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Research article

Single Nucleotide Polymorphism array analysis for fetuses from balanced translocation carriers at the second trimester

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ABSTRACT

Prenatal diagnosis is crucial for pregnancies from couples with a carrier of a balanced translocation. We retrospectively reviewed 195 pregnancies from 189 couples with a balanced translocation carrier. Of these, 126 were from natural conception, while 69 were conceived through assisted reproductive technology (ART) with preimplantation genetic diagnosis (PGD). Both single nucleotide polymorphism (SNP) array analysis and conventional karyotyping were conducted on all pregnancies, and karyotype-visible imbalances and pathogenic/likely pathogenic copy number variations (CNVs) were categorized as clinically significant abnormalities. In natural conception group, couples with a female carrier experiencing more than two miscarriages accounted for 30.2 %, significantly higher than the 14.0 % in male carrier couples ($p < 0.05$). In the PGD group, similar difference was observed between female and male carrier couples ($p < 0.05$). In the natural pregnancies, SNP array analysis yielded additional 12 cases of CNVs, including two cases of pathogenic (P)/likely pathogenic (LP) aberrations, four variants with uncertain significance (VUS), and six likely benign variants. Only two CNVs were found to be associated with translocation breakpoints, which were finally confirmed to be of parental inheritance. In the PGD pregnancies, two cases of VUS unrelated to the translocation breakpoints were revealed. Taken together, repeated miscarriage was more frequently observed in couples where the carrier was female than male. Conventional SNP array analysis in prenatal diagnosis

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indicated insufficient evidence to support the notion that balanced translocations increase the likelihood of fetuses having clinically significant CNVs.

1. Introduction

Balanced translocation stands out as the most frequently reported chromosomal rearrangement in human beings, with an incidence of approximately 0.2 % in the general population [1]. This frequency escalates to more than 2.2 % in patients experiencing pregnancy loss [2]. Generally, gene expression is not severely influenced because of the balance of gene number, and carriers may remain asymptomatic [3]. However, the production of unbalanced gametes and, subsequently, embryos from individuals with balanced translocations can lead to infertility, early miscarriages, fetuses with congenital anomalies and the birth of infants with genetic abnormalities [4,5].

Preimplantation genetic diagnosis (PGD) serves as an alternative prenatal diagnostic method for couples with translocations. It allows the selection of balanced/normal euploid embryos for embryo transfer, enabling the couples to have a healthy baby using their own gametes. Current 24-chromosome testing methods, such as array-based comparative genomic hybridization (aCGH) [6] and next-generation sequencing (NGS) [7] have been applied in PGD to detected embryos of aneuploidy and clinically significant copy number variations (CNVs). However, due to financial, convenience, or personal circumstances issues, many carriers still opt for natural conception [8]. Regardless of whether conception occurs naturally or with assistance, invasive prenatal diagnosis during the first or second trimester should be strongly recommended to confirm the fetal chromosome profile and prevent the birth of fetuses with unbalanced chromosomal abnormalities derived from balanced translocation carriers.

Conventional karyotyping remains the most cost-effective means for detecting chromosomal structural variation with a resolution of 5–10 Mb, regardless of whether the translocation is balanced or imbalanced. With the advancement of molecular detection technology, there is a growing focus on uncovering hidden imbalances within seemingly balanced translocations or normal chromosomes [9,10]. In the field of prenatal diagnosis, the high-resolution single nucleotide polymorphism array (SNP array) has gained widespread use. It is instrumental in identifying microdeletions and microduplications that may go undetected by traditional karyotyping [11–13]. While there is a wealth of literature on SNP array applications in pregnancies exhibiting ultrasound abnormalities, advanced maternal age, abnormal first-trimester screening results, and de novo apparently balanced translocations [14–16], limited studies have addressed the efficacy of SNP array analysis in pregnancies involving couples with balanced translocations [17]. Therefore, the primary aim of this study was to evaluate the genetic profiles of fetuses from couples with balanced translocation using both traditional karyotyping and SNP array analysis, with a specific emphasis on pregnancies conceived naturally.

