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Effective detection of 148 cases chromosomal mosaicism by karyotyping, chromosomal microarray analysis and QF-PCR in 32,967 prenatal diagnoses

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Abstract

Background Detection of mosaicism has always been difficult in prenatal diagnosis, which is to assess the value of karyotyping combined with three different molecular genetic tests for prenatal diagnosis. Retrospective review of chromosomal mosaicism (CM) was conducted in 32,967 pregnant women from January 2015 to December 2022.

Methods A total of 148 fetuses diagnosed with chromosomal mosaicism by karyotyping with copy number variant sequencing (CNV-seq)/ chromosomal microarray analysis (CMA) and quantitative fluorescent polymerase chain reaction (QF-PCR) were selected, and the results from three the methods were compared and further analyzed. The χ2 test for multiple group rates was for the 5 clinical prenatal diagnostic indication groups was used to do multiple comparison tests for statistical analysis. Inconsistent results between methods were identified and further analyzed.

Results A total of 148 CM cases was detected (0.45%, 148/32967), of which karyotyping was detected in combination with CMA in 73 cases (73/85), with CNV-seq in 5 cases (5/11), and with QF-PCR in 35 cases (35/52) and the mosaic conformity rates of the three methods compared with karyotyping were 85.9% (CMA), 67.3% (QF-PCR), and 45.5% (CNV-seq), respectively. There were 49 cases of autosomal mosaicism (49/148, 33.1%) and 99 cases of sex CM (99/148, 66.9%). There were 9 cases of small supernumerary marker chromosome (sSMC)with CMA detection clarified the origin of chromosome fragments. The non-invasive prenatal testing (NIPT) group and the ultrasound abnormality group had the highest detection rates, accounting for 35.1% and 22.3%.

Conclusions In chromosomal mosaicism, there are inconsistent results between different detection methods. Therefore, karyotyping combined with CMA/CNV-seq and FISH methods significantly improves the detection rate of chromosomal mosaicism and also confirms experimental data in the literature, which is of great value for prenatal diagnosis.

Keywords Chromosomal mosaicism, Karyotyping, Chromosomal microarray analysis, Copy number variant sequencing, Prenatal diagnoses

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Background

In prenatal diagnostics to detect cultured or uncultured fetal cells obtained from amniocentesis, fetal cell lineages are evaluated by classical cytogenetic methods and DNA-based molecular techniques [1].Amniotic fluid cells(AF) are considered to be the best specimen for fetal confirmation because it consists mainly of cells from fetal anatomical regions, including the urogenital tract, respiratory tract, and epithelial system, and represents different embryological layers, which can reflect the true mosaic type of the fetus. Chromosomal mosaicism (CM) is defined by the presence of two or more chromosomally distinct cell lines in an individual [1, 2].The main underlying mechanisms leading to mosaicism formation involve mitotic or meiotic non-disjunction errors result in chromosome non-segregation in zygote oocytes, anaphase lagging and trisomy rescue, endoreplication events, and uniparental diploidy (UPD) associated with trisomy rescue [3].

Human CM is an inherently complex phenomenon. Although the mechanisms of fetal mosaicism have been elucidated theoretically, fetal mosaicism itself remains a challenge to diagnose and counsel. Karyotyping and chromosomal microarray analysis (CMA) have both advantages and limitations. Conventional cytogenetics has been used to detect abnormalities in chromosome number and structure. For more than 50 years, karyotyping has been considered the gold standard for identifying chromosomal abnormalities in prenatal diagnosis and is the best candidate for detecting mosaics in terms of the cytogenetic, although some mosaic chromosome aberrations may be caused by culture processes. Conlin LK et al. studied 2019 pregnant women using CMA and found that mosaic aneuploidy accounted for 1% of all patients in the cytogenetic laboratory [4].

Recently, quantitative fluorescent polymerase chain reaction (QF-PCR) and CMA are two commonly used molecular techniques for detecting CM that bypass the need for culture and provide rapid results. CMA can only detect a low percentage of mosaics in 30%~50% of cases. Rapid QF-PCR using uncultured amniotic cells can be used as an adjunct to routine medium-term cytogenetics. The main limitation of QF-PCR is that it can only detect specific chromosomes 13/18/21/X/Y and cannot detect mosaic and structural abnormalities in the five chromosomes if they are below 15 ~ 20%. More recently, the technology of low-coverage massively parallel copy number variant sequencing (CNV-seq) has emerged as a widely used technique for the detection of chromosomal mosaic copy number variants with the advantages of high resolution and low cost [5, 6, 7, 8, 9].

However, there are limited retrospective studies on the accuracy and validity of CMA/CNV-seq, QF-PCR and karyotyping comparisons in the literature. Therefore, we conducted a study to evaluate the diagnostic outcomes and technical limitations of karyotyping, CMA/CNV-seq and QF-PCR in detecting mosaicism.

Methods

Study design

This study retrospectively analyzed 32,967 pregnant women who underwent genetic counseling and signed informed consent to receive interventional prenatal diagnosis at the department of Medical Genetics and Prenatal Diagnosis of the Sichuan Provincial Maternity and Child Health Care Hospital from January 2015 to December 2022. There were 148 cases of chromosomal mosaic fetuses diagnosed by the combined application of karyotyping and molecular testing techniques. Patients enrolled in the study ranged in age from 17 to 41years, with an average age of 29.5 years. The gestational age of the samples was 18 to 31^{+2}weeks, with an average age of 21^{+3}weeks.

In our study, inclusion criteria: clear indications for prenatal diagnosis, including: advanced age, NIPT, ultrasound abnormality, high risk of serum screening, adverse reproductive history and other relevant indications. Exclusion criteria: (1) gestational weeks < 9 weeks; (2) preeclampsia in recent months; (3) other contraindications to invasive prenatal diagnosis. This study did not count the mosaic fetuses detected by molecular biology alone and the non-mosaic fetuses analyzed by G-banding karyotyping.

Sample preparation

30 mL of amniotic fluid was collected under transabdominal ultrasound-guided amniocentesis was performed. Karyotyping was analyzed using 20 mL amniotic fluid cell cultures, and 10 mL was used for CNV-seq/CMA or QF-PCR. For AF samples with maternal cell contamination visible blood cell contamination before centrifugation or abnormal QF-PCR results, CMA was performed after AF cell culture.

Karyotyping

Karyotyping was performed independently by two individuals using two cell culture systems. BIO-AMF-2 Medium (Biological Industries, Israel) and Amniotic Fluid Cells Medium(He NENG BIO, China)was used for cell culture. Then, G-banding (300–400 bands) karyotyping analyses were performed on metaphases cells according to standard protocols. Karyotyping was performed following the International System for Human Cytogenetic Nomenclature (ISCN2020) standard, using a fully automated scanning system (Zeiss, Germany). The 20 metaphases were counted, and 5 karyotypes were analyzed. Chromosome karyotype mosaicism increased the count to 50 metaphases. Only level II and III mosaicism

were recorded. Level II mosaicism is defined as the presence of two or more cells with the same chromosome abnormality in a culture. Level III mosaicism is defined as the presence of two or more cells with the same chromosome abnormality in two independent cultures.

