Clin. Lab. 2026;72:XXX-XXX

ORIGINAL ARTICLE

Karyotype Analysis and Chromosomal Microarray Analysis in 568 Couples with Balanced Translocation for Prenatal Diagnosis

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SUMMARY

Background: **Chromosomal balanced translocation is a prevalent structural chromosomal abnormality. Carriers typically exhibit no phenotypic differences, as the genetic material remains unchanged. However, during germ cell meiosis, unbalanced gametes may be produced, leading to genetic effects in offspring and potentially resulting in adverse outcomes. This study aims to explore the prenatal diagnostic value of combining chromosome karyotype analysis with chromosomal microarray analysis (CMA) for carriers of balanced chromosomal translocations, and to provide a reference for clinical genetic counselling.**

Methods: **A total of 568 pregnant women who underwent prenatal diagnosis at our hospital from January 2018 to December 2023, in which one of the spouses was a balanced translocation carrier, were included. Amniocentesis was performed for karyotype analysis and CMA to detect abnormal chromosomes and assess the risk of adverse pregnancy outcomes in the fetus.**

Results: **Among the 568 examinees, karyotype analysis identified 236 cases of abnormal fetuses (41.55%), whereas CMA identified 200 cases (35.21%). The combination of both methods detected a total of 265 cases of abnormal fetuses (46.65%). Karyotype analysis identified 163 cases (28.70%) as high risk for adverse pregnancy outcomes, whereas CMA and the combined use of both methods identified 183 cases (32.22%) as high risk. These differences were attributed to each method's adaptability and limitations. Follow-up revealed a 100% rate of adverse outcomes among those at high and moderate risk.**

Conclusions: **Couples with balanced chromosomal translocations face an increased risk of adverse pregnancy outcomes. While karyotyping is effective in identifying diverse chromosomal abnormalities, its ability to detect minor fragments is limited. Conversely, CMA excels at identifying chromosomal abnormalities with small fragments but struggles with detecting balanced structural variations. The concurrent application of both technologies enhances the precision of diagnosing the risk of adverse pregnancy outcomes.**

(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250667)

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Manuscript accepted July 8, 2025

KEYWORDS

prenatal diagnosis, chromosomal karyotype, chromosomal microarray analysis, balanced chromosomal translocations

INTRODUCTION

Chromosomal balanced translocation is a common type of chromosomal structural abnormality characterized by the exchange of fragments resulting from the breakage and reconnection of two or more chromosomes at breakpoints, leading to the formation of new derivative

chromosomes [1,2]. Because there are no genetic material changes, carriers of chromosomal balanced translocations typically do not exhibit discernible differences in intelligence, appearance, or physiological functions compared with individuals with normal karyotypes [3,4]. However, during germ cell meiosis in carriers, the production of gametes with unbalanced genetic material may occur, potentially resulting in genetic effects in offspring and contributing to adverse pregnancy outcomes such as miscarriage, stillbirth, fetal malformations, and birth defects [5].

Prenatal diagnosis plays a pivotal role in reducing birth defects, with chromosome karyotype analysis and chromosomal microarray analysis (CMA) being the primary diagnostic modalities, but both methods have their own limitations [6]. Karyotype analysis is regarded as the gold standard in prenatal diagnosis because of its effectiveness in identifying chromosomal numerical abnormalities and structural variations, which encompass both balanced and unbalanced structural anomalies. Nevertheless, this method has drawbacks such as a prolonged cell culture cycle, a cumbersome experimental process, and potential risk including culture contamination or failure. Most importantly, it is unable to detect minor copy number variations smaller than 5 - 10 Mb [7,8]. CMA utilizes high-density and specific probes for the high-throughput analysis of genome-wide copy number variations. This method eliminates the need to culture amniotic fluid cells, employs simple experimental protocols, and has a high rate of efficacy. This technology can detect ≥ 200 kb for gains and ≥ 100 kb for losses of unbalanced copy number variations and can accurately pinpoint the chromosomal sites involved in abnormal chromosomes. However, owing to their equivalence in terms of gene quantity and genetic content to those of normal chromosomes, CMA is unable to identify balanced variations such as translocations, inversions, and insertions. Additionally, CMA may overlook diagnoses of low-proportion mosaicism less than 20% [9,10]. This study performs a retrospective analysis of the prenatal diagnosis results and pregnancy outcomes of couples who are carriers of balanced chromosomal translocations and who underwent prenatal diagnosis at our hospital, aiming to explore the application value of karyotype analysis and CMA in prenatal diagnosis for carriers of balanced chromosomal translocations.