2. Materials and methods

2.1. Patients and samples

Between June 2017 and June 2023, 203 consecutive pregnancies from couples with a balanced translocation carrier were reviewed. The translocations were confirmed through cytogenetic analysis of peripheral blood lymphocytes. Pregnancies that underwent both conventional karyotyping and SNP array analysis in parallel were included, while cases with incomplete information, such as chromosomes involved in translocation and conception mode, were excluded. Consequently, a total of 195 pregnancies from 189 couples (including six couples who underwent invasive prenatal diagnosis twice) were included in the study. These balanced translocation carriers have no other abnormal phenotypes except fertility problems. Among these pregnancies, 126 occurred naturally, and 69 were conceived through assisted reproductive technology (ART) following preimplantation genetic diagnosis (PGD) with Fluorescence in situ hybridization (FISH), Array-based comparative genomic hybridization analysis (aCGH), or Next-Generation

Table 1
Demographic characteristics.

Characteristic	Value
Gestational age at invasive prenatal diagnosis (weeks): mean ± SD	19.3 ± 1.9
Maternal age (years): mean ± SD	30.1 ± 5.4
Adverse reproductive history[a] (gravidity)	1.5 ± 1.2
Translocation type	
Reciprocal translocation (n, %)	161, 82.6 %
Robertsonian translocation (n, %)	34, 17.4 %
Gender of carrier	
Female carrier (n, %)	100, 51.3 %
Male carrier (n, %)	95, 48.7 %
Conception mode	
Natural conception	125, 64.1 %
Assisted conception	70, 35.9 %

[a] Including early pregnancy losses, stillbirths, or the birth of a child with congenital anomalies and an unbalanced karyotype.

Sequencing (NGS). The cohort comprised 33 couples with Robertsonian translocation carriers and 156 couples with reciprocal translocation carriers. Specimens included one case of chorionic villi sampled at 13 weeks of gestation, 192 cases of amniotic fluid sampled between 18 and 24 weeks of gestation, and two cases of cord blood sampled after 24 weeks of gestation. Based on ultrasound findings up to the time of prenatal diagnosis, the cohort was categorized into normal ultrasound and abnormal ultrasound groups. Key baseline characteristics are presented in Table 1.

2.2. Methods

The following methodology details have been described in our previous publications [16,18].

2.2.1. Conventional karyotyping

Conventional karyotyping involved cell culture, and G-banded karyotyping was performed on peripheral blood lymphocytes, chorionic villi, amniotic fluid, and fetal cord blood using standard protocols in our laboratory. The karyotype was determined at a resolution of 320–500 bands and described following the International System for Human Cytogenetic Nomenclature (ISCN) 2020 guidelines.

2.2.2. SNP array testing and results interpretation

Genomic DNA was extracted from uncultured amniotic fluid, fetal cord blood, or cultured chorionic villi samples using the QIAamp DNA Blood Mini kit (QIAGEN, Germany) following the manufacturer's instructions. A total of 250–300 ng of fetal DNA was analyzed using the Affymetrix CytoScan 750K array (Affymetrix Inc., Santa Clara, CA, USA), which includes 200,000 SNP markers and 550,000 copy number variation (CNV) markers. The Chromosome Analysis Suite (CHAS) 3.2 software (Affymetrix, Santa Clara, CA, USA) was utilized for raw data analysis. All identified CNVs were cross-referenced with in-house (Based on our years of work experience, we have established a database based on submicroscopic chromosomal abnormalities and postnatal phenotypes). and national public CNVs databases, including the Database of Genomic Variants (DGV: https://www.ncbi.nlm.nih.gov/clinvar/), the Database of Chromosome Imbalance and Phenotype in Humans Using Ensemble Resources (DECIPHER: https://www.deciphergenomics.org/), and the Online Mendelian Inheritance in Man (OMIM: https://www.ncbi.nlm.nih.gov/omim).

The results from CMA were classified into five levels based on the American College of Medical Genetics (ACMG) [19] definitions and the local database: pathogenic, benign, likely pathogenic, likely benign, and variants of uncertain significance (VUS). Parental CMA was advised to ascertain the inheritance of CNVs. The laboratory reported microdeletions of ≥500 kb and microduplications of ≥1 Mb. CNVs, including deletions <500 kb or duplications <1 Mb, were also reported if they involved known pathogenic genes.