Copy number variant sequencing

Genomic DNA was extracted from AF using a QIAamp DNA Blood Mini Kit (Dusseldorf, Germany) according to the manufacturer's instructions. DNA content and quality were determined using nanodrop and agarose gel electrophoresis, respectively. Genomic DNA (10 ng) was fragmented, the DNA library was constructed, and low-depth high-throughput sequencing (Chigene Medical Laboratory, Beijing, China) was performed. The sequencing data were compared to the hg19 reference genome, and chromosomal aneuploidy variation and copy number variations (CNVs) above 100 kb were recorded and analyzed. The analysis of CNVs primarily involves the use of databases such as the Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home), the Database of Genomic Variation and Phenotype in Humans (DECIPHER, https://decipher.sanger.ac.uk), and the Online Mendelian Inheritance in Man (OMIM, https://omim.org). Additionally, the gene dosage effect database ClinGen (https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen) was consulted to analyze the significance of chromosom al deletions or duplications.

Chromosomal microarray analysis

Affymetrix CytoScan 750 K chips were used for CMA analysis following the CytoScanTM Assay Manual Protocol (Affymetrix, 2015). The chip contains 200,000 SNP probes and 550,000 CNV probes that cover all International Standard Cytogenomic Array (ISCA) CNVs and can discern micro deletion/duplication with size > 100 kb.

Quantitative fluorescent polymerase chain reaction

Amniotic fluid DNA extraction was performed using nucleic acid extraction kit (TIANGEN, China). The six STR sites on the chromosome were selected as AMEL, SRY, DXS1187, DS6809, DXS8377, and DYS981. Use 25ul, 23ul primer mixture and 2ul of DNA template (about 20 ng of DNA) as the amplification system. The PCR reaction was denaturation for 5 min at 95 C°, 95 C°30s, and 58 C°40s, 72 C°50s for 25 cycles, and stored at 72 C°10 min.The PCR products were detected using a 3500DX capillary electrophoresis instrument, and GeneMarkerV4.1 was used for result analysis.

Fluorescence in situ hybridization

Interphase FISH analysis of uncultured amniotic cells was performed using a CEP20 probe and 100 cells were counted using an Olympus Fluorescence Microscope-Model BX53/Leica Cyto Vision Analysis System for specific loci only.

Statistical analysis

Using case count versus percentage line counts, descriptive statistical analysis, statistical analyses were performed with SPSS software 25.0. The chi-square test for multiple comparisons were applied to analyze the statistical data. Differences were considered statistically significant when $P < 0.01$.

Results

Overall mosaicism data

To the best of our knowledge, this is the retrospective study in a Chinese population to assess the effectiveness of CNV-seq, CMA and QF-PCR methods. In our study, a total of 148 CM cases were detected in 32,967 pregnant women (0.45%, 148/32,967), and the mosaic conformity rates of karyotyping combined with CMA, CNV-seq and QF-PCR were 85.9%, 45.5% and 67.3%, respectively in Fig. 1. 26,833 amniotic fluid specimens with 5 prenatal diagnostic indications were analyzed, and the 148 cases of detected amniotic fluid mosaicism were classified into 5 groups (amniotic mosaicism not detected in other relevant indications).The non-invasive prenatal testing (NIPT)group detection mosaicism 52 cases, the highest proportion, mainly for sex chromosome abnormalities, followed by ultrasound abnormality group, 33 cases of mosaicism, high risk of serum screening detected 32 cases, advanced age group detected 25 cases, and adverse reproductive history group detected 6 cases. The R*2 table chi-square test for multiple group rates for the 5 prenatal diagnosis indications, and the results showed that all 5 groups exhibited statistically significant differences ($P < 0.01$), Table 1.

CNV-seq

Of the 11 pregnant women selected for CNV-seq, 5 (45.5%) were consistent with karyotyping. In one case, karyotype analysis suggested chromosome 21 mosaicism (7.1% mosaic ratio) but the CNV-seq test result was normal; 4 cases of karyotype analysis suggested sex chromosome low-proportional mosaicism, but the CNV-seq test results were also normal; another case of karyotype analysis suggested trisomy 20 (8% mosaic ratio), and CNV-seq results indicated deletion of chromosome 17, which was later verified by the fluorescence in situ hybridization(FISH)before amniotic fluid cell culture was verified to be a low-proportional mosaic trisomy 20, Table 2.

CMA

Among the 85 pregnant women selected for CMA, 73 cases had test results consistent with karyotyping, of

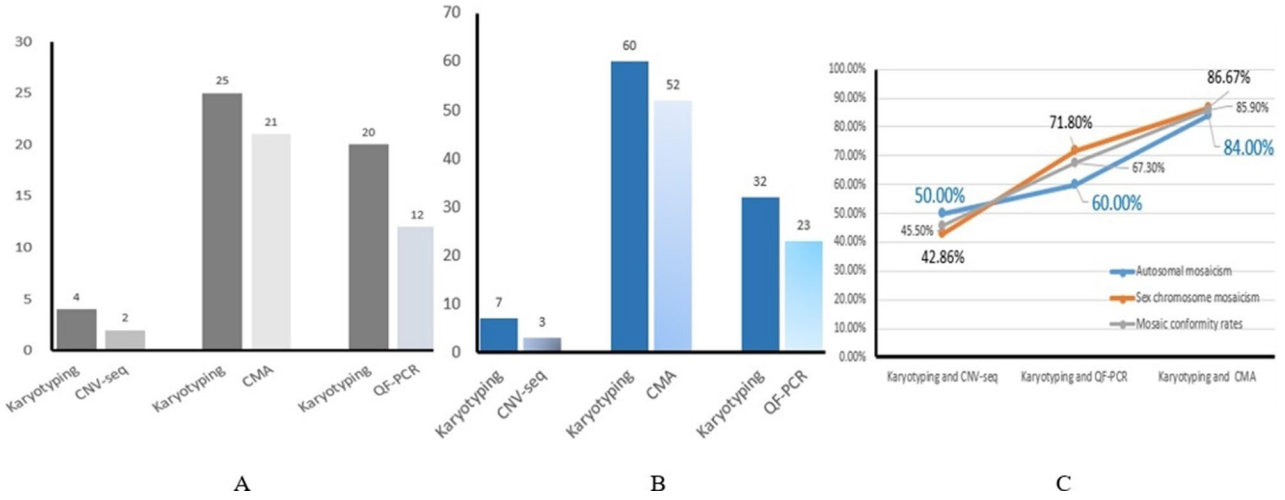

Fig. 1 (**A**) Autosomal mosaicism. X-axis: methods. Y-axis: patient number. (**B**) Sex chromosome mosaicism. X-axis: methods. Y-axis: patient number. (**C**) Mosaic conformity rates of the three methods. X-axis: methods. Y-axis: mosaic conformity rates. Supplement. CNV-seq, Copy number variant sequencing; CMA, Chromosomal microarray analysis; QF-PCR, Quantitative fluorescent polymerase chain reaction