MATERIALS AND METHODS

Patient sample collection

This study was a retrospective investigation that was reviewed and approved by the Medical Ethics Committee of our hospital (Approval No. 2018-335). A total of 568 pregnant women who underwent prenatal diagnosis at our outpatient department from January 2018 to December 2023, with at least one spouse being a carrier of a balanced chromosomal translocation, were included as study subjects. The inclusion criteria were as follows: 1) aged between 20 and 45 years; 2) gestational age between 16 and 24 weeks; 3) confirmed carrier status of one spouse for a balanced chromosomal translocation, without the presence of other chromosomal abnormalities; 4) complete clinical data, with both amniotic fluid karyotype analysis and CMA performed. The exclusion criteria were as follows: 1) individuals with a history of blood transfusion or bone marrow transplantation within 6 months prior to pregnancy; 2) individuals with infections caused by viruses such as mycoplasma or chlamydia during pregnancy; 3) individuals with immune diseases or severe intrauterine conditions; 4) individuals with nonnatural pregnancies; 5) individuals with failed amniotic fluid karyotype analysis or CMA testing; 6) individuals with incomplete pregnancy outcomes.

Amniotic fluid specimen collection

The pregnant woman was placed in the supine position for ultrasound assessment to determine the placental location and amniotic fluid volume, facilitating identification of the optimal puncture site. The abdomen was disinfected routinely and draped, after which amniocentesis was conducted under ultrasound guidance. A sterile syringe aspirated 30 mL of amniotic fluid in one attempt, which was then divided into three sterile tubes, each containing 10 mL. The samples were promptly transferred to the laboratory, with two tubes designated for karyotype analysis and one for CMA.

Karyotype analysis

In the conventional protocol, amniotic fluid is centrifuged at 150 rpm for 10 minutes, after which the supernatant is discarded. The resulting sediment was meticulously resuspended and transferred to a cell culture flask with 5 mL of amniotic fluid medium. The flask was subsequently incubated at 37°C with 5% carbon dioxide for 7 - 10 days until substantial proliferation of adherent amniotic fluid cells and colony formation were observed. Following a 3-hour incubation with amniotic fluid medium containing colchicine, the amniotic fluid cells were harvested. Chromosome karyotyping was conducted after sequential steps, including digestion, hypotonic treatment, prefixation, fixation, slide preparation, and banding. Twenty metaphases were assessed from each amniotic fluid sample's dual-line cultures, with a minimum of 5 karyotypes analyzed. In cases of abnormal karyotypes, the number of metaphases counted and karyotypes analyzed increased. Karyotypes were designated in accordance with the International System for Human Cytogenetic Nomenclature (ISCN 2020).

Chromosomal microarray analysis

Genomic DNA was isolated from 10 mL of uncultured amniotic fluid cells via a standard DNA extraction kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's protocol. CMA was conducted via the CytoScan 750K array (Affymetrix, CA, USA), which

involves several steps: digestion, ligation, PCR amplification, purification of PCR products and quantification, fragmentation, labelling, array hybridization, washing, and scanning. Genome-wide screening thresholds were established at ≥ 200 kb for gains and ≥ 100 kb for losses via the accompanying software ChA2.0. The pathogenicity of copy number variations was interpreted with reference to several databases, including DECIPHER (https://decipher.sanger.ac.uk/), DGV (http://dgv.tcag.ca/dgv), OMIM (https://omim.org/), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), and our laboratory's internal database. Copy number variations pathogenicity was classified according to the American College of Medical Genetics (ACMG) standards into five categories [11]: pathogenic (P), likely pathogenic (LP), variants of unknown significance (VOUS), likely benign (LB), and benign (B).

Risk assessment and follow-up of pregnancy outcomes

Conducting a risk assessment for the fetus on the basis of the combined detection of karyotype analysis and CMA was essential. High risk is indicated by the presence of severe genetic disorders, significant malformations, or defects. Moderate risk is characterized by mild malformations or defects, which may have a good prognosis achievable through surgical intervention or appropriate treatment measures after birth. Low risk refers to the presence of chromosomal abnormalities, but there is no previous evidence indicating pathogenicity. No risk is determined by the absence of chromosomal abnormalities.

Statistical analysis

Statistical analysis of the data was performed with SPSS 26.0 software. Count data are presented as frequencies and rates (%), and the chi-squared test was used for comparisons between groups. $p < 0.05$ was considered statistically significant.