In couples with balanced translocations, fetuses inheriting balanced translocations or a normal karyotype were considered acceptable results. Therefore, in present study, only unbalanced translocations and pathogenic/likely pathogenic variants were regarded as clinically significant genetic findings.

2.3. Statistical analysis

Statistical analysis was carried out using SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA).

Categorical data were comparatively assessed using Pearson's chi-squared test or Fisher's exact test, and a p-value of <0.05 was considered statistically significant.

For stratified analysis, subgroups were analyzed based on variables such as the type of chromosomal translocation (Reciprocal vs. Robertsonian) and carrier gender (female vs. male). Stratified chi-squared tests were conducted to assess the significance of differences within these subgroups. In addition, subgroup analysis was performed to compare the rates of recurrent pregnancy loss, early miscarriages, and cytogenetic abnormalities between different conception groups (natural conception vs. PGD).

Table 2
The early miscarriage history in 189 couples of different translocation type and different carrier sex.

Previous early miscarriage	Natural pregnancy group				PGD group			
	Translocation type		Carrier gender		Translocation type		Carrier gender	
	Robertsonian translocation (%)	Reciprocal translocation (%)	Male carriers (%)	Female carriers (%)	Robertsonian translocation (%)	Reciprocal translocation (%)	Male carriers (%)	Female carriers (%)
0	20.0 % (4/20)	26.0 % (26/100)	35.1 % (20/57)	15.9 % (10/63)	46.2 % (6/13)	28.6 % (15/56)	39.4 % (13/33)	25.0 % (9/36)
1	20.0 % (4/20)	18.0 % (18/100)	15.8 % (9/57)	20.6 % (13/63)	15.4 % (2/13)	30.4 % (17/56)	27.3 % (9/33)	27.8 % (10/36)
2	55.0 % (11/20)	30.0 % (30/100)	35.1 % (20/57)	33.3 % (21/63)	38.5 % (5/13)	25.0 % (14/56)	27.3 % (9/33)	27.8 % (10/36)
≥3	5.0 % (1/20)[a]	26.0 % (26/100)[b]	14.0 % (8/57)[e]	30.2 % (19/63)[f]	0.0 % (0/13)[c]	16.1 % (9/56)[d]	3.0 % (1/33)[g]	22.2 % (8/36)[h]

Note: a vs. b, $p > 0.05$; c vs. d, $p > 0.05$; e vs. f, $p < 0.05$; g vs. h, $p < 0.05$.

3. Results

3.1. Reproductive history analysis for 189 couples

In the natural conception group, 120 couples experienced 387 conceptions, with 196 (50.6 %) resulting in early miscarriage. In the PGD group, 69 couples experienced 183 conceptions, and 84 (45.6 %) of them ended in early spontaneous abortion. The total of 387 pregnancies and 183 pregnancies included cases of spontaneous miscarriage, previous live births, and pregnancies that have progressed to the midterm in this study. The proportion of couples experiencing recurrent pregnancy loss in the two groups were 56.7 % (68/120) and 40.6 % (28/69), respectively ($p < 0.05$). Table 2 illustrated that in both the natural conception and PGD conception groups, the rate of couples experiencing more than two early miscarriages in Reciprocal translocation couples was higher than but without significant difference compared to that in Robertsonian translocation couples ($p > 0.05$). In the natural conception group, couples with female carrier having more than two miscarriages accounted for 30.2 %, significantly higher than 14.0 % in male carrier couples ($p < 0.05$). In the PGD conception group, significant difference was also found between female and male carrier couples (22.2 % vs. 3.0 %, $p < 0.05$).

