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Characteristics and mechanisms of mosaicism in prenatal diagnosis cases by application of SNP array

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Abstract

Background With the application of chromosome microarray, next-generation sequencing and other highly sensitive genetic techniques in disease diagnosis, the detection of mosaicism has become increasingly prevalent. This study involved a retrospective analysis of SNP array testing on 4512 prenatal diagnosis samples, wherein the characterization of mosaicism was explored and insights were gained into the underlying mechanisms thereof.

Results Using SNP array, a total of 44 cases of mosaicism were identified among 4512 prenatal diagnostic cases; resulting in a detection rate of approximately 1.0%. The prevalence of mosaicism was 4.1% for chorionic villus sample, 0.4% for amniotic fluid, and 1.3% for umbilical cord blood. Of these cases, 29 were mosaic aneuploidy and 15 were mosaic segmental duplication/deletion. Three cases of mosaic trisomy 16 and three cases of mosaic trisomy 22 were diagnosed in the CVS samples, while four cases of mosaic trisomy 21 were detected in amniotic fluid and umbilical cord blood samples. The distribution pattern of mosaicism suggested trisomy rescue as the underlying mechanism. Structurally rearranged chromosomes were observed, including three cases with supernumerary marker chromosomes, three cases with dicentric chromosomes, and one case with a ring chromosome. All mosaic segmental duplication/deletion cases were the result of mitotic non-disjunction, with the exception of one case involving mosaic 11q segmental duplication.

Conclusion Improved utilization of SNP arrays enables the characterization of mosaicism and facilitates the estimation of disease mechanisms and recurrence.

Keywords Mosaicism, SNP array, Mechanism, Prenatal diagnosis

Background

Mosaicism occurs when a single fertilized egg develops into an embryo containing two or more populations of cells with distinct genotypes [1]. According to the variation types, chromosomal abnormalities can be divided into aneuploidy, polyploidy, segmental duplications/deletions, translocations, inversions, ring chromosomes,

isochromosomes, etc. The most frequent type of aneuploidy mosaicism is gonosomal aneuploidy, while mosaicism involving abnormal chromosome structure are relatively uncommon. The clinical phenotype of mosaicism is variable, ranging from mild mental retardation in genetic syndromes such as Pallister-Killian syndrome and Ito, to embryonic lethality [2–4].

During prenatal diagnosis, the incidence of mosaicism in chorionic villus samples is about 1%~2%. However, in most cases, it is confined placental mosaicism (CPM) accounting for 86.5%, while true fetal mosaicism (TFM) accounts for only 13.5% [5, 6]. In amniotic fluid samples, the incidence of TFM is about 0.1%~0.3%.

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Both fetal mosaicism and placental mosaicism can lead to prenatal or perinatal complications [7, 8]. However, the mechanisms behind these conditions have vastly different impacts on fetal development. In cases of mosaic aneuploidy originating from meiosis, the aneuploid constitution likely occurs in the very early stages of embryo development, where correct chromosome number might be vital [9, 10]. Conversely, mitotic-originated cases may proceed with normal early cleavage but could potentially affect a subset of tissues. Differentiation between a mitotic and meiotic origin of trisomies is necessary for determining recurrence risks and for proper counseling; this is because mosaic trisomy arising from meiotic non-disjunction is associated with a higher risk of recurrence, especially in younger women [11].

Mosaicism can be identified by various methods, including chromosome karyotyping, fluorescence in situ hybridization, chromosome microarray, and next-generation sequencing. However, single nucleotide polymorphism (SNP) array analysis offers several advantages over traditional chromosome karyotyping for detecting mosaicism. With SNP array analysis, a large number of cells can be detected simultaneously and culture bias can be eliminated by analyzing interphase cells [12, 13]. For cases of complex mosaicism, SNP array can provide crucial information on the characteristics of the mosaicism (including content, origin, and mechanism), which are essential for accurate fetal prognosis assessment and genetic counselling.

Here, we conducted a study on 4512 pregnant women referred for prenatal diagnosis, utilizing genome-wide SNP arrays and karyotype analysis to investigate the characteristics and mechanisms of mosaicism.

Results

Distribution of mosaicism by sample type and mosaic type

A total of 44 cases of mosaicism were initially detected among the 4512 patients using genome-wide SNP array; the overall prevalence of mosaicism was 1.0% (44/4512 cases). Of these, 21 cases were identified from chorionic villus sample, 11 cases from amniotic fluid, and 12 cases from umbilical cord blood. The prevalence of mosaicism was 4.1% for chorionic villus sample, 0.4% for amniotic fluid, and 1.3% for umbilical cord blood. In this cohort, 29 cases were diagnosed as mosaic aneuploidy and 15 cases were mosaic segmental duplication/deletion (Tables 1 and 2). Among the cases exhibiting mosaic aneuploidy, fifteen were diagnosed as mosaic trisomy, one as mosaic monosomy, six as gonosomal aneuploidy mosaicism, six as double trisomy, and one as near-diploid. By comparing the percentage of mosaicism in each case between the array data and

conventional karyotype analysis, a total of 18 mosaic aneuploidy cases showed differential results with a discordance rate of 66.7% (18/27).

Mosaic trisomy

Fifteen cases of mosaic trisomy were detected. Comparison between the array and karyotype analysis indicated that the eight cases exhibited concordant results (a 10% deviation was deemed to be within acceptable limits), while six cases showed discordance; there was a failure of cultural testing in one case of mosaic trisomy 4.

In our cohort, seven cases of mosaic trisomy arose by mitotic non-disjunction (Figs. 1 and 2). Four cases of mosaic trisomy arose by meiotic I non-disjunction, including one case of mosaic trisomy 22 and three cases of mosaic trisomy 16, with additional haplotypes visible near the centromere. Those three cases of mosaic trisomy 16 were determined by the visible patterns of recombination with 3–5 crossovers (Fig. 1). The crossovers were in 16p12.3 and 16q22.1, indicating that these loci were hot spots of recombination. Four cases arose by meiotic II non-disjunction, including one case each of mosaic trisomy 18 and 22, as well as two cases of mosaic trisomy 21; these cases exhibited additional haplotypes in close proximity to the telomeres.

Three cases of mosaic trisomy 16 and three cases of mosaic trisomy 22 were diagnosed in the CVS samples, both of which can be lethal during the first trimester. On the other hand, four cases of mosaic trisomy 21 were detected in amniotic fluid and umbilical cord blood samples, which can be better tolerated by the developing embryo. The distribution pattern of mosaicism suggested trisomy rescue as the underlying mechanism.

Gonosomal aneuploidy mosaicism

Six cases of gonosomal aneuploidy mosaicism were detected. Comparison between the SNP array and karyotype analyses revealed differential results for the gonosomal aneuploidy mosaicism cases, except for two cases of 45,X/46,XX. Overall, the mosaic frequencies of gonosomal aneuploidy mosaicism in the array data were unclear.