RESULTS

General information

This study involved a total of 568 couples with balanced chromosomal translocations. The average age of the participants was 28.4 ± 6.2 years, with an average gestational age of 20.7 ± 2.2 weeks and an average of 2.6 ± 1.5 pregnancies per couple. In addition, 266 cases presented chromosomal abnormalities in male partners, whereas 302 cases presented chromosomal abnormalities in female partners.

Results of karyotype analysis and chromosomal microarray analysis

Among the 568 examined fetuses, chromosomal abnormalities were detected via karyotype analysis in 236 cases, resulting in an abnormality detection rate of 41.55%. CMA identified abnormalities in 200 cases, yielding a detection rate of 35.21% (Table 1). The difference in the detection rates for abnormal chromosomes between the two methods was statistically significant ($\chi^2 = 4.824$, $p < 0.05$). Combined detection identified chromosomal abnormalities in 265 patients, resulting in a detection rate of 46.65%. There was no statistically significant difference compared with single karyotype analysis ($\chi^2 = 3.003$, $p > 0.05$). However, a statistically significant difference was found compared with a single CMA ($\chi^2 = 13.575$, $p < 0.001$). The detection rates for numerical abnormalities of autosomal and sex chromosomes were identical between karyotype analysis and CMA. The differences in detection rates for both methods are attributed primarily to balanced structural abnormalities, unbalanced structural abnormalities, and mosaicism. Among the fetuses identified with chromosomal abnormalities through combined detection, 216 fetuses had abnormal chromosomes associated with balanced chromosomal translocations in their parents, whereas 49 fetuses had abnormal chromosomes not linked to parental balanced chromosomal translocations.

The results of risk assessment

This study conducted a risk assessment for patients who presented with abnormal results. On the basis of karyotype analysis, 163 cases were identified as high risk for adverse pregnancy outcomes, yielding a high risk rate of 28.70%. CMA and combined detection identified 183 cases as high risk, resulting in a high risk rate of 32.22% (Table 2). There was no statistically significant difference in the high risk assessment results for adverse pregnancy outcomes in fetuses when karyotype analysis was compared with CMA/combined detection ($\chi^2 = 1.662$, $p > 0.05$). In the results of the combined detection, all balanced structural variations were classified as low risk for adverse pregnancy outcomes. Among all cases of sex chromosome numerical abnormalities and mosaics, only one case of Turner syndrome (karyotype 45,X) and one case of sex chromosome mosaicism were deemed to have a moderate risk for adverse pregnancy outcomes, whereas the remaining cases were classified as low risk. All cases of trisomies and other unbalanced structural variations, except for 8 cases of VOUS assessed as having a low risk for adverse pregnancy outcomes, were classified as having a high risk for adverse pregnancy outcomes.

Pregnancy outcome follow-up

The pregnancy outcomes of fetuses diagnosed by the joint detection categorized by varying risk levels are presented in Table 3. Among the 183 fetuses classified as high risk, the incidence of adverse pregnancy outcomes was 100.00%. Among the 2 fetuses whose parents chose to continue the pregnancy and give a live birth normally, one diagnosed with a 21-trisomy karyotype was born with Down syndrome, whereas the other, diagnosed with 1q21.1 duplication syndrome, exhibited developmental delays after birth. Among the 2 fetuses classified as medium risk, the rate of adverse pregnancy

Table 1. Comparison of karyotype analysis and CMA in detecting fetal chromosomal abnormalities.

	Groups	Types	Karyotype analysis		CMA		Combined detection	
			numbers	detection rates	numbers	detection rates	numbers	detection rates
Not related	autosomal aneuploidy	trisomy 21/18/13	12	2.11%	12	2.11%	12	2.11%
	sex chromosome	47,XXY/47,XXX/ 45,XO	5	0.88%	5	0.88%	5	0.88%
	balanced structural abnormality	balanced translocation	3	0.53%	0	0.00%	3	0.53%
		inversion	2	0.35%	0	0.00%	2	0.35%
		insertion	1	0.18%	0	0.00%	1	0.18%
	unbalanced structural abnormality	deletion	1	0.18%	7	1.23%	7	1.23%
		duplication	2	0.35%	8	1.41%	8	1.41%
		isochromosome	3	0.53%	3	0.53%	3	0.53%
		small marker chromosomes	2	0.35%	1	0.18%	2	0.35%
	mosaicism	numerical mosaicism	6	1.06%	4	0.70%	6	1.06%
	total		37	6.51%	40	7.04%	49	8.63%
Related	autosomal aneuploidies	trisomy 21	7	1.23%	7	1.23%	7	1.23%
	balanced structural abnormality	balanced translocation	52	9.15%	0	0.00%	52	9.15%
		insertion	4	0.70%	0	0.00%	4	0.70%
	unbalanced structural abnormality	deletion	59	10.39%	68	11.97%	68	11.97%
		duplication	51	8.98%	59	10.39%	59	10.39%
		deletion and duplication	26	4.58%	26	4.58%	26	4.58%
	total		199	35.04%	160	28.17%	216	38.03%
Total			236	41.55%	200	35.21%	265	46.65%