3.2. Cytogenetic and CMA findings in 195 pregnancies

In the analysis of 126 natural pregnancies, conventional karyotyping identified 7 cases (5.6 %) of unbalanced structural abnormalities (Table 3), 53 cases (42.1 %) of balanced translocations, and 66 cases (52.4 %) of normal chromosomes. Five cases of unbalanced translocations resulted from reciprocal translocations, involving adjacent-1 and 3:1 segregation types. These cases exhibited distinct patterns in their SNP array results: two cases revealed segmental duplications and deletions in different regions (case 1 and case 2), one case exhibited a duplication in a single region (case 3), one case displayed two partial duplications in different regions (case 4), and one case showed regular trisomy (Case 5). In total, SNP array analysis identified an additional 12 cases (6.4 %) of copy number variations (CNVs) ranging in size from 462 kb to 3.3 Mb, including 1 likely pathogenic CNVs (case 8), 1 pathogenic CNVs (case 9), 4 VUS, and 6 likely benign CNVs. Specific details are presented in Table 4. The two cases (Case 8 and Case 9) of clinically significant CNVs were both detected in fetuses with inherited balanced translocations, contributing to an incremental yield of 1.6 % (2/126) for clinically significant CNVs in the natural conception group. Neither of these cases was associated with the parental translocation breakpoint. In Case 8, a de novo 2.8 Mb duplication on 22q11.2 was identified in an apparently balanced t(6; 9) translocation (Fig. 1). The fetus was born live and exhibited normal development at the 2-year-old follow-up. In Case 9, karyotyping revealed a paternal t(4;

Table 3
Details of 7 cases with unbalanced rearrangements derived from parental balanced translocation.

Case number	Parental karyotype	Ultrasound findings before invasive prenatal diagnosis	Fetal karyotype	Segregation type	CMA result	Size	Pregnancy outcome
1	46,XY,t(2; 4)(p25.1; p15.2)	Normal	46,XY,der(4)t(2; 4)(p25.1; p15.2)dpat	adjacent-1	arr[GRCh37] 2p25.3(12,770–918,533)x3, 4p16.3p15.32(68,345–15,668,426)x1	906 Kb; 15.6 Mb	TOP
2	46,XY,t(11; 13)(q23; p13)	Normal	46,XY,der(13)t(11; 13)(q23; p11)dpat	adjacent-1	arr[GRCh37] 11q23.1q25(111,164,403–134,937,416)x3	23.7 Mb	TOP
3	46,XX,t(7; 14)(p14.3q32.3)	Bilateral choroid plexus cyst	46,XY,der(14)t(7; 14)(p14.3q32.3)dmat	adjacent-1	arr[GRCh37] 7p22.3p14.3(43,376–31,039,092)x3, 14q32.33(105,090,669–106,257,269)x1,	30.9 Mb, 1.1 Mb, 579 Kb	TOP
4	46,XX,t(11; 22)(q23; q11)	Dilation of the fourth ventricle	47,XY,+der(22)t(11; 22)(q23; q11)dmat	3:1 disjunction	arr[GRCh37] 11q23.3q25(116,683,754–134,937,416)x3, 22q11.1q11.21(16,888,899–20,312,661)x3	18.2 Mb, 3.4 Mb	TOP
5	46,XX,t(9; 21)(q22; q21)	Endocardial cushion defects, bilateral choroid plexus cyst, increased nuchal translucency, echogenic bowel, nasal bone dysplasia	47,XX,t(9; 21)(q22; q21)mat,+21	3:1 disjunction	arr[GRCh37] (21)x3		TOP
6	45,XX,rob(14; 21)(q10; q10)	choroid plexus cyst	46,XX,rob(14; 21)(q10; q10)mat,+21	adjacent-1	arr[GRCh37] (21)x3		TOP
7	45,XX,rob(13; 14)(q10; q10)	Cardiac malformation	46,XX,+13,rob(13; 14)(q10; q10) mat	adjacent-1	arr[GRCh37] (13)x3		TOP

TOP, termination of pregnancy.

Table 4
Details of 14 copy number variants (CNVs) detected by SNP array analysis.