All mosaic gonosomal aneuploidies arose by mitotic non-disjunction, with the exception of one case of 47,XXY/46,XY which arose from meiotic I non-disjunction. With the exception of this particular case resulting in intrauterine fetal death during the first trimester, all other cases survived until the second or third trimester. This suggests that chromosome X monosomy is associated with less embryonic lethality than euchromosome monosomy when non-disjunction occurs mitotically.

Table 1 Results of 29 cases with mosaic aneuploidy

Case No	Specimen	Type of aneuploidy	Mosaic% by SNP array	Karyotype	Mosaic% by karyotype	Origin	Indication
1	Villus	T 4	30	–	–	Mitosis	IFD
2	UCB	T 8	20	47,XY,+8[3]/46,XY[16]	15	Mitosis	MR in mother
3	Villus	T 8	80	47,XX,+8[8]/46,XX[2]	80	Mitosis	IFD
4	Villus	T 15	60	46,XY,+15,rob(15;15)(q10;q10)	100	Mitosis	IFD
5	Villus	T 16	20	47,XX,+16[8]/46,XX[2]	80	M I	IFD
6	Villus	T 16	30	47,XX,+16[3]/46,XX[7]	30	M I	IFD
7	Villus	T 16	20	47,XY,+16[3]/46,XY[7]	30	M I	IFD
8	AF	T 18	80	47,XX,+18[38]/46,XX[8]	82.61	M II	high T18 risk
9	AF	T 21	20	46,XY,+21,rob(21;21)(q10;q10)[3]/46,XY[16]	15	Mitosis	AMA
10	AF	T 21	20	47,XY,+21[1]/46,XY[49]	2	M II	abnormal NIPT
11	AF	T 21	70	47,XX,+21[32]/46,XX[9]	76.19	M II	high T 21 risk
12	UCB	T 21	60	45,XY,rob(15;21)(q10;q10)	0	Mitosis	ventriculomegaly
13	Villus	T 22	80	47,XX,+22[8]/46,XX[2]	80	Mitosis	IFD
14	Villus	T 22	30	47,XX,+22[5]/46,XX[5]	50	M II	IFD
15	Villus	T 22	15	47,XX,+22[4]/46,XX[7]	36.36	M I	IFD
16	Villus	T 2	100	47,XY,+2	100	Meiosis	IFD
		T 20	60		0	Mitosis	
17	Villus	T 7	50	48,XX,+7,+21[11]/47,XX,+21[2]	85.71	Mitosis	IFD
		T 21	70		100	M I	
18	Villus	T 13	20	46,XY,rob(13;14)(q10;q10),+rob(13;14)(q10;q10)	100	Mitosis	IFD
		T 14	20		100	Mitosis	
19	Villus	T 13	15	48,XY,+13,+14[4]/46,XY[1]	80	M II	IFD
		T 14	20		80	M I	
20	Villus	T 14	30	48,XY,+14,+20	100	Mitosis	IFD
		T 20	30		100	Mitosis	
21	Villus	T 18	100	48,XX,+18,+22[2]/47,XX,+18[8]	100	M II	IFD
		T 22	15		20	M I	
22	Villus	T 13	15	50,XX,+13,+18,+19,+21	100	M II	IFD
		T 18					
		T 19					
		T 21					
23	Villus	M 14	60	–	–	Mitosis	IFD
24	AF	X	20	45,X[6]/46,XX[13]	30	Mitosis	abnormal NIPT
25	AF	X	20	45,X[7]/46,XX[14]	31.82	Mitosis	AMA
26	AF	X	50	45,X[16]/47,XXX[6]	74	Mitosis	AMA
		XXX	50		26		
27	UCB	X	50	45,X[7]/46,XY[39]	15.22	Mitosis	abnormal NIPT
28	AF	XXY	60	47,XXY[4]/46,XY[17]	18.18	Mitosis	AMA
		XY	40		81.82		
29	Villus	XXY	20	47,XXY[14]/46,XY[6]	40	M I	IFD
		XY	80		60		

Mosaic double trisomies and near-diploid

Six cases of mosaic double trisomies were identified. Comparison between the array and karyotype analyses revealed differential results for the double trisomy cases, except for one case of +18/+22. All cases resulted in intrauterine fetal death during the first trimester,

indicating that these mosaic double trisomies cannot be tolerated by the embryo.

One case with mosaic near-diploid of +13/+18/+19/+21 was detected. This rare abnormality arose from simultaneous meiosis II, in which four chromosomes exhibited the same mosaic frequency of

Table 2 Results of 15 cases with mosaic segmental duplication/deletion

Case No	Specimen	SNP array	Type	Size (Mb)	Karyotype	Indication
30	UCB	arr 1q31.3q44x2~3	Dup	52.24	46,XX,add(1)(q44)[6]/46,XX[20]	Neural tube malformation
31	UCB	arr 2p25.2p24.2x1~2	Del	11.42	46,XY,del(2)(p24.2p25.2)[6]/46,XY[14]	Unknown
32	AF	arr 15q11.2q13.1x2~6	Dup	7.61	48,XX,+2mar[30]/47,XX,+mar[28]/46,XX[7]	AMA
33	AF	arr 21q11.2q21.1x2~4	Dup	4.18	47,XY,+mar[13]/46,XY[15]	High T 21 risk
34	UCB	arr (12)x2~3, 12p11.23p11.1x3	Dup Dup	133.56 7.23	47,XX,+mar	Choroidal fissure cyst
35	UCB	arr Xp22.33p22.31x1	Del	8.46	46,X,psu idic(X)(p22.3)[38]/45,X[3]	Unknown
36	AF	arr Yp11.32q11.222x0~2, Yq11.222q11.223x0	Del Dup	2.9 20.76	45,X[20]/46,X,idic(Y)(q11.22)[5]/46,XY[18]	Abnormal NIPT
37	UCB	arr 4p16.3p14x1, 4p14q35.2x2~3, (22)x2~3	Del Dup Dup	37.12 153.36 34.17	45,XX,dic(4;22)(p11;p11.2)	UMM
38	UCB	arr 5p15.33p13.3x1, 5p13.3q35.3x2~3	Del Dup	33.34 147.34	46,XX,r(5)(p13q35)	Intrauterine growth retardation
39	UCB	arr 1q32.2q44x3, 3q11.1q29x2~3	Dup Dup	38.79 99.87	46,XY,add(6)(p25)	Enlarged posterior cranial fossa
40	Villus	arr Xp22.33q21.33x1~2, Xq21.33q28x1	Del Del	95.60 59.33	45,X	IFD
41	UCB	arr 11q23.3q25x2~3	Dup	15.92	46,XX	Enlarged pelvis
42	UCB	arr 11q13.4q25x2~3	Dup	63.24	46,XX	PCF
43	Villus	arr 11q14.3q24.1x2~3, 11q24.1q25x1~2	Dup Del	31.54 13.27	46,XY	IFD
44	Villus	arr 3p26.3p26.1x1, 3p26.1p21.31x2~3, 6p25.3p22.1x2~3	Del Dup Dup	4.98 39.78 28.02	46,XY	IFD

UCB Umbilical cord blood; AF Amniotic fluid; T Trisomy; M Monosomy; MI Meiosis I; MII Meiosis II; IFD Intrauterine fetal death; PCF Posterior cranial fossa communicates with the lateral ventricle; MR Mental retardation; AMA Advanced maternal age; UMM Ultrasound multiple malformation

15%; however, the result of karyotype analysis indicated a non-mosaic near-diploid with 50,XX,+13,+18,+19,+21 (Fig. 3).