Not related: Abnormal chromosomes of the fetus not related to the parents' balanced translocation.
Related: Abnormal chromosomes of the fetus related to the parents' balanced translocation.

outcomes was also 100.00%. One patient exhibited intersexuality due to sex chromosome mosaicism, whereas the other presented with congenital absence of the uterus, which was attributed to Turner syndrome. In a cohort of 80 subjects categorized as low risk, the rate of adverse pregnancy outcomes was 35.00%. Among these, 25 fetuses identified as carriers of balanced chromosomal translocations were aborted due to concerns regarding potential future fertility issues in adulthood, and one normally delivered fetus experienced developmental delay attributed to postnatal injury. In the group of 303 subjects classified as no risk, the rate of adverse pregnancy outcomes was 5.28%, with 4 cases of developmental delay in fetuses for which the etiology was unidentified.

Molecular characteristics and pregnancy outcomes of patients with normal karyotypes but abnormal chromosomal microarray analysis results

This study identified 29 cases with copy number variations through CMA, revealing abnormal chromosomal segments ranging from 226.0 kb to 5.2 Mb, despite normal karyotype analysis results. Among these cases, 17 exhibited abnormal segment loci related to the chromosomal balanced translocation points in their parents, including 12 pathogenic variations and 5 variants of VOUS. The remaining 12 cases presented abnormal segment loci that were not related to their parents' chromosomal balanced translocation points, comprising 9 pathogenic variations and 3 VOUS. The syndromes resulting from these variations and the associated pregnancy outcomes are detailed in Tables 4 and 5.

Table 2. Comparison of karyotype analysis and CMA in detecting high-risk adverse pregnancy outcomes.

Groups	Types	Karyotype analysis		CMA		Combined detection	
		numbers	high-risk	numbers	high-risk	numbers	high-risk
Autosomal aneuploidy	trisomy 21/18/13	19	19 (3.35%)	19	19 (3.35%)	19	19 (3.35%)
Sex chromosome	47,XXY/47,XXX/45,XO	5	0 (0.00%)	5	0 (0.00%)	5	0 (0.00%)
Balanced structural abnormality	balanced translocation	55	0 (0.00%)	0	0 (0.00%)	55	0 (0.00%)
	inversion	2	0 (0.00%)	0	0 (0.00%)	2	0 (0.00%)
	insertion	5	0 (0.00%)	0	0 (0.00%)	5	0 (0.00%)
Unbalanced structural abnormality	deletion	60	60 (10.56%)	75	72 (12.68%)	75	72 (12.68%)
	duplication	53	53 (9.33%)	67	62 (10.92%)	67	62 (10.92%)
	deletion and duplication	26	26 (4.58%)	26	26 (4.58%)	26	26 (4.58%)
	isochromosome	3	3 (0.53%)	3	3 (0.53%)	3	3 (0.53%)
	small marker chromosomes	2	2 (0.35%)	1	1 (0.18%)	2	1 (0.18%)
Mosaicism	numerical chimerism	6	0 (0.00%)	4	0 (0.00%)	6	0 (0.00%)
Total		236	163 (28.70%)	200	183 (32.22%)	265	183 (32.22%)

Table 3. Statistics of follow-up results of pregnancy outcomes [n (%)].