Table 1 Results of multiple comparisons of 5 prenatal diagnostic indications groups

Prenatal diagnostic indications	Non-Mosaicism(*n*)	Mosaicism(*n*/%)	Total(*n*)
High risk of serum screening	6946	32(21.6)	6978
Adverse reproductive history	1448	6(4.1)	1454
NIPT	1326	52(35.1)	1378
Ultrasound abnormality	7926	33(22.3)	7959
Advanced age	9039	25(16.9)	9064
Total	26,685	148(100)	26,833

Abbreviations. NIPT, the non-invasive prenatal testing

which 11 cases had karyotyping suggestive of chromosomal structural abnormalities, and the results of each karyotype and the corresponding CMA are shown in Table 3. There were 9 cases with karyotype suggestive of supernumerary marker chromosome (sSMC), and the CMA results were clear about the origin of chromosome fragments in Table 4. Except for 7 cases with low-proportional karyotype mosaicism and normal CMA results, there were another 5 cases (5.9%) were inconsistent with the results of karyotyping. Including karyotyping analysis suggested that 1 case was a chromosomal balanced translocation and 2 case was mosaic trisomy 20,1 case of mos 45,X/46,XX, 1 case of sSMC, but no abnormality was found in CMA. In order to further validate the experimental specimens, the remaining amniotic fluid cells were collected from the culture, and the CNV-seq test was performed to verify the results, which showed that the whole chromosome 20 was mosaic duplicated with a proportion of 12%, which was consistent with the results of the karyotyping analysis, Table 5.

Autosomal mosaicism

In our study, a total of 49 cases (33.1%) of autosomal mosaicism were detected, including 40 cases of autosomal numerical abnormalities and 9 cases of autosomal structural abnormalities. Among the number abnormalities, mosaicism of chromosome 8,9,17,18,20, 21and marker chromosome were detected, at most 24 cases of trisomy 21, followed by 6 cases of sSMC, and 4 cases each of trisomy 9 and 20. Among the structural abnormalities, there were 4 cases of chromosomal translocations, 2 cases of equal arms, 1 case of deletions, 1 case of inversions, and 1 case of ring chromosomes. Comparing the three methods with karyotyping, CMA had the highest mosaic conformity rate of 84%, QF-PCR and CNV-seq were 60% and 50%, respectively, Fig. 2.

Sex chromosome mosaicism

A total of 99 cases (66.9%) of sex CM were detected, including 85 cases of sex chromosome numerical abnormality and 14 cases of sex chromosome structural abnormality, respectively. Among the sex chromosome number abnormalities, there were 42 cases of X mosaicism, 32 cases of Y mosaicism and 11 cases of marker mosaicism, and the mosaic conformity rates of the three methods compared to karyotyping were 73.8%, 75% and 100%, respectively. The mosaic fraction determined by FISH for all involved sex chromosomal mosaicism ranged from 7 to 42%. Of the structural abnormalities of the sex chromosomes, 6 cases were Y chromosome deletions, 3 each of X chromosome deletions and isochromosome X, as well as 1 each of X chromosome rearrangements and pseudodicentric chromosome X, Table 6.

Table 2 Karyotype analysis and CNV-seq results on 11 mosaic cases

Case ID	Indication(s)	Karyotype	CNV-seq	Consistency of the two approaches
1	Advanced age	mos 47,XY,+21[8]/46,XY[104]	Normal	Inconsistent
2	Advanced age	mos 45,X[15]/46,XY[35]	Normal	Inconsistent
3	Adverse reproductive history	mos 45,X[3]/46,XX[47]	Normal	Inconsistent
4	High risk of serum screening	mos 47,XY,+20[45]/46,XY[5]	seq[GRCh37]dup(20)(p13q13.33)mos chr20:g.60001_62960000dup	Consistent
5	NIPT	mos 46,X,+mar[11]/45,X[9]	seq[GRCh37]dup(Y)(p11.31q11.221)chrY: g.2660001_19560000dup, seq[GRCh37]del(Y)(q11.222q11.23) chrY: g.20610001_28060000del	Consistent
6	Advanced age	mos 47,XY,+17[2]/46,XY[48]	seq[GRCh37]dup(17)(p13.3q25.3)mos chr17:g.1_81195210dup	Consistent
7	NIPT	mos 45,X[46]/46,XY[4]	seq[GRCh37]del(Y)(p11.32q12)mos chrY: g.10001-59360000del.	Consistent
8	High risk of serum screening	mos 45,X[6]/46,XX[44]	Normal	Inconsistent
9	Ultrasound abnormality	mos 47,XYY[1]/48,XYYY[2]/46,XY[47]	seq[GRCh37]dup(Y)(p11.32q12)mos chrY: g.10001_59360000dup	Consistent
10	Ultrasound abnormality	mos 45,X, t(3;19)(q21;q13.1)[3]/46,XX, t(3;19)(q21;q13.1)[47]	Normal	Inconsistent
11	Ultrasound abnormality	mos 47,XX,+20[4]/46,XX[46]	seq[GRCh37]del(17)(q12q12)chr17:g.34800001_36300000del	Inconsistent

Abbreviations. CNV-seq, copy number variant sequencing; NIPT, non-invasive prenatal testing

Discussion

CM can be defined as the coexistence of normal cells with abnormal cells or multiple types of abnormal cells. Studies have now demonstrated that mosaic embryos are common and involve almost all types of chromosomal alterations, including trisomies, monosomies, duplications, deletions, inversions, translocations, rings, homologous chromosomes and other rare alterations [10].

Microdeletions/microduplications caused by chromosome copy number abnormalities have been found to be the main cause of congenital growth retardation, structural malformations, and various other genetic syndromes. However, the prenatal diagnosis of some chromosomal abnormalities, especially the presence of true and false mosaicism, the correlation between the clinical phenotype and the percentage of mosaicism, as well as the organizational distribution of genetic changes, has become a hot topic and a challenge for clinical practice in recent years. In this study, out of 32,967 pregnant women, 0.45% of the fetuses were diagnosed with CM using karyotyping, CNV-seq/CMA and QF-PCR at our center in a seven-year period between January 2015 and December 2022.This is close to the chromosomal mosaic detection rate of 0.42% reported by Zhang Lifang et al. [11]. Therefore, rational selection of karyotyping combined with other molecular genetic testing methods is an important factor in improving the detection rate of CM.