Mosaic segmental duplication/deletion cases

Among the fifteen cases of mosaic segmental duplication/deletion, twelve cases involved euchromosomes and three cases involved sex chromosomes. In terms of mosaic types, seven cases were characterized by duplication, two cases by deletion and six cases by a combination of both. Nevertheless, three cases resulted in intrauterine fetal death during the first trimester, while the remaining twelve cases survived until the second or third trimester. Among these twelve cases, seven cases (58.3%) with euchromosomes exhibited abnormal ultrasonographic findings. Thus, mosaic segmental duplication/deletion resulted in lower embryonic lethality than mosaic aneuploidy. Additionally, mosaic segmental duplication/deletion had a high incidence of ultrasound malformations, especially in cases of mosaic segmental duplication.

In our cohort, there were three cases of small supernumerary marker chromosomes (sSMC), three cases of dicentric chromosomes, and one case of a ring chromosome (Figs. 4 and 5). Further, there were three cases of mosaic 11q segmental duplication with normal karyotypes (Fig. 6).

Here, eleven cases exhibited abnormal karyotypes and four cases displayed normal karyotypes. A comparison between the array and karyotype analyses revealed that only two cases showed concordant results, which involved simple mosaic duplication or deletion greater than 10 Mb. The remaining thirteen cases had differential results, with a coincidence rate of only 13.3%.

Discussion

With the application of chromosome microarray, next-generation sequencing and other highly sensitive genetic techniques in disease diagnosis, the detection of mosaicism has become increasingly prevalent. In our study, a total of 44 cases of mosaicism were initially detected among the 4512 patients using genome-wide SNP array;

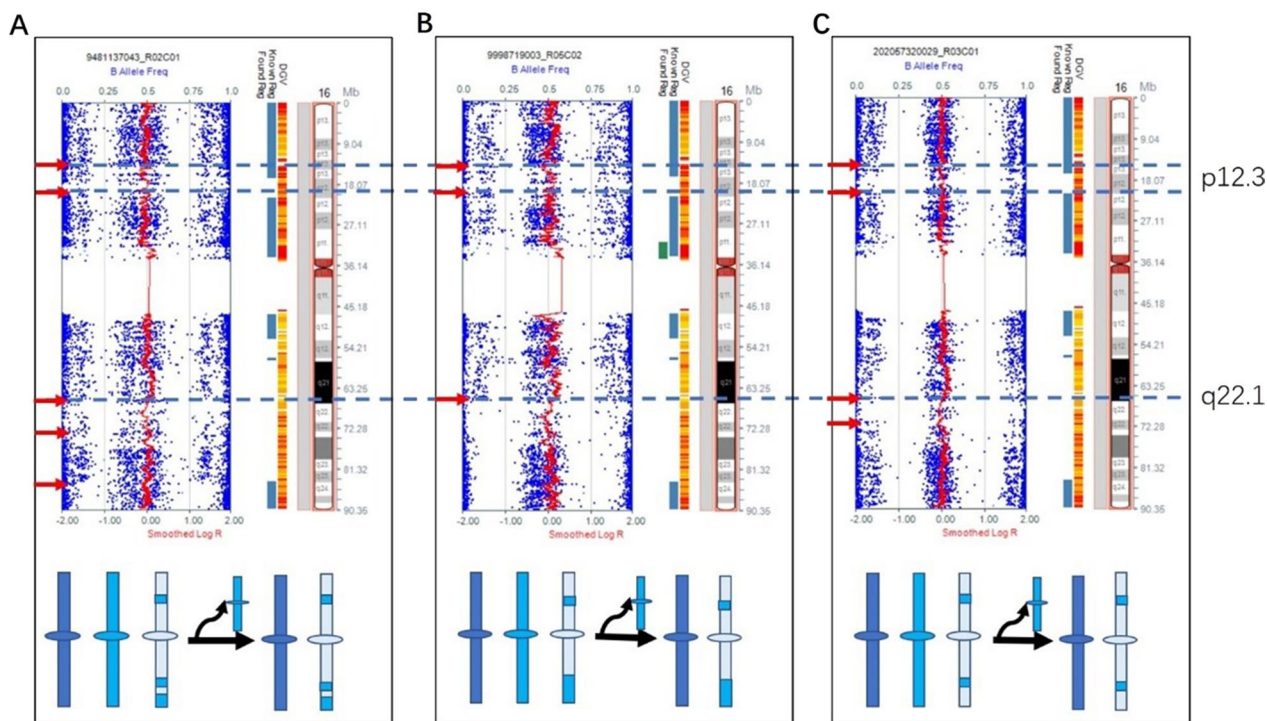


Fig. 1 SNP array results and mechanisms for mosaic trisomy 16. The log R ratio indicates an increase in copy number, between two and three copies. Additional shifts in the B allele frequency are observed, corresponding to a shift in B allele frequency from 0% towards 33% (in the case of AA in the euploid cell line and AAB in the trisomic cell line), and a shift from 100% toward 66% (in the case of BB in the euploid cell line and ABB in the trisomic cell line). The additional haplotypes are visible near the centromere, consistent with a meiosis I nondisjunction. The crossovers were in 16p12.3 and 16q22.1, indicating that these loci were hot spot of recombination. **A** Mosaic trisomy 16 in case 5 arose from meiosis I with the presence of five visible crossovers. The proportion of cells exhibiting mosaic trisomy 16 was 20%. **B** Mosaic trisomy 16 in case 6 arose from meiosis I with 3 crossovers. The proportion of cells exhibiting mosaic trisomy 16 was 30%. **C** Mosaic trisomy 16 of case 7 arose from meiosis I with 4 crossovers. The proportion of cells exhibiting mosaic trisomy 16 was 20%

the overall prevalence of mosaicism was 1.0% (44/4512 cases).