Pregnancy outcomes	High risk (n = 183)	Moderate risk (n = 2)	Low risk (n = 80)	No risk (n = 303)
Induced abortion	146 (79.78%)	0 (0.00%)	25 (32.05%)	2 (0.66%)
Spontaneous abortion	32 (17.49%)	0 (0.00%)	2 (2.56%)	8 (2.64%)
Stillbirth	2 (1.09%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Neonatal death	1 (0.55%)	0 (0.00%)	0 (0.00%)	2 (0.66%)
Infants with birth defects	1 (0.55%)	2 (50.00%)	0 (0.00%)	0 (0.00%)
Developmental delay	1 (0.55%)	0 (0.00%)	1 (1.28%)	4 (1.32%)
Adverse pregnancy outcomes	183 (100.00%)	2 (100.00%)	28 (35.00%)	16 (5.28%)

DISCUSSION

Fetal chromosomal abnormalities account for approximately 0.1% to 1.0% of live births and are a primary cause of birth defects, including developmental abnormalities, tissue malformations, and organ deformities in newborns [12,13]. In fact, the incidence rate of fetal chromosomal abnormalities is substantially higher than that of live births, representing a significant factor contributing to abnormal pregnancies. Genetic factors are a primary cause of fetal chromosomal abnormalities. Chromosomal balanced translocation is one of the most common forms of chromosomal abnormalities, accounting for approximately 0.16% to 0.25% of live births [14,15], 1.1% of infertile patients [16], and 3 - 4% of couples with recurrent miscarriage [17,18]. Balanced translocation carriers can theoretically produce 18 distinct types of gametes through the formation of quadrivalents during meiosis in germ cells. When these gametes combine with normal gametes, they can generate

Table 4. Abnormal CMA results in 17 fetuses with normal karyotypes, related to parental balanced translocation.

Karyotypes of parents	CMA					Pregnancy outcome
	Region	Sizes of CNVs	CNVs type	Pathogenicity	Known syndrome	
46,XX,t(1;4) (1q21;p16)	1q21. 1	676.7 kb	deletion	P	TAR syndrome	induced abortion
46,XX,t(1;16) (1q21;q24)	1q21. 1q21. 2	1. 4 Mb	duplication	P	1q21.1 duplication syndrome	born, developmental delay
46,XY,t(2;3) (p16;q29)	3q29	1.6 Mb	deletion	P	3q29 deletion syndrome	induced abortion
46,XX,t(7;20) (q11.2;p11.2)	7q11.23	1.4 Mb	deletion	P	Williams-Beuren syndrome	induced abortion
46,XX,t(5;8) (q34;p23)	8p23.1	3.8 Mb	duplication	P	8p23.1 duplication syndrome	induced abortion
46,XY,t(7;15) (q22;q11.2)	15q11.2q 13.1	5.2 Mb	duplication	P	15q11-q13 duplication	induced abortion
46,XY,t(10;15) (p11.2;q11.2)	15q11.2	342.0 kb	deletion	P	15q11.2 deletion syndrome	induced abortion
46,XX,t(X;16) (q22;p13.1)	16p13.11	1.8 Mb	duplication	P	16p13.11 duplication syndrome	induced abortion
46,XY,t(16;18) (p11.2;q21)	16p11.2	761.0 kb	deletion	P	16p11.2 deletion syndrome	induced abortion
46,XX,t(3;17) (p24;q12)	17q12	1.4 Mb	deletion	P	RCAD syndrome	induced abortion
46,XY,t(1;22) (q21;q11.2)	22q11.21	3.1 Mb	deletion	P	22q11.2 deletion syndrome	induced abortion
46,XY,t(X;7) (q26;p15)	Xq26. 2	226. 0 kb	duplication	P	Simpson-Golabi-Behmel syndrome	induced abortion
46,XY,t(2;11) (p16;q22)	2p16.3	286.0 kb	deletion	VOUS	/	inherited from parents, no abnormalities.
46,XX,t(X;6)t (p21;q16)	6q16.1	963.0 kb	duplication	VOUS	/	de novo variant, induced abortion.
46,XY,t(7;12) (q35;q14)	7q35	878.0 kb	duplication	VOUS	/	de novo variant, induced abortion
46,XY,t(8;10) (p21;q24)	10q24.2	805.0 kb	deletion	VOUS	/	no parental CMA
46,XX,t(X;9) (p22.3;q21)	Xp22.31	1.69 Mb	duplication	VOUS	/	no parental CMA

P pathogenic, VOUS variants of unknown significance, TAR thrombocytopenia absent radius, RCAD renal cysts and diabetes.