To the best of our knowledge, this is the retrospective study evaluating the effectiveness of CNV-seq, CMA, QF-PCR methods in detecting mosaicism, benchmarking against karyotyping as a reference. Based on the microarray platform and the type of copy number variation detected, CMA technology can be divided into two main categories: array comparative genomic hybridization (aCGH) and single nucleotide polymorphism arrays (SNP arrays). aCGH is a lower-density microarray compared to SNP arrays, capable of completing the amplification process within 12 ~ 15 h, which is significantly faster than SNP arrays that require 30 ~ 40 h. However, aCGH is unable to detect polyploidy or chromosomal rearrangements such as translocations, inversions, and UPD. Additionally, the resolution of this technique and its ability to detect mosaicism are limited [12]. In our study, 2450 cases were detected for karyotyping and CNV-seq, with a detection rate of 0.2% (5/2450); 18,934 cases for karyotyping and CMA, with a detection rate of 0.39% (73/18934); 11,583 cases for karyotyping and QF-PCR, with a detection rate of 0.29% (34/11583). This rate is similar to the 0.1 ~ 0.5% chromosomal mosaicism rate in in amniotic fluid samples. Therefore, this study chose SNP arrays, which are usually located in non-exotic segments of the genome and compared with human reference genes [13, 14].

This method can detect CNVs, but also to identify polyploids of meiotic origin, uniparental diploids, and low proportional mosaicism. Karyotyping and CMA have their own advantages, and their combined application can compensate for their respective shortcomings.

Large segment translocations, deletions, and duplications can be visualized morphologically, but the long

Table 3 Karyotype analysis and CMA results on 73 mosaic cases

Case ID	Karyotype	CMA
1	mos 47,XY,+21[4]/46,XY[46]	arr[GRCh37] (21)x2 ~ 3
2	mos 47,XY,+21[5]/46,XY[95]	arr[GRCh37] (21)x2 ~ 3
3	mos 47,XY,+21[6]/46,XY[86]	arr[GRCh37]13q31.3(92142069_94990520)x3 21q11.2q22.3(15484314_48091215)x2 ~ 3
4	mos 47,XX,+21[6]/46,XX[44]	arr[GRCh37] (21)x2 ~ 3
5	mos 47,XY,+21[8]/46,XY[42]	arr[GRCh37] (21)x2 ~ 3
6	mos 47,XX,+21[10]/46,XX[40]	arr[GRCh37] (21)x2 ~ 3.
7	mos 47,XY,+21[11]/46,XY[37]	arr[GRCh37] (21)x2 ~ 3
8	mos 47,XX,+21[11]/46,XX[39]	arr[GRCh37] (21)x2 ~ 3
9	mos 46,XY,+21,rob(21;21)(q10;q10)[11]/46,XY[55]	arr[GRCh37] (21)x2 ~ 3
10	mos 47,XY,+21[26]/46,XY[6]	arr[GRCh37] (21)x2 ~ 3
11	mos 47,XX,+21[47]/46,XX[3]	arr[GRCh37] (21)x2 ~ 3
12	mos 47,XY,+18[12]/46,XY[8]	arr[GRCh37] (18)x2 ~ 3
13	mos 47,XX,+9[10]/46,XX[40]	arr[GRCh37] (9)x2 ~ 3
14	mos 47,XX,+9[3]/46,XX[47]	arr[GRCh37] (9)x2 ~ 3
15	mos 47,XX,+9[6]/46,XX[14]	arr[GRCh37] (9)x2 ~ 3.
16	mos 46,XY, r(14)(p11.2q32)[11]/45,XY,-14[10]	arr[GRCh37]14q11.2q32.33(20457781_104968743) x1 ~ 2, 14q32.33(104968743_107349540) x1,15q11.2(22822019_23085218) x1
17	mos 47,XX,+i(12)(p10)[14]/46,XX[6]	arr[GRCh37]12p13.33-p11.1(189216-34060203)×4 33,871 kb
18	mos 47,XY, i(12)(p10)[14]/46,XY[6]	arr[GRCh37]12p13.33p11.1(190,462 – 34,078,152) x3 ~ 4.
19	mos 47,XX,+i(12)(p10)[5]/46,XX[45]	arr[GRCh37]12p13.33p11.1(173,787 – 34,835,641) x2-3 34.6 Mb, 12q11q12(37,857,931 – 38,728,412) x4 870.4Kb
20	mos 47,XX,+dup(15)(pter→q11.2::q13.2→pter)[8]/46,XX[42]	arr[GRCh37]15q11.2q13.2(22822019_30351527)x3
21	mos 47,XX,+dup(18)(pter→q21.2::q21.2→pter)[11]/47,XX,+18[9]	arr[GRCh37]18p11.3q21.2(136228-49,871,597) x3, 18q21.2qter (49,871,597 – 77,989,124) x2 ~ 3.
22	mos 45,X[5]/46,XX[15]	arr[GRCh37] arr(X)x1 ~ 2.
23	mos 45,X[6]/46,XX[44]	arr[GRCh37] (X)x1 ~ 2
24	mos 45,X[6]/46,XX[44]	arr[GRCh37] (X)x1 ~ 2
25	mos 45,X[6]/46,XY[14]	arr[GRCh37] (Y)x0 ~ 1
26	mos 45,X[6]/46,XX[39]	arr[GRCh37] (X)x1 ~ 2.
27	mos 45,X[7]/46,XX[43]	arr[GRCh37] (X)x1 ~ 2
28	mos 45,X[7]/46,XY[13]	arr[GRCh37] (Y)x0 ~ 1
29	mos 45,X[7]46,XX[43]	arr[GRCh37] (X)x1-2; Xp22.33(484,177–602,731) x1
30	mos 45,X[8]/46,XY[12]	arr[GRCh37] (X)x1 ~ 2
31	mos 46,X,+mar[41]/45,X[9]	arr [GRCh37] (Yp)x1 ~ 2, (Yq)x0
32	mos 45,X[10]/46,XX[10]	arr[GRCh37] (X)x1 ~ 2
33	mos 45,X[10]/46,XX[10]	arr[GRCh37] (X)x1 ~ 2
34	mos 45,X[11]/46,XX[9]	arr[GRCh37] Xp22.33p21.2(168,552 – 31,365,680) x1-2, Xp21.2(31,365,681 – 31,445,411) x1, Xp21.2q28(31,445,412 – 15 5,233,098) x1-2
35	mos 45,X[11]/46,XX[39]	arr[GRCh37] (X)x1 ~ 2.
36	mos 45,X[11]/46,XX[39]	arr[GRCh37] (X)x1 ~ 2.
37	mos 45,X[13]/46,XX[37]	arr[GRCh37] (X)x1 ~ 2
38	mos 45,X[15]/46,XY[36]	arr[GRCh37] (Y)x0 ~ 1
39	mos 45,X[17]/46,XX[13]	arr[GRCh37] (X)x1 ~ 2
40	mos 45,X[19]/46,XX[31]	arr[GRCh37] (X)x1 ~ 2
41	mos 45,X[19]/46,XX[31]	arr[GRCh37] (X)x1 ~ 2.
42	mos 45,X[20]/46,XX[30]	arr[GRCh37] (X)x1 ~ 2
43	mos 45,X[21]/46,X,+mar[9]	arr[GRCh37]Xp22.33p11.22(296520_53256474) x1, Xp11.22q21.1(53284533_83969013) x1 ~ 2, Xq21.1q28(84015976_155221912) x1
44	mos 45,X[22]/46,XY[28]	arr[GRCh37] (Y)x0 ~ 1