For different prenatal diagnostic samples, mosaic aneuploidy was found in about 1–2% of chorionic villus samples and 0.2% of amniocentesis samples obtained for prenatal diagnosis [14]. In our survey, the frequency of mosaicism was 4.1% in chorionic villus samples, 0.4% in amniotic fluid samples, and 1.3% in umbilical cord blood samples. Thus, the frequency of these events exceeded our initial expectations. We hypothesize that mosaicism may be more prevalent than previously anticipated, particularly in fetal tissue obtained from abortions. The high frequency of mosaicism observed in chorionic villus samples may be subject to bias due to the potential presence of confined placental mosaicism, which have not been excluded by the second prenatal testing. The incidence of mosaicism is relatively low in karyotype analysis of the umbilical cord blood, and there is insufficient large-scale research data available in this area. In our paper, we found that only three cases of the umbilical cord blood were mosaic aneuploidy, while nine cases were mosaic segmental duplication/deletion. These findings suggest

that the incidence of mosaicism in umbilical cord blood may be higher than previously reported, and structurally rearranged chromosomes of mosaicism were readily observed in umbilical cord blood.

We calculated the percentage of mosaicism in each case from the array data and compared it with the findings from karyotype analysis. The results of eighteen cases with mosaic aneuploidy exhibited a differential outcome, with a discordance rate of 66.7% (18/27). Although mosaicism can be identified cytogenetically, the metaphase may provide a biased view due to culture influences or specific abnormalities such as Pallister Killian syndrome [4]. The SNP array presents advantages in detecting mosaicism, as it eliminates culture bias and provides insight into underlying mechanisms. However, it is not suitable for the detection of gonosomal mosaic aneuploidies. SNP array tests mixtures of DNA, thus it cannot confirm the actual type and frequencies of gonosomal mosaic aneuploidy. Six cases of gonosomal mosaic aneuploidy were detected in this study, and comparison between the array and karyotype analyses revealed discrepancies in their results, except for two cases of

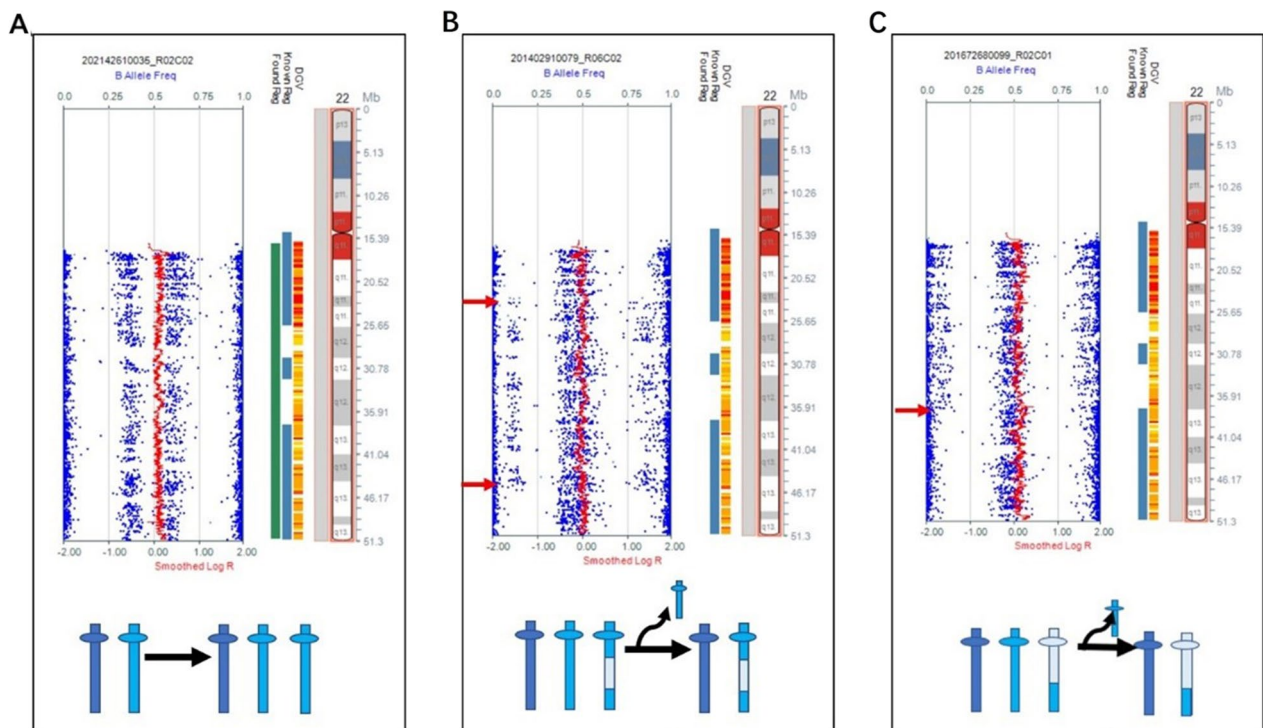


Fig. 2 SNP array results and mechanisms for mosaic trisomy 22. **A** Mosaic trisomy 22 in case 13 arose from mitotic nondisjunction without crossover, with mosaic trisomy 22 cells accounting for 80% of the total. A mitotic origin was suggested by the absence of a third haplotype, indicated on the SNP array by shifts in the B allele frequency, corresponding to a shift in B allele frequency from 50% towards 33%, and a shift from 50% toward 66%. **B** Mosaic trisomy 22 in case 14 arose from meiotic II nondisjunction with a frequency of 30%. The additional haplotypes are observable near the telomeres, but not at the centromere, which is consistent with a meiosis II origin, where sister chromatids undergo non-disjunction with two crossovers. **C** Mosaic trisomy 22 in case 15 arose from meiotic I nondisjunction with a frequency of 15%. The additional haplotypes are visible proximal to the centromere with one crossover