18 types of zygotes, among which only one is normal, one is a balanced translocation carrier, and the remaining are either partial monosomies or partial trisomies [19,20]. Theoretically, carriers of nonhomologous balanced chromosomal translocations have a 1/9 chance of having phenotypically normal offspring [21]. However, numerous studies indicate that this chance is actually much greater than 1/9. Li et al. [22] reported that a carrier of balanced translocation can generate normal gametes, with a theoretical probability of producing phenotypically normal offspring estimated at 1/9, and an actual probability of approximately 1/3. Kochhar et al. [23] reported that approximately 2/3 of individuals carrying balanced chromosomal translocations were expected to have normal offspring. The findings of this study are consistent with those reported in the literature. In this study, the incidence of chromosomally abnormal fetuses among couples in which one partner was a carrier of a balanced chromosomal translocation was found to be 46.65% (265/568). In these cases, only 38.03% (216/568) of the fetuses inherited abnormal gametes from parents, indicating that these fetuses presented chromosomal abnormalities associated with the balanced translocation chromosomes of their parents. The proportion of abnormal chromosomes not related to the parents' balanced translocations was 8.63% (49/568), indicating that the fetus obtained normal gametes but subsequently developed new mutations. The underlying mechanism may involve the fetus inheriting normal gametes from the parents, followed by environmental

Table 5. Abnormal CMA results in 12 fetuses with normal karyotypes, unrelated to parental balanced translocation.

Karyotypes of parents	CMA					Pregnancy outcome
	region	sizes of CNVs	CNVs type	pathogenicity	known syndrome	
46,XX,t(4;10)(q31.3;q22)	1q21.1q21.2	1.7 Mb	deletion	P	1q21.1 deletion syndrome	induced abortion
46,XY,t(3;20)(q26;p12)	7q11.23	1.4 Mb	duplication	P	7q11.23 duplication syndrome	induced abortion
46,XX,t(5;7)(q11.2;q11.2)	9q34.3	3.5 Mb	deletion	P	Kleefstra Syndrome	induced abortion
46,XY,t(7;12)(p21;q21)	15q11.2	312.0 kb	deletion	P	The 15q11.2 deletion syndrome	induced abortion
46,XX,t(6;17)(q22;q24)	15q11.2q13.1	5.2 Mb	deletion	P	PWS/AS	induced abortion
46,XX,t(7;12)(p21;q14)	16p11.2	522.0 kb	duplication	P	16p11.2 duplication syndrome	spontaneous abortion
46,XY,t(5;6)(p14;p22)	16p13.11	1.4 Mb	deletion	P	16p13.11 deletion syndrome	induced abortion
46,XY,t(10;13)(p11.2;q32)	22q11.21	2.9 Mb	duplication	P	22q11.2 duplication syndrome	induced abortion
46,XX,t(3;6)(p21.3;q21)	Xq28	447.0 kb	duplication	P	Xq28 duplication syndrome	induced abortion
46,XY,t(4;7)(p15;q21)	3p26.3	1.1 Mb	deletion	VOUS	/	inherited from parents, no abnormalities
46,XX,t(5;10)(q14;q21)	9q21.13	1.2 Mb	duplication	VOUS	/	no parental CMA
46,XX,t(X;18)(p21;q12)	16q23.3	885.0 kb	duplication	VOUS	/	inherited from parents, no abnormalities

P pathogenic, VOUS variants of unknown significance, PWS Prader-Willi syndrome, AS Angelman syndrome.

influences during the formation of germ cells or the early stages of embryonic development, resulting in variations in chromosome number or structure. Therefore, the probability of balanced translocation carriers producing normal gametes was 61.97% (352/568) in this study, rather than the theoretical value of 1/18. In addition, phenotypically normal fetuses, classified as low risk or no risk for adverse pregnancy outcomes, constituted 67.43% (383/568) of the total, which is significantly higher than the theoretical ratio of 1/9.

The discrepancy between the theoretical and actual probabilities of a balanced translocation carrier couple producing normal gametes when having offspring may be attributed to the following factors: 1) Although chromosomal balanced translocation can result in various types of gametes, the primary segregation patterns during meiosis are 2:2, 3:1, and 4:0. In 2:2 segregation, two chromosomes are distributed to one cell and two to the other; a 3:1 ratio indicates that three chromosomes are assigned to one cell and one to the other; a 4:0 ratio signifies the transfer of all quadrivalent chromosomes to one cell and none to the other. Among them, 2:2 segregation, especially alternate segregation, is the most common [24]. Therefore, the actual probability of producing normal gametes is greater than the theoretical value. 2) Sperm or eggs with unbalanced translocations have a low fertilization ability and may be eliminated during natural selection, making it difficult to form zygotes. 3) During meiosis, unbalanced products are more likely to segregate into polar bodies. 4) Unbalanced zygotes have a greater chance of being lost in the early embryonic stage. The subjects of this study were all second-trimester pregnant women, and early miscarriages of unbalanced fetuses were not included in this study.