Table 3 (continued)

Case ID	Karyotype	CMA
45	mos 45,X[23]/46,X,+mar[7]	arr[GRCh37]Xp22.33p11.3(168552_44764775) x1, Xp11.3q22.1(44999230_100779320) x1-2, Xq22.1q28(100897399_155233098) x1.
46	mos 45,X[30]/46,X,+mar[25]	arr[GRCh37] Xp22.33p11.21(296,520 – 55,976,925) x1, Xp11.21 q26.2(56,151,838 – 133,279,271) x1 ~ 2, Xq26.2q28(133,382,540 – 155,086,462) x1.
47	mos 45,X[35]/46,X,+mar[8]/46,X, del(Y)(q11.22q12)[7]	arr[GRCh37] Yq11.222q12(21,035,228 – 59,324,918) x0 38.29 Mb
48	mos 45,X[43]/46,XX[7]	arr[GRCh37] (X)x1 ~ 2.
49	mos 45,X[46]/46,XY[4]	arr[GRCh37] (Y)x0 ~ 1.
50	mos 45,X[48]/46,XX[2]	arr[GRCh37] (X)x1 ~ 2
51	mos 45,X[48]/46,XX[2]	arr[GRCh37]16p13.3(215499_232685)x1 17.19 kb, Xp22.33q28(1_155270560) x1 ~ 2
52	mos 45,X[48]/47,XX,+mar[2]	arr[GRCh37] (X)x1 ~ 2
53	mos 45,X[68]/46,X,+mar[5]/46,XY[23]	arr[GRCh37]Yq11.223q12(24377969_59324918)x0
54	mos 47,XXX[2]/45,X[48]	arr[GRCh37] (X)x1 ~ 2
55	mos 47,XXX[5]/46,XX[49]	arr[GRCh37] (X)x2 ~ 3.
56	mos 47,XXY[6]/46,XY[44]	arr[GRCh37] (X)x1 ~ 2.
57	mos 47,XXY[10]/46,XY[40]	arr[GRCh37] (X)x1 ~ 2.
58	mos 47,XXX[11]/46,XX[39]	arr[GRCh37] (X)x2 ~ 3.
59	mos 47,XYY[11]/46,XY[39]	arr[GRCh37] (Y)x1 ~ 2.
60	mos 47,XXY[12]/46,XY[8]	arr[GRCh37] (X)x1 ~ 2.
61	mos 47,XXX[26]/45,X[24]	arr[GRCh37] (X)x2 ~ 3
62	mos 47,XXY[26]/46,XY[24]	arr[GRCh37] (X)x1 ~ 2, (Y)x1
63	mos 47,XXY[48]/46,XY[2]	arr[GRCh37] (X)x1 ~ 2, (Y)x1
64	mos 47,XYY[48]/46,XY[2]	arr[GRCh37] (Y)x1 ~ 2
65	mos 45,X[8]/46,X, del(X)(q22)[23]	arr[GRCh37]Xp22.33q21.31(1_88233437)x1 ~ 2, Xq21.32q22.2(92433472_103158324) x2 ~ 3, Xq22.2q28(103409821_155221912) x1
66	mos 45,X[32]/46,X, del(X)(q23)[18]	arr[GRCh37] Xp22.33q23(296,520 – 114,124,293) x1 ~ 2, Xq23q2 8(114,220,735 – 155,221,912) x0 ~ 1.
67	mos 45,X[15]/46,X, i(X)(q10)[7]	arr[GRCh37]Xp22.33p11.21(92811_55433086)x1 ~ 2, Xp11.21q28(55507789_155086462) x1 ~ 2
68	mos 45,X[31]/46,X, i(X)(q10)[4]	arr[GRCh37]Xp22.33p11.1(296520_58189366)x1, Xq11.1q28(61935385_155221912) x1 ~ 3
69	mos 45,X, [73]/46,X, psu dic(Y)(q12)[10]	arr[GRCh37] (Y)x0 ~ 1
70	mos 45,X[5]/46,X, Yqh-[45]	arr[GRCh37]Yp11.32-q11.221(292758-18926224)×2, Yq11.221-q11.223(18983088–24445033) ×0
71	mos 45,X[44]/46,X, Yqh-[6]	arr[GRCh37] Xp22.33(168,552-2,690,819) x1-2 or Yp11.3 2p11.31(118,552-2,640,819) x1-2, Yp11.31q11.221(2,650,425 – 1 8,163,889) x0-1, Yq11.221q11.23(18,192,897 – 28,799,654) x0
72	mos 45,X[48]/46,X, Yqh-[2]	arr[GRCh37] Yq11.222q12(20,618,888-59373566) x0
73	mos 47,X, Yqh-,Yqh-[4]/45,X[3]/46,X, Yqh-[13]	arr[GRCh37] Yp11.32q11.221(246,520 – 19,523,995) x1 ~ 2, Yq11. 222q12(21,035,228 – 59,373,566) x0

Abbreviations. CMA, chromosomal microarray analysis

amniotic fluid cell culture cycle, the selective growth of cells after artificial culture, and the ability of different methods to detect a low percentage of CM may contribute to the inconsistency of these results. In this study, karyotype analysis revealed a total of 49 cases (33.1%) of autosomal mosaicism, among the number abnormalities, the most was trisomy 21 mosaicism, and 4 cases each mosaicism of trisomy 9 and trisomy 20. Comparison of the three methods with the karyotypic conformity rate showed that for trisomy 21/18/9 mosaicism CMA with karyotypic conformity reached 100%, but the two cases of trisomy 20 CMA were suggested to be normal. In order to further determine the accuracy of the experimental samples, the amniotic fluid cells remaining after culture were collected and verified by CNV-seq, and the results showed that the whole chromosome 20 was mosaic with a proportion of 12%, which was consistent with the results of karyotype analysis. This also confirms literature reports that CNV-seq can detect aneuploid mosaicism in 5–10% of cases and that CMA can only detect

Table 4 9 cases of karyotyping to sSMC mosaicism, CMA to clarify the origin of chromosome segments