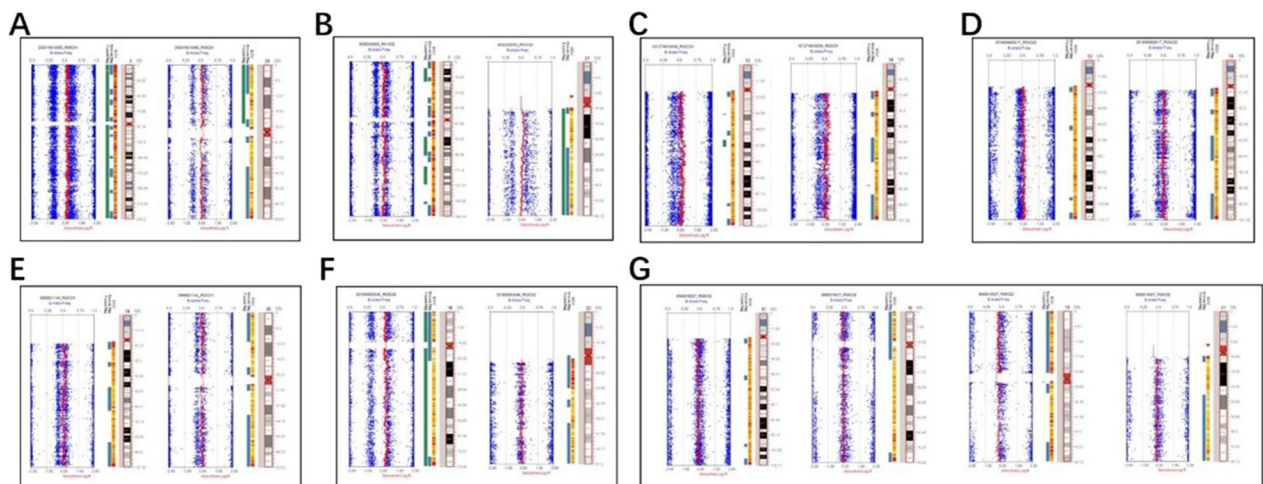


Fig. 3 SNP array of six cases with mosaic double trisomies and one case with mosaic near-diploid. The mechanisms of mosaic double trisomies and near-diploid are intricate; with the possibility of either congruous or incongruous origins for the affected chromosomes. The occurrence of errors in **A**, **B**, **D**, and **F** is characterized by varying mosaic frequencies across different chromosomes. The origin of **C** and **E** were from mitotic non-disjunction errors. **G** was from a meiosis II non-disjunction error with simultaneous occurrence at the same mosaic frequencies

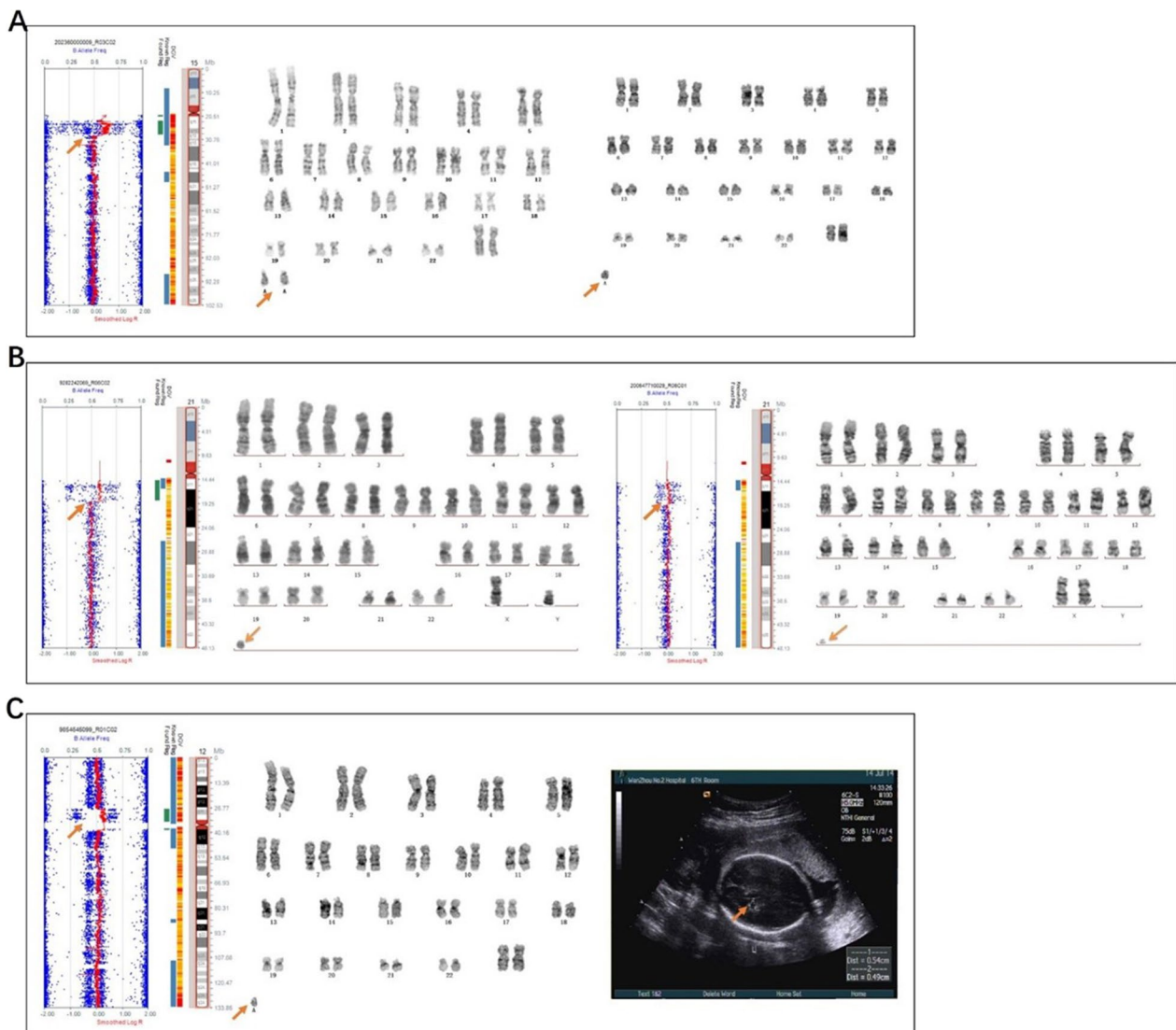


Fig. 4 SNP array and karyotype results of small supernumerary marker chromosomes. **A** SNP array revealed 15q11.2q13.1×2~6 and karyotype was 48,XX,+2mar[30]/47,XX,+mar[28]/46,XX[7] for case 32. **B** SNP array showed 21q11.2q21.1×2~4 and karyotype was 47,XY,+mar[13]/46,XY[15] for case 33. In addition, the SNP array of the mother was 21q11.2q21.1×2~3 and her karyotype was 47,XX,+mar[10]/46,XX[39]. **C** SNP array was (12)×2~3, 12p11.23p11.1×3 and karyotype was 47,XX,+mar for case 34. The presence of a choroidal fissure cyst malformation was detected via ultrasound examination

45,X/46,XX. We hypothesized that the SNP array tests DNA mixtures, thus it is unable to confirm the precise types and frequencies of gonosomal mosaicism.

In our study, SNP array analysis revealed a total of fifteen mosaic segmental duplication/deletion cases, including three cases with small supernumerary marker chromosomes (SSMCs), three cases with dicentric chromosomes, and one case with a ring chromosome. All mosaic segmental duplication/deletion cases were the result of mitotic non-disjunction, with the exception of one case involving mosaic 11q segmental duplication.