To elucidate the application value of karyotype analysis and CMA in prenatal diagnosis for couples with balanced chromosomal translocations, this study compared the detection rates of these two techniques for fetuses presenting with chromosomal abnormalities. As demonstrated in Table 1, the detection rates for both methods were identical in the 24 cases of numerical abnormalities involving autosomes and sex chromosomes. Karyotype analysis identified 62 cases of balanced structural variations, including balanced chromosomal translocations, inversions, and insertions. Owing to the absence of changes in genetic material, CMA is unable to detect these variations. This underscores the advantage of karyotype analysis in identifying cases with balanced structural abnormalities. A total of 144 unbalanced structural variants were identified through karyotype analysis,

whereas 172 cases were detected via CMA. Among these cases, 29 exhibited abnormal chromosomal fragments smaller than 5 - 10 Mb, which were undetectable by karyotype analysis but were successfully identified by the higher resolution and sensitivity of CMA. In addition, karyotype analysis revealed two cases of small supernumerary marker chromosomes (sSMCs). One case was identified through CMA as a duplication of approximately 15.0 Mb in the region from p11.32 to p11.21 on chromosome 18, confirming the case's diagnosis of 18p tetrasomy syndrome. The other patient did not exhibit any copy number variations upon CMA testing and may represent a partial tetrasomy karyotype composed of the short arms of acrocentric chromosomes. This finding indicates that, in contrast to karyotype analysis, CMA offers the advantage of identifying the origin of sSMCs while also determining their pathogenicity. In addition, CMA failed to detect two cases of chromosomal number mosaicism with a mosaicism ratio less than 20%. In contrast, karyotype analysis demonstrated superiority in detecting low-proportion mosaics.

In this investigation, we found that karyotype analysis had a higher detection rate of chromosomal abnormalities than did CMA (41.55% vs. 35.21%), which is inconsistent with the findings of previous studies. As reported in the literature, the investigators reported a 13% [25], 4 - 6.1% [26], 8.1% [27], and 8% [28] increase in the detection rate of chromosome abnormalities via CMA compared with conventional karyotyping, respectively. The reason is that in the cases included in this study, one parent was identified as a carrier of a balanced translocation. The probability of the fetus also being a carrier of a balanced translocation was significantly elevated compared with that of fetuses conceived by the general population. Given that CMA is unable to detect balanced structural variations, the detection rate of abnormalities through karyotyping in this study was higher than that achieved by CMA technology. These findings also demonstrate that CMA and karyotype analysis differ in the detection of chromosomal balanced translocations. In addition, our findings indicate that CMA has a higher detection rate than does karyotyping for high risk adverse pregnancy outcomes (32.22% vs. 28.70%), which is consistent with the literature [29]. This can be attributed to the high sensitivity of the CMA.

In this investigation, CMA identified copy number variations in 29 cases with apparently normal karyotypes. These variations encompassed 21 pathogenic copy number alterations and 8 VOUS, involving abnormal chromosomal segments ranging from 226.0 kb to 5.2 Mb in size. Among these cases, 17 exhibited abnormal chromosomes associated with balanced chromosomal translocations observed in their parents. The underlying cause may be attributed to disruptions in gene structure or positional effects at the breakpoints during gametogenesis in parents who are carriers of balanced chromosomal translocations. Such disruptions can lead to a partial loss of gene function, resulting in microdeletions or microduplications at the breakpoints during inheritance, thereby causing abnormal phenotypes in the offspring. Among these 29 cases, 8 cases of VOUS were detected, with an overall detection rate of 1.4% (8/568), which was similar to that reported in previous studies [30,31]. Managing VOUS poses challenges in clinical counselling due to the current limitations in definitively interpreting their clinical implications. A comprehensive assessment requires integrating CMA findings with ultrasound examination results and investigating whether similar variations are present in the parents. This uncertainty often causes distress among pregnant women and their families and may lead to unwarranted pregnancy terminations.