Case ID	Indication(s)	Karyotype	CMA
1	High risk of serum screening	mos 45,X[21]/46,X,+mar[9]	arr[GRCh37]Xp22.33p11.22(296520_53256474) x1, Xp11.22q21.1(53284533_83969013) x1 ~ 2, Xq21.1q28(84015976_155221912) x1
2	Advanced age	mos 46,X,+mar[41]/45,X[9]	arr[GRCh37] (Yp)x1 ~ 2, (Yq)x0
3	Ultrasound abnormality	mos 45,X[48]/47,XX,+mar[2]	arr[GRCh37] (X)x1 ~ 2
4	NIPT	mos 45,X[68]/46,X,+mar[5]/46,XY[23]	arr[GRCh37]Yq11.223q12(24377969_59324918)x0
5	NIPT	mos 47,XX,+dup(15)(pter→q11.2::q13.2→pter)[8]/46,XX[42]	arr[GRCh37]15q11.2q13.2(22822019_30351527)x3
6	NIPT	mos 47,XX,+i(12)(p10)[5]/46,XX[45]	arr[GRCh37]12p13.33p11.1(173,787 – 34,835,641) x2-3,12q11q12(37,857,931 – 38,728,412) x4
7	NIPT	mos 45,X[30]/46,X,+mar[25]	arr[GRCh37]Xp22.33p11.21(296,520 – 55,976,925) x1, Xp11.21q26.2(56,151,838 – 133,279,271) x1 ~ 2, Xq26.2q28(133,382,540 – 155,086,462) x1.
8	Advanced age	mos 47,XX,+dup(18)(pter→q21.2::q21.2→pter)[11]/47,XX,+18[9]	arr[GRCh37]18p11.3q21.2(136228-49,871,597) x3,18q21.2qter (49,871,597 – 77,989,124) x2 ~ 3.
9	NIPT	mos 45,X[23]/46,X,+mar[7]	arr[GRCh37]Xp22.33p11.3(168552_44764775) x1, Xp11.3q22.1(44999230_100779320)x1-2, Xq22.1q28(100897399_155233098) x1.

Abbreviations. sSMC, small supernumerary marker chromosome; CMA, chromosomal microarray analysis; NIPT, non-invasive prenatal testing

Table 5 5 cases inconsistent mosaicism by CMA and karyotyping

Case ID	Indication(s)	Karyotype	CMA
1	Adverse reproductive history	mos 47,XX,+20[3]/46,XX[97]	Normal
2	High risk of serum screening	mos 45,X[8]/46,XX[42]	Normal
3	Ultrasound abnormality	mos 46,XY, t(3;9)(q21;p24)[4]/46,XY[16]	Normal
4	NIPT	mos 47,XX,+der(?)cenh ps[15]/46,XX[5]	Normal
5	NIPT	mos 47,XY,+20[16]/46,XY[4]	Normal

Abbreviations. CMA, chromosomal microarray analysis; NIPT, non-invasive prenatal testing

a low percentage of mosaicism in less than 30% of cases [15, 16].The sensitivity of CNV-seq is higher than that of CMA, and CNV-seq is used as a first-line prenatal diagnostic method for pregnant women to make informed choices [17]. However, CNV-seq cannot identify chromosomal structural abnormalities and uniparental diploidy [18].Therefore, in the study of autosomal structural abnormalities, the three methods were compared with karyotyping, and CMA had the highest conformity rate of 84%, while QF-PCR and CNV-seq were 60% and 50%, respectively. Due to the specificity of probe-based methods, QF-PCR is useful for mosaicism detection on chromosomes 21 and X, but it cannot identify less than 15-20% of mosaicism and chromosomal structural abnormalities, despite its simplicity and short turnaround time [19], Table 7.

In our study, there were 85 cases of sex chromosome numerical abnormality, with the highest of X chromosome number mosaicism (42/85,49.4%), which existed in the form of mos 45,X/47,XXX, due to the fact that autosomal monosomy cells are essentially non-viable in the late stages of division, and the X chromosome can be present as a monosomy [20, 21].This is also a common reason for the high prevalence of X-monosomy and X-triploid mosaicism in cell cultures, and therefore no abnormality was detected in the CMA assay. For detection of marker mosaicism, X deletion, isochromosome X, and pseudodicentric chromosome X and Y deletion, the mosaic conformity rates of karyotyping combined with CMA was 100% [22, 23]. This also demonstrates the advantages of CMA in detecting sex chromosome mosaicism.

Due to the differences in the detection rate of amniotic fluid mosaicism among different prenatal diagnostic indications, it is necessary to rationally select detection methods according to different indications. In this study, amniotic fluid specimens from pregnant women were divided into 5 groups according to different prenatal diagnostic indications, and the NIPT group and the ultrasound abnormality group accounted for the largest proportion, with 35.1% and 22.3%, respectively. The NIPT group is based on second-generation sequencing technology, and theoretically, even in low-coverage whole-genome sequencing studies, NIPT can still detect CNVs smaller than 5 Mb, so the percentage of positive mosaicism in the NIPT group will be high, providing data for clinicians to choose a reasonable test. This is consistent with the finding by Li et al. that individuals with a higher proportion of autosomal mosaicism are significantly more common in the group with ultrasound abnormalities compared to other groups [24].CMA has a higher resolution than traditional karyotyping, can detect

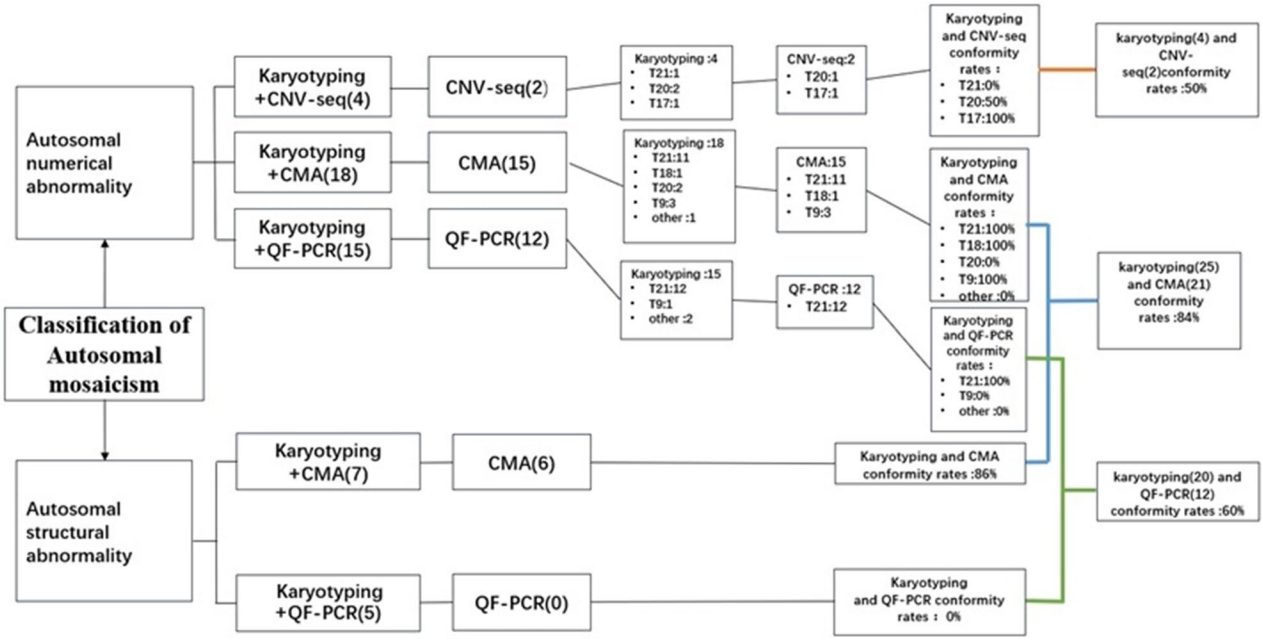

Fig. 2 Classification of autosomal mosaicism. **Abbreviations**: CNV-seq, copy number variant sequencing; CMA, Chromosomal microarray analysis; QF-PCR, Quantitative fluorescent polymerase chain reaction. T21, trisomy 21; T18, trisomy 18; T20, trisomy 20; T17, trisomy 17; T9, trisomy 9