It is known that those structurally rearranged chromosomes often lead to mosaicism during mitotic disjunction. The clinical consequences of instability may surpass those related to the chromosomal abnormalities themselves [15–18]. For instance, the karyotype was 45,XX,dic(4;22)(p11;p11.2) for case 37, while SNP array showed 4p16.3p14×1, 4p14q35.2×2~3, (22)×2~3. Two thirds of retained dicentrics undergo epigenetic centromere inactivation, followed by a breakage event results in the formation of two monocentric parental chromosomes [19]. The SNP array analysis revealed

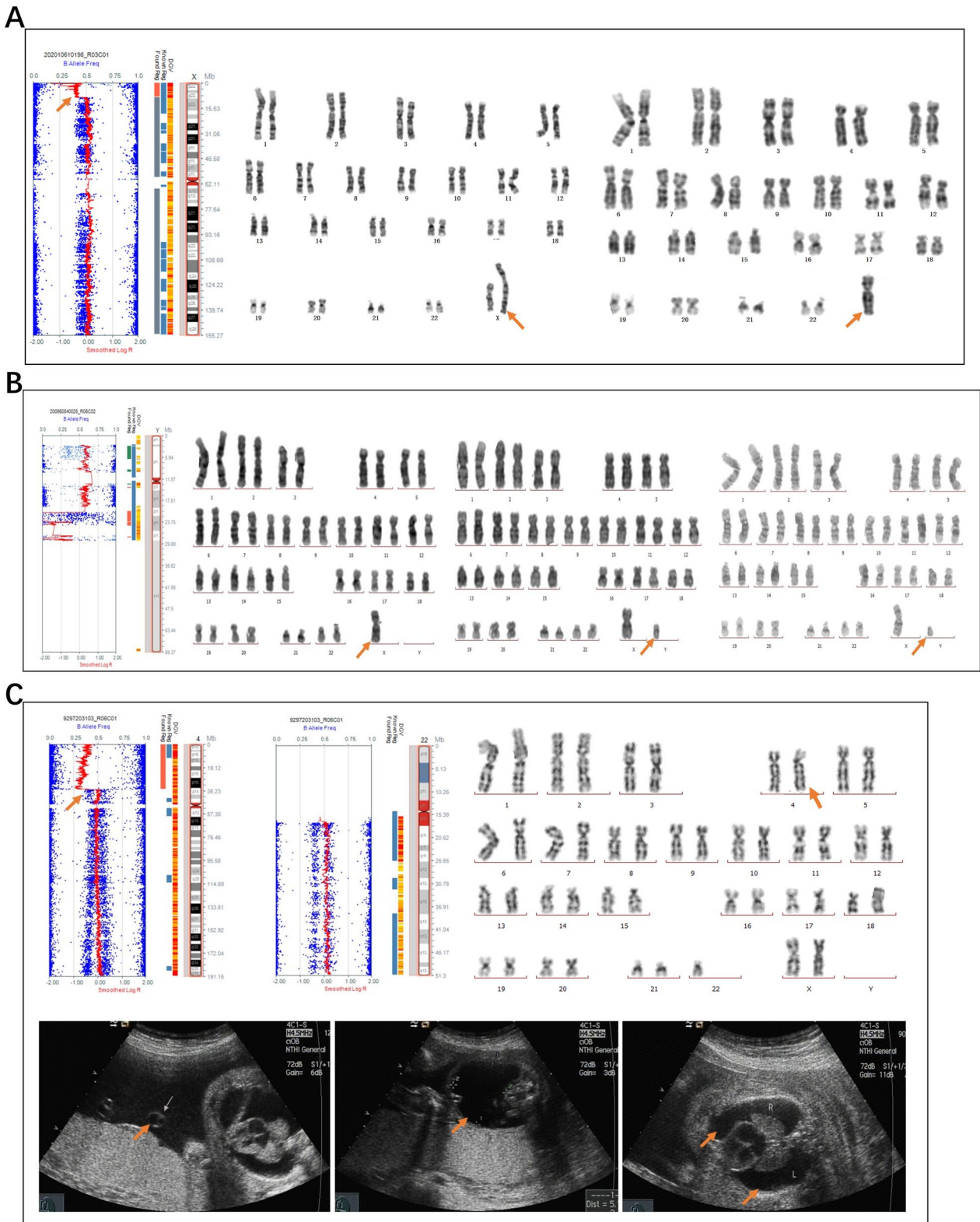


Fig. 5 SNP array and karyotype of dicentric chromosomes. **A** SNP array showed Xp22.33p22.31x1 and karyotype was 46,X,psu idic(X)(p22.3)[38]/45,X[3] for case 35. **B** SNP array showed Yp11.32q11.222x0~2, Yq11.222q11.222x0 and karyotype was 45,X[20]/46,X,idic(Y)(q11.22)[5]/46,XY[18] for case 36. **C** SNP array showed 4p16.3p14x1, 4p14q35.2x2~3, (22)x2~3 and karyotype was 45,XX,dic(4;22)(p11;p11.2) for case 37. Multiple ultrasound malformations were observed in case 37, including a single umbilical artery, ascites, and bilateral pleural effusion

