A, et al[16]	Maternally inherited triplication	DXS1187 (Xq26.2)	46,XY	A 358 kb duplication at Xq26.2 (130,725,010–131,083,764)×3	A healthy newborn male

TABLE 1 (Continued)

Reference	Abnormal Type	STR	Karyotype analysis (uncultured cells)	CMA/NGS	Outcome and phenotype
Luo H, et al.[17]	Duplication at 18p of approximately 15.38 Mb	GATA178F11	47,XN,+mar	46,XN,dup (18p11.21p11.32). seq [GRCh37/ hg19](10,001–15,378,887)×4, with the duplicated fragment spanning approximately 15.38 Mb	The cardiac malformation of the fetus may be attributed to the partial duplication of chromosome 18p.
The present case 2	Deletion at Yq11.2; mosaic deletion at Yp11.32–q11.221	AMXY (Yq11.2); DXYS267 (Yp11.31); DYS448 (Yq11.2)	mos 45,X[19]/46,X,Yqh-[96]	10.1 Mb deletion at Yq11.221–q11.23 (chr Y: 18,674,015–28,799,654) and a 16 Mb mosaic deletion at Yp11.32–q11.221 (chr Y: 2,650,424–18,641,290)	a male infant with normal external genital
The present case 1	Mosaic trisomy 21	D21S1433 (21q21.1); 21q11.2; D21S1411 (21q22.3); D21S1414 (21q21.1); D21S1412 (21q22.2); D21S1445 (21q22.11)	mos 47,XY,+21,der(21;21) (q10;q10)[13]/46,XY[103]		Pregnancy termination
The present case 3	Deletion at Xp11.23–pter; 1.7 Mb duplication at 22q11.1–q11.21	DXY218 (Xp22.3)	46,X,?psu dic(X;22) (p11.23;q11.21)	A 1.7 Mb duplication at 22q11.1–q11.21 (16,888,900–18,630,593); a 56 Mb deletion at Xp11.23–pter Xp22.33p11.23 (168,552–48,183,279)	Pregnancy termination

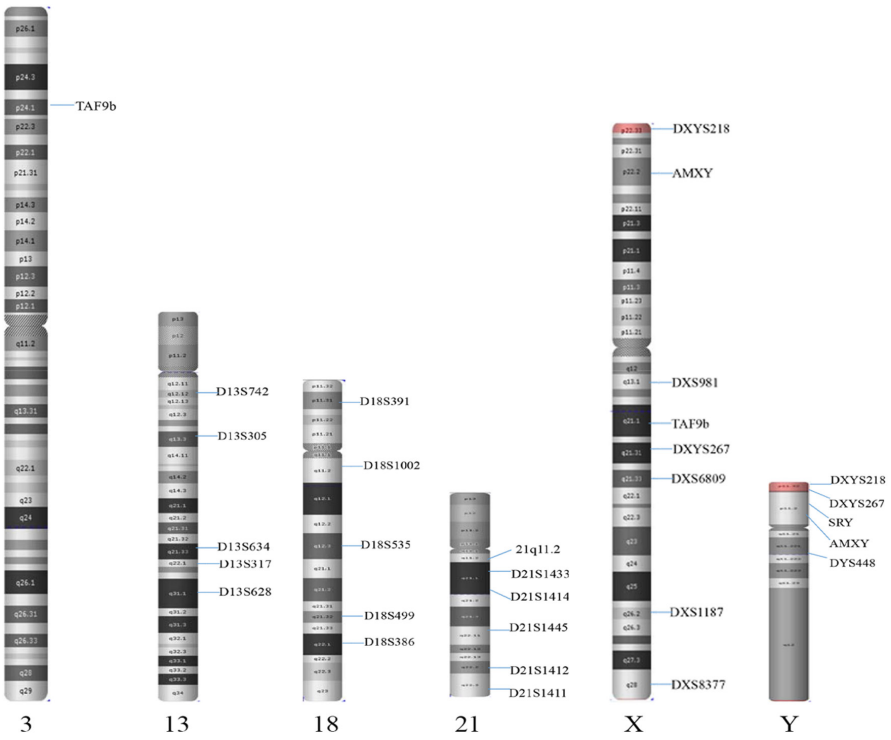

FIGURE 4 The marker locations of short tandem repeat (STR) markers on chromosomes 3, 13, 18, 21, X, and Y

the deletion/duplication region between the two STR loci will be missed. This study suggests that when developing multiple STR typing markers, the selected STR sites should be evenly distributed along the whole chromosome. Therefore, further analysis by NGS or microarray to confirm the diagnosis is necessary for those doubted cases.[6,10,14,15,18] QF-PCR cannot be used as a routine method to detect chromosome deletion or duplication, but only as a supplemental method for the discovery of deletions or duplications in the detection of 13/18/21/X/Y chromosome aneuploidy. In conclusion, QF-PCR can aid the discovery of deletion or duplication within STR loci, but further analysis by NGS or microarray to confirm the diagnosis is necessary.

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CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article are reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CONSENT FOR PUBLICATION

Written and informed consent was obtained from the patients for the publication of this case report and any accompanying images.

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