Table 6 Classification of sex chromosomal mosaicism

Abnormal number of sex chromosomes(*n*/%)				Structural abnormality of the sex chromosomes(*n*/%)				
Detection method	X mosaicism	Y mosaicism	sSMC	X deletion	X rearrangements	isochromosome X	pseudodicentric chromosome X	Y deletion
Karyotyping /CNV	3/0(0%)	3/2(66.7%)	1/1(100%)	0	0	0	0	0
Karyotyping /CMA	26/23(88.5%)	18/13(72.2%)	7/7(100%)	2/2(100%)		2/2(100%)	1/1(100%)	4/4(100%)
Karyotyping /QF-PCR	13/8(61.5%)	11/9(81.8%)	3/3(100%)	1/1(100%)	1/1(100%)	1/0(0%)	0	2/1(50%)
Total	42/31(73.8%)	32/24(75%)	11/11(100%)	3/3(100%)	1/1(100%)	3/2(66.7%)	1/1(100%)	6/5(83.3%)

Abbreviations. CNV-seq, copy number variant sequencing; CMA, chromosomal microarray analysis; QF-PCR, quantitative fluorescent polymerase chain reaction; CM, chromosomal mosaicism; sSMC, small supernumerary marker chromosome

deletions and duplications of < 50 kb, and can identify chromosomes of unknown origin, and is now widely used in prenatal diagnosis [25]. Among them, karyotypic analysis revealed 9 cases of sSMC, such as case P5 in Table 4. The CMA test results confirmed the duplication of the 15q11.2-q13.2 region with a length of about 7.14 Mb, indicating the presence of partial trisomy 15q in the fetus, which is similar to the presence of chromosome segment copy number, locus, and range of variation in the cases reported by LU et al. and CHEN et al. [26, 27]. 15q11-q13 duplication syndrome (OMIM # 608636) is associated with neurodevelopmental disorders (hypotonia, developmental delays, speech delays, and seizures) and autism spectrum disorder(ASD), but is characterized by variable expressivity and reduced penetrance, features that make genetic counseling a complex process especially in prenatal cases [28]. This suggests that CMA is able to clearly mark the origin of chromosomes, and when performing karyotyping, if sSMCs and chromosomal structural abnormalities such as translocations and deletions involving gene copy number imbalances are identified, CMA can help clinicians determine the site of the gene breaks, the origins and sizes of the sSMCs and the unbalanced fragments, and their possible clinical implications, which can help provide more accurate genetic counseling and pregnancy decisions [29].

In addition, karyotyping of case P17 and case P18 in Table 3 showed that the mosaicism were similar in shape and size to the chromosome 21 duplication, which was mistaken by the cytogenetic group for a mosaic trisomy 21, but the CMA results indicated the presence of a duplication at 12p13.33-p11.1 (189216-34060203) ×4.Upon literature search, the latter is associated with the rare Pallister-Killian syndrome(PKS, OMIM #601803), often in the form of mosaic tetrasomy of 12p with wide neurological involvement [30, 31]. The 12p duplicated region appears to be enriched for genes associated with neuropathological features, as reported by Poulton et al.

Table 7 Karyotype analysis and QF-PCR results on 11 mosaic cases

Number	Indication(s)	Karyotype	QF-PCR
1	High risk of serum screening	mos 45,X[2]/46,XX[39]	Normal
2	NIPT	mos 45,X[6]/46,XX[23]	Normal
3	NIPT	mos 45,X[8]/46,XX[42]	Normal
4	High risk of serum screening	mos 45,X[8]/46,XX[42]	Sex chromosome aneuploid
5	High risk of serum screening	mos 45,X[10]/46,XX[13]	Sex chromosome aneuploid
6	Ultrasound abnormality	mos 45,X[38]/46,XX[12]	Sex chromosome aneuploid
7	Adverse reproductive history	mos 45,X[47]/46,XX[3]	Sex chromosome aneuploid
8	High risk of serum screening	mos 45,X[10]/46,XY[40]	Sex chromosome aneuploid
9	Ultrasound abnormality	mos 45,X[11]/46,XY[39]	Normal
10	High risk of serum screening	mos 45,X[12]/46,XY[14]	Sex chromosome aneuploid
11	High risk of serum screening	mos 45,X[16]/46,XY[24]	Sex chromosome aneuploid
12	Ultrasound abnormality	mos 45,X[17]/46,XY[21]	Sex chromosome aneuploid
13	High risk of serum screening	mos 45,X[34]/46,XY[16]	Sex chromosome aneuploid
14	High risk of serum screening	mos 45,X[35]/46,XY[15]	Sex chromosome aneuploid
15	NIPT	mos 45,X[10]/47,XXX[40]	Sex chromosome aneuploid
16	High risk of serum screening	mos 45,X[14]/47,XXX[6]	Normal
17	Advanced age	mos 45,X[14]/47,XXX[6]	Sex chromosome aneuploid
18	High risk of serum screening	mos 45,X[16]/47,XXX[4]	Sex chromosome aneuploid
19	High risk of serum screening	mos 45,X[17]/47,XYY[3]	Sex chromosome aneuploid
20	High risk of serum screening	mos 47,XXX[3]/46,XX[35]	Normal
21	Ultrasound abnormality	mos 47,XXX[33]/45,X[17]	Sex chromosome aneuploid
22	High risk of serum screening	mos 47,XXY[15]/46,XY[10]	Sex chromosome aneuploid
23	NIPT	mos 47,XXY[6]/46,XY[14]	Sex chromosome aneuploid
24	Advanced age	mos 47,XXY[6]/46,XY[17]	Normal
25	High risk of serum screening	mos 45,X[12]/46,X,+mar[8]	Sex chromosome aneuploid
26	Advanced age	mos 47,XY,+mar[10]/46,XY[10]	Normal
27	Adverse reproductive history	mos 47,XX,+mar[13]/46,XX[37]	Normal
28	Ultrasound abnormality	mos 46,X,+mar[30]/45,X[20]	Sex chromosome aneuploid
29	High risk of serum screening	mos 47,XX,+9[14]/46,XX[36]	Normal
30	Advanced age	mos 47,XY,+21[1]/46,XY[35]	Trisomy 21
31	NIPT	mos 47,XX,+21[3]/46,XX[47]	Sex chromosome aneuploid
32	Advanced age	mos 47,XX,+21[3]/46,XX[47]	Sex chromosome aneuploid
33	High risk of serum screening	mos 47,XX,+21[4]/46,XX[16]	Sex chromosome aneuploid
34	NIPT	mos 47,XY,+21[5]/46,XY[45]	Trisomy 21
35	NIPT	mos 47,XX,+21[8]/46,XX[42]	Sex chromosome aneuploid
36	Advanced age	mos 47,XY,+21[9]/46,XY[11]	Trisomy 21
37	NIPT	mos 47,XX,+21[11]/46,XX[9]	Sex chromosome aneuploid
38	High risk of serum screening	mos 47,XX,+21[21]/46,XX[29]	Trisomy 21
39	High risk of serum screening	mos 47,XX,+21[36]/46,XX[14]	Sex chromosome aneuploid
40	Advanced age	mos 47,XX, t(1;13)(p32;q34),+21[3]/47,XX,+21[18]	Trisomy 21
41	Advanced age	mos 45,X[11]/46,X, add(X)(p22.1)[9]	Sex chromosome aneuploid
42	High risk of serum screening	mos 45,X[11]/46,X, del(X)(q21)[14]	Sex chromosome aneuploid
43	Ultrasound abnormality	mos 45,X[34]/46,X, i(X)(q10)[7]/46,XX[9]	Normal
44	High risk of serum screening	mos 45,X[42]/46,X,+r[12]	Sex chromosome aneuploid
45	Ultrasound abnormality	mos 45,X,22pstk+[26]/46,X, Yqh-,22pstk+[18]	Normal
46	NIPT	mos 45,X[20]/46,X,?Yqh-[30]	Sex chromosome aneuploid
47	High risk of serum screening	mos 46,XX, del(11)(q14)[2]/46,XX[48]	Normal
48	NIPT	mos 45,XY, rob(21;21)(q10;q10)[38]/46,XY,+21,rob(21;21)(q10;q10)[6]/46,XY[62]	Trisomy 21
49	Advanced age	mos 46,XX, t(15;16)(q11.2;p11.2)[2]/46,XX[53]	Normal
50	High risk of serum screening	mos 46,XY, inv(7)(p15q32)t(6;7)(p21.1;q32)[5]/46,XY[47]	Normal
51	High risk of serum screening	mos 46,XY, t(1;14)(q21q32)[13]/46,XY[53]	Normal
52	High risk of serum screening	mos 46,XY, t(11;14)(q13;q14)[2]/46,XY[18]	Normal