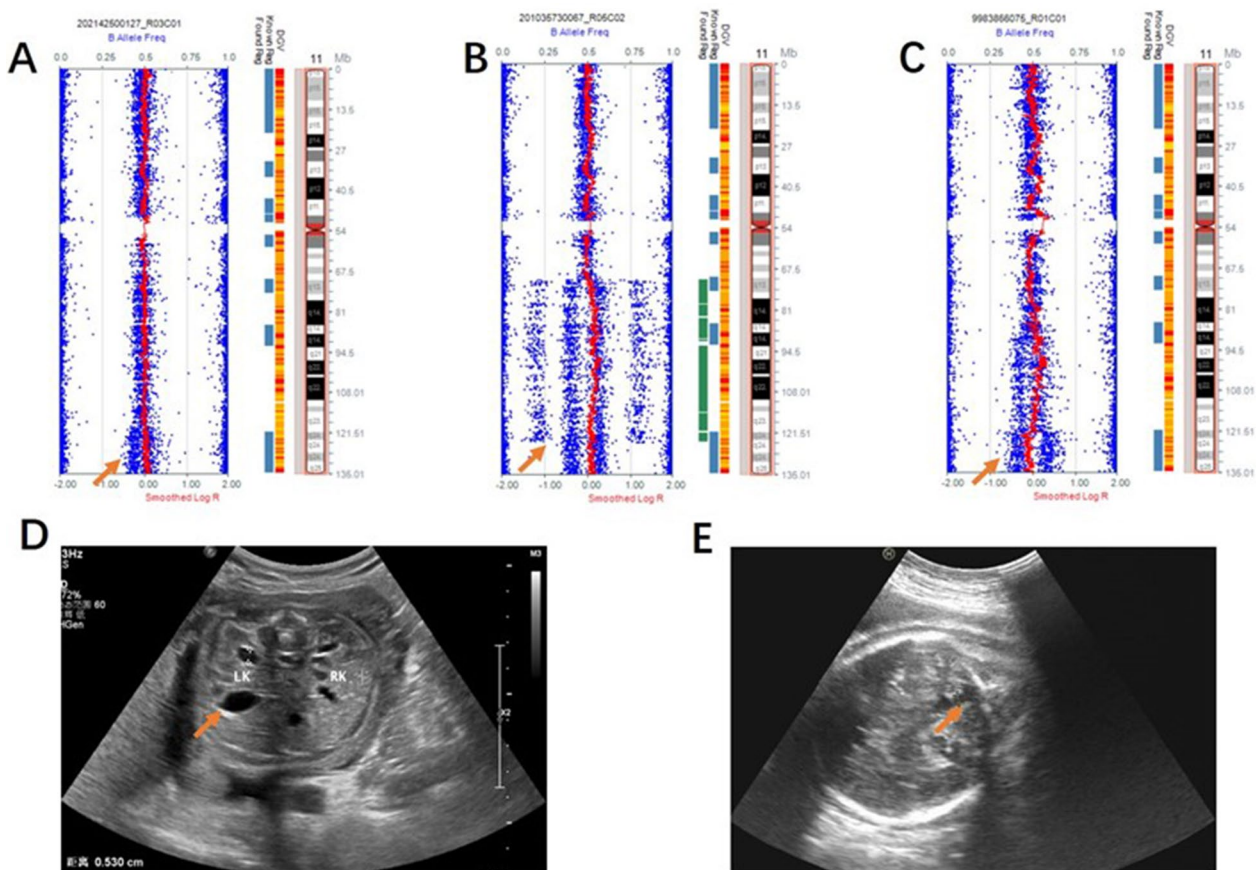


Fig. 6 SNP array and ultrasound malformations of mosaic 11q segmental duplication. **A** Case 41 arose by mitotic non-disjunction with mosaic duplication of 11q23.3q25. **B** Case 42 arose by meiotic I non-disjunction with mosaic duplication of 11q13.4q25. **C** Case 43 arose by mitotic non-disjunction with mosaic duplication of 11q14.3q24.1 combined with mosaic deletion of 11q24.1q25. **D** Enlarged pelvis malformation was observed on ultrasound for case 41. **E** The ultrasound malformation of case 42 was a posterior cranial fossa communicating with the lateral ventricle

additional genomic abnormalities beyond the deletion of the short arm of chromosome 4, providing insights into the pathogenic cause and underlying mechanism.

Previous work has demonstrated that there is a chromosome-specific bias in the ratio of meiotically to mitotically occurring non-disjunctions [9, 10]. For instance, the vast majority of trisomy 16 cases are linked to errors occurring during maternal meiotic I, while trisomy 18 is frequently linked to meiotic II errors and both trisomy 21 and trisomy 22 are commonly linked to meiotic I errors [20]. Our findings are consistent with prior research. Four cases of mosaic trisomy arose by meiotic I non-disjunction, including one case of mosaic trisomy 22 and three cases of mosaic trisomy 16; Four cases arose by meiotic II non-disjunction, including one case each of mosaic trisomy 18 and 22 and two cases of mosaic trisomy 21. Surprisingly, specific recombination hotspots were identified in 16p12.3 and 16q22.1, indicating that these loci are highly prone to cross-exchange.

Six cases with mosaic double trisomies were identified in our study. All cases resulted in intrauterine fetal death during the first trimester, indicating that these mosaic double trisomies are incompatible with embryonic development. Mosaic double trisomies are present in 0.21%~2.8% of early spontaneous abortions, which represent a selective pressure against embryonic development [21, 22]. The lethality of double aneuploidies depends on the specific chromosomes involved, and in certain cases, liveborns have aneuploidies involving chromosomes 8, 13, 18, 21, X, and Y [23]. The mechanism underlying mosaic double trisomies is intricate; the origin of the two chromosomes may be identical or distinct [24, 25]. Here, two cases were from mitotic errors where the mistakes occurred simultaneously with two chromosomes having identical mosaic frequency. Conversely, in the remaining four cases, errors occurred successively with varying mosaic frequencies for different chromosomes. These cases suggest that the assumption of equal mosaic frequency in double trisomies arise from the

same mechanisms. On the contrary, if double trisomies arises from different mechanisms, their mosaic frequency are likely to differ.

Conclusion

We identified 44 cases of mosaicism by the use of SNP array analyses. The utilization of SNP arrays allows for the characterization of mosaicism and provides valuable data for estimating disease mechanisms and recurrence. It is recommended to employ a combination of different technologies for detecting mosaicism.

Methods

Study subjects

This study was approved by the institutional research ethics committee of Wenzhou Central Hospital. All parents agreed to participate in the study and provided written informed consent. A total of 4512 pregnant women were referred for genome-wide SNP array testing for prenatal diagnosis at our prenatal diagnosis center in Wenzhou, China, between 2012 and 2018. All pregnant women underwent CVS, amniocentesis, or venipuncture, one of the three options. The indications for prenatal diagnostic testing included: advanced maternal age, high-risk serological screening, abnormal non-invasive prenatal DNA test, abnormal ultrasonographic indications, mother/father carrying chromosomal structural rearrangement, history of intrauterine fetal death or aborted fetuses, and other necessary situations. Peripheral venous blood was collected from the parents if necessary.

SNP array analysis

DNA was extracted from villi, amniotic fluid, or cord blood. Chromosomal microarray analysis was performed using the Illumina Human CytoSNP-12 array, according to the manufacturer's instructions. The results were analyzed with Illumina BeadStudio software.

Mosaic changes were detected by assessing for aberrations in probe intensities (log R ratios) along with shifts in genotype frequencies of the SNP probes (B allele frequencies) [26]. Mosaic trisomy is diagnosed when the log R ratio shows an increase in copy number, with between two and three copies; in addition, the B allele frequency must appear to be altered. In the case of mosaic monosomy, the log R ratio indicates a decrease in copy number, between one and two copies.

Karyotype analysis

Samples of CVS, amniotic fluid, and cord blood were cultured and conventional G-banded karyotyping, according to the standard methods. Karyotypes were analyzed by two physicians, according to the International System for Human Cytogenetic Nomenclature (ISCN) 2020

standard. Additionally, we conducted a comparative analysis of SNP array and conventional karyotype results for each mosaic case.

Abbreviations

CPM	Confined placental mosaicism
TFM	True fetal mosaicism
SNP	Single nucleotide polymorphism
CVS	Chorionic villus sampling
sSMCs	Small supernumerary marker chromosomes
ISCN	International System for Human Cytogenetic Nomenclature

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Author contributions

All authors contributed to the study conception and design. Clinical data collection, genetic counseling and follow-up were performed by LZ and XX. SNP array data analyses was performed by HL and CX. Karyotype analysis was performed by ZZ. The first draft of the manuscript was written by LZ and ST, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

This research was approved by the Ethics Committee of Wenzhou Central Hospital. It is a retrospective study without any identifiers related with patients. All patients participating in the study wrote informed consent.