The 21 cases of copy number variation were associated with various microdeletion/microduplication syndromes. Deletions and duplications of the 1q21.1-q21.2 segment, encompassing the 1q21.1 recurrent region (BP3-BP4, distal) and including GJA5, lead to 1q21.1 deletion syndrome and 1q21.1 duplication syndrome, respectively. Both syndromes show incomplete penetrance and variable expressivity, manifesting as developmental delays, craniofacial abnormalities, and cardiac anomalies. Notably, 1q21.1 deletion syndrome is more frequently associated with microcephaly and schizophrenia, whereas 1q21.1 duplications are linked to macrocephaly and autism [32]. Deletion of the 1q21.1 proximal region leads to thrombocytopenia-absent radius (TAR) syndrome, which is caused by the absence of the RBM8A gene. TAR syndrome is characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia while both thumbs are retained [33]. Copy number variation of a 1.4 - 1.7 Mb segment on chromosome 7q11.23, containing 25 - 27 genes, causes two distinct neurodevelopmental disorders. Deletion of this segment results in Williams-Beuren syndrome (WBS), whereas duplication leads to 7q11.23 duplication syndrome. Both conditions feature mild to moderate intellectual disability, speech sound disorders, facial dysmorphisms, and related cardiovascular and neurological diseases. Moreover, anxiety disorders are more prevalent in patients with 7q11.23 duplication syndrome than in those with WBS [34]. The 15q11.2 deletion is located within the critical region implicated in 15q11.2 BP1-BP2 deletion syndrome, also known as Burnside-Butler syndrome. This syndrome is connected to neurodevelopmental disorders characterized by changes in brain morphology, behavior, and cognition [35]. Alterations at the 15q11-q13 locus give rise to two distinct neurodevelopmental disorders: Prader-Willi syndrome (PWS) and Angelman syndrome (AS), which are imprinting disorders resulting from the absence or reduced expression of paternal or maternal genes in the chromosome 15q11-q13 region, and 15q11-q13 duplication syndrome, caused by the duplication of the 15q11-q13 region [36,37]. Variations in the 16p11.2 region covering the 16p11.2 recurrent region (proximal, BP4-BP5) (including TBX6) lead to the development of 16p11.2

deletion syndrome 16p11.2 duplication syndrome. The former is commonly associated with neurodevelopmental disorders, autism spectrum disorders, and an increased risk of obesity. Conversely, the latter typically displays characteristics such as low body weight, microcephaly, and developmental delays [38].

Deletions and duplications of the 16p13.11 segment, which encompasses the recurrent 16p13.11 region (including MYH11), lead to 16p13.11 deletion syndrome and 16p13.11 duplication syndrome. Studies have indicated that individuals with 16p13.11 deletion exhibit microcephaly, developmental delay, various mental disorders, and a propensity for epilepsy, whereas those with 16p13.11 microduplication present features such as mental retardation, autism, epilepsy, and distinctive physical characteristics [39]. The 22q11.21 fragment encompasses the recurrent 22q11.2 region (proximal, A-D) and includes the TBX1 gene, leading to 22q11.2 deletion syndrome and 22q11.2 duplication syndrome, respectively. Common features of 22q11.2 deletion syndrome include congenital heart disease, immune deficiency, distinctive facial characteristics, and learning difficulties. In contrast, the incidence of 22q11.2 duplication syndrome is relatively low, with clinical manifestations primarily consisting of growth retardation, intellectual disability, autism, and hypotonia [40]. Additionally, this study covers the following syndromes: 3q29 deletion syndrome, 8p23.1 duplication syndrome, Kleefstra syndrome, renal cysts and diabetes (RCAD) syndrome, Xq28 duplication syndrome, and Simpson-Golabi-Behmel syndrome.

CONCLUSION

Couples who are carriers of balanced chromosomal translocations have an increased likelihood of producing unbalanced gametes during the conception of their next generation, thereby necessitating prenatal diagnosis. While karyotype analysis has limitations in detecting small segmental chromosomal duplications and deletions, CMA is unable to identify balanced chromosomal structural variations. The integration of these two techniques for prenatal diagnosis provides greater accuracy than employing either technique in isolation does, facilitating a more precise evaluation of the risk of adverse pregnancy outcomes in carriers of balanced chromosomal translocations.

Acknowledgment:
We thank all the participants and the families in this study for their cooperation.

Source of Funds:
This research was funded by the Natural Science Foundation of Ningxia Hui Autonomous Region (2023AAC03616) and the Ningxia Health System Scientific Research Project (2022-NWKY-065).

Ethics Approval and Consent to Participate:
This study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University (2018-335).

Declaration of Interest:
The authors declare no competing interests.

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