Abbreviations. QF-PCR, Quantitative fluorescent polymerase chain reaction; NIPT, non-invasive prenatal testing

and Izumi et al. The minimal critical region responsible for PKS is spanning 12p13.33 to 12p13.2 region [32, 33].

Karyotyping of case 3 in Table 4 showed mos 45, X[48]/47, XX, +mar [2], since the proportion was too small the CMA could not detect the presence of 4% sSMC mosaics and only monosomy X could be detected.

However, there were also 5 discordant cases which karyotyping detected mosaicism, but CMA showed a normal. 1 case had a balanced chromosomal translocation and 2 cases had mosaic trisomy 20, and the CMA results were suggestive of normal. It is also worth noting that despite the very high risk of abnormal outcomes 47, +20/46 (>60%) in a review conducted by Wallerstein et al., others have reported that about 90–93% of cases of mosaic trisomy 20 are diagnosed prenatally with a normal phenotype [34, 35, 36, 37].

Furthermore, these genetic anomalies of the chromosomal mosaicism and UPD arise from errors in meiosis and/or mitosis and can occur independently or in combination. The detection of chromosomal and UPD mosaicism is often hampered by low levels of abnormal cell lines, and detection is also affected by the sensitivity of the applied method. Whereas FISH can detect less than 2% of aneuploid mosaicism levels, the sensitivity of SNP assays was 5% [4].

Seven out of 148 cases were lost to follow-up. Of the 46 cases patients with autosomal mosaicism, 20 continued their pregnancies during the follow-up period and 26 chose to terminate their pregnancies after receiving genetic counseling. The majority of patients with sex chromosome mosaicism continued their pregnancies at follow-up, and 17 chose to terminate their pregnancies. This retrospective study may have limitations in terms of clinical practice and prognosis due to the long and incomplete collection of pregnancy outcomes in cases of chromosomal mosaicism. Because of the limitations of the laboratory equipment from 2015, karyotyping was combined with QF-PCR, while from 2019, due to the update of molecular genetics techniques in laboratories, more use was made of karyotyping combined with CNV-seq and CMA. Therefore, the major limitation of this study is that the same specimens were not subjected to CMA/CNV-seq and QF-PCR due to the pregnant consideration of the economic benefits and the impact of the high volume of amniotic fluid extracted, which does not allow for a real comparison of the detection capabilities of these methods.

Currently, optical genome mapping (OGM) and long-read sequencing technologies (LRS) are new tools used for detecting copy number variations (CNVs) and chromosomal structural variations (SVs) [38]. Although OGM and LRS are capable of assessing the clinically relevant SVs in all cases, the limitations of the isolated use of these techniques could still interfere with successfully making a molecular diagnosis. Moreover, OGM remains demanding on prenatal samples and does not allow direct DNA extraction using uncultured amniotic fluid samples. We recommend that future studies continue with larger groups of individuals and that the database of common chromosomal structural variants be expanded to filter out irrelevant variants. However, with the refinement of the technology and optimization of the algorithm OGM is expected to become the first line of prenatal SVs detection and the diagnostic utility of these techniques will be further investigated in cases where SV is present by CMA and karyotyping. Nevertheless, whether they will replace karyotyping and CMA remains controversial.

Conclusions

In summary, the diagnosis of CM is difficult and complex. These findings suggest that the increased risk of genetic counseling is due to inconsistent CM from different specimens or different testing methods. In order to improve the accuracy and reliability of the diagnosis, specialized knowledge and clinical experience of the physician are required, as well as a combination of advanced genetic techniques and multidisciplinary collaboration. Through our study, the advantages of the combined use of different cytogenetic and molecular detection techniques have been reconfirmed, especially the combined karyotyping and CMA combination modality to detect amniotic fluid cell mosaicism with reliable results and to test the amniotic fluid cells for reliable results before making any irreversible pregnancy decisions.

Abbreviations

AF	Amniotic fluid
CM	Chromosomal mosaicism
CMA	Chromosomal microarray analysis
CNV	seq-Copy number variant sequencing
FISH	Fluorescence in situ hybridization
NIPT	Non-invasive prenatal testing
QF	PCR-Quantitative fluorescent polymerase chain reaction

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Author contributions

ZL and DY conceptualized the review; ZL and DY wrote the original draft; ZL, WJ and YML reviewed and edited the draft; CC, WP, WDN, DGM and ZSY administrative support. All authors reviewed the article critically for intellectual content and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study protocol (no. 20230414-061) was approved by the Institutional Ethics Committee of the Sichuan Provincial Hospital for Women and Children, and all patients signed informed consent forms.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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