Consent for publication

All patients in this study provided their consent for publication.

Competing interests

The authors declare that they have no conflict of interest.

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References

1. Strachan T, Read AP. Human molecular genetics. Garland: Science; 2011.
2. Youssoufian H, Pyeritz RE. Mechanisms and consequences of somatic mosaicism in humans. *Nat Rev Genet.* 2002;3(10):748–58. <https://doi.org/10.1038/nrg906>.
3. Taibjee SM, Bennett DC, Moss C. Abnormal pigmentation in hypomelanosis of Ito and pigmentary mosaicism: the role of pigmentary genes. *Br J Dermatol.* 2004;151(2):269–82. <https://doi.org/10.1111/j.1365-2133.2004.06057.x>.

4. Priest JH, Rust JM, Fernhoff PM. Tissue specificity and stability of mosaicism in Pallister-Killian +i(12p) syndrome: relevance for prenatal diagnosis. *Am J Med Genet.* 1992;42(6):820–4. <https://doi.org/10.1002/ajmg.1320420615>.
5. Grati FR, Malvestiti F, Branca L, et al. Chromosomal mosaicism in the fetoplacental unit. *Best Pract Res Clin Obstet Gynaecol.* 2017;42:39–52. <https://doi.org/10.1016/j.bpobgyn.2017.02.004>.
6. Grati FR, Grimi B, Frascoli G, et al. Confirmation of mosaicism and uniparental disomy in amniocytes, after detection of mosaic chromosome abnormalities in chorionic villi. *Eur J Hum Genet.* 2006;14(3):282–8. <https://doi.org/10.1038/sj.ejhg.5201564>.
7. Hahnemann JM, Vejerslev LO. European collaborative research on mosaicism in CVS (EUCROMIC)—fetal and extra fetal cell lineages in 192 gestations with CVS mosaicism involving single autosomal trisomy. *Am J Med Genet.* 1997;70(2):179–87. [https://doi.org/10.1002/\(sici\)1096-8628\(19970516\)70:2%3c179::aid-ajmg15%3e33.0.co;2-g](https://doi.org/10.1002/(sici)1096-8628(19970516)70:2%3c179::aid-ajmg15%3e33.0.co;2-g).
8. Breed AS, Mantingh A, Vosters R, et al. Follow-up and pregnancy outcome after a diagnosis of mosaicism in CVS. *Prenat Diagn.* 1991;11(8):577–80. <https://doi.org/10.1002/pd.1970110814>.
9. Robinson WP, Bernasconi F, Lau A, McFadden DE. Frequency of meiotic trisomy depends on involved chromosome and mode of ascertainment. *Am J Med Genet.* 1999;84(1):34–42 (PMID: 10213044).
10. Robinson WP, Binkert F, Bernasconi F, et al. Molecular studies of chromosomal mosaicism: relative frequency of chromosome gain or loss and possible role of cell selection. *Am J Hum Genet.* 1995;56:444–51 (PMID: 7847381).
11. Warburton D, Dallaire L, Thangavelu M, et al. Trisomy recurrence: a reconsideration based on North American data. *Am J Hum Genet.* 2004;75(3):376–85. <https://doi.org/10.1086/423331>.
12. Vissers LE, de Vries BB, Osoegawa K, et al. Array-based comparative genomic hybridization for the genome-wide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet.* 2003;73(6):1261–70. <https://doi.org/10.1086/379977>.
13. Rodr guez-Santiago B, Malats N, Rothman N, et al. Mosaic uniparental disomies and aneuploidies as large structural variants of the human genome. *Am J Hum Genet.* 2010;87(1):129–38. <https://doi.org/10.1016/j.ajhg.2010.06.002>.
14. Levy B, Hoffmann ER, McCoy RC, et al. Chromosomal mosaicism: origins and clinical implications in preimplantation and prenatal diagnosis. *Prenat Diagn.* 2021;41(5):631–41. <https://doi.org/10.1002/pd.5931>.
15. Vundinti BR, Korgaonkar S, Ghosh K. De novo origin of multiple small supernumerary marker chromosomes (sSMCs) in a child with intellectual disability and dysmorphic features. *Gene.* 2012;498(1):128–30. <https://doi.org/10.1016/j.gene.2012.01.066>.
16. T mer Z, Harboe TL, Blennow E, et al. Molecular cytogenetic characterization of ring chromosome 15 in three unrelated patients. *Am J Med Genet A.* 2004;130A(4):340–4. <https://doi.org/10.1002/ajmg.a.30035>.
17. Purandare SM, Lee J, Hased S, et al. Ring chromosome 9 [r(9)(p24q34)]: a report of two cases. *Am J Med Genet A.* 2005;138A(3):229–35. <https://doi.org/10.1002/ajmg.a.30382>.
18. Guilherme RS, Meloni VF, Kim CA, et al. Mechanisms of ring chromosome formation, ring instability and clinical consequences. *BMC Med Genet.* 2011;12:171. <https://doi.org/10.1186/1471-2350-12-171>.
19. Stimpson KM, Matheny JE, Sullivan BA. Dicentric chromosomes: unique models to study centromere function and inactivation. *Chromosome Res.* 2012;20(5):595–605.
20. Hassold T, Hall H, Hunt P. The origin of human aneuploidy: where we have been, where we are going. *Hum Mol Genet.* 2007;16:R203–8. <https://doi.org/10.1093/hmg/ddm243>.
21. Reddy KS. Double trisomy in spontaneous abortions. *Hum Genet.* 1997;101(3):339–45. <https://doi.org/10.1007/s004390050638>.
22. Kalousek DK, Pantzar T, Tsai M, Paradise B. Early spontaneous abortion: morphologic and karyotypic findings in 3,912 cases. *Birth Defects Orig Artic Ser.* 1993;29(1):53–61 (PMID: 8280893).
23. Diego-Alvarez D, Ramos-Corrales C, Garcia-Hoyos M, et al. Double trisomy in spontaneous miscarriages: cytogenetic and molecular approach. *Hum Reprod.* 2006;21(4):958–66. <https://doi.org/10.1093/humrep/dei406>.
24. Kato T, Kawai M, Miyai S, et al. Analysis of the origin of double mosaic aneuploidy in two cases. *Cytogenet Genome Res.* 2020;160(3):118–23. <https://doi.org/10.1159/000507177>.
25. Li QY, Tsukishiro S, Nakagawa C, et al. Parental origin and cell stage of non-disjunction of double trisomy in spontaneous abortion. *Congenit Anom.* 2005;45(1):21–5. <https://doi.org/10.1111/j.1741-4520.2005.00056.x>.
26. Conlin LK, Thiel BD, Bonnemann CG, et al. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet.* 2010;19(7):1263–75. <https://doi.org/10.1093/hmg/ddq003>.

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