Case number	Gestational age (weeks)	Ultrasound finding	Karyotype	CMA results	Size	Classification	Inheritance
8	19	Transient mitral regurgitation	46,XX,t(6; 9) (p21.3p23)mat	arr[GRCh37] 22q11.21 (18,648,855–21,461,017)x3	2.8 Mb	LP	De novo
9	24	Increased nuchal translucency, mild tricuspid regurgitation	46,XX,t(4; 11) (q35; q22.3)pat	arr[GRCh37] 1q21.1q21.2 (145,829,473–148,520,164)x1	2.7 Mb	P	De novo
10	19	Normal	46,XY	arr[GRCh37] 16p13.11 (14900042–16538596)x3	1.6 Mb	VOUS	Unknown
11	20	Normal	46,XX	arr[GRCh37] 1q21.1q21.2 (146,106,723–147,933,973)x1	1.8 Mb	LB	Paternal
12	19	Normal	46,XX	arr[GRCh37] 16p13.11 (14,920,864–16,538,596)x3	1.65 Mb	VOUS	Unknown
13	18	Normal	46,XX	arr[GRCh37] 2p25.3p25.2 (4,131,412–4,635,997)x1	505 kb	LB	Paternal
14	18	Normal	46,XX	arr[GRCh37] 15q13.3 (31,981,843–32,444,043)x3	462 kb	VOUS	Unknown
15	20	Normal	46,XX	arr[GRCh37] 15q26.3 (98,696,237–99,394,164)x3	898 kb	VOUS	De novo
16	23	Normal	46,XX,t(20; 21) (p11.2; q21)mat	arr[GRCh37] 21q21.2q21.3 (26,501,564–27,409,999)x3	908 kb	LB	Maternal
17	19	Normal	46,XX,t(4; 5) (q31; q23)pat	arr[GRCh37] 5q23.1 (116,826,156–117,856,345)x1	1.0 Mb	LB	Paternal
18	18	Normal	46,XY,t(1; 15) (q44; q14)mat	arr[GRCh37] Xp22.33p22.32 (2,372,667–5,718,525)x2,(XY)x1	3.3 Mb	LB	Paternal
19	19	Normal	46,XY,t(3; 19) (q21; p13.2)mat	arr[GRCh37] 16q23.1 (77,400,820–78,439,099)x3	1.0 Mb	LB	Maternal
20	19	Normal	46,XX	arr[GRCh37]16p13.11 (14,920,864–16,538,596)x3	1.65 Mb	VOUS	Unknown
21	18	Normal	46,XX	arr[GRCh37] 15q13.3 (31,981,843–32,444,043)x3	462 Kb	VOUS	Unknown

LP, likely pathogenic; P, pathogenic; VOUS, variants of unknown significance; LB, likely benign.

11) translocation, while SNP array analysis uncovered a de novo 2.7 Mb deletion on the 1q21.1q21.2 region (Fig. 2). This deletion was categorized as a pathogenic aberration, and the pregnancy was terminated. Among the 12 CNVs, only two (Cases 16 and 17) were related to the translocation breakpoints, both of which were inherited from phenotypically normal parents and were thus classified as likely benign CNVs.

Following preimplantation selection, all 69 PGD fetuses were found to be euploid, comprising 26 cases of balanced translocation and 43 cases of normal karyotype. Two VUS unrelated to translocation breakpoints were confirmed by SNP array analysis.

We analyzed fetal ultrasound findings for all pregnancies, revealing that 16 fetuses exhibited abnormal ultrasounds. Among these, 37.5 % (6/16) displayed clinically significant aberrations. The results of these 6 cases with ultrasound abnormalities and genetic abnormalities were presented in Table 5. In contrast, among the 179 fetuses with normal ultrasounds, the rates of clinically significant aberrations identified by karyotype and SNP array analysis were 1.7 % (3/179) and 0 % (0/179), respectively. Both rates were significantly higher than those observed in cases with abnormal ultrasounds ($p < 0.05$).

4. Discussion

Couples with a balanced translocation carrier face a high risk of fertility problems, encompassing issues such as infertility, early pregnancy losses, stillbirths, or the birth of a child with congenital anomalies and an unbalanced karyotype [20–22]. The present study included 189 couples with balanced translocation carrier. Reviewing their reproductive history, it was evident that approximately half of the pregnancies ended in early miscarriage, emphasizing the prevalent risk of early pregnancy loss following successful conception. All invasive prenatal diagnoses for the studied pregnancies were conducted in the second trimester, primarily around 19-week gestation. Notably, only 5.6 % of natural pregnancies were found to be imbalanced translocations. This suggests that if the pregnancy persisted into the second trimester, it was considerably more likely to exhibit a normal karyotype or a balanced translocation.

Various studies have delved into the impact of carrier gender on the risk of embryonic abnormalities, often scrutinizing blastocysts during pre-implantation diagnosis [23,24]. Some research suggested that paternal carriers were much less likely to be associated with unbalanced translocations compared to maternal carriers [25], while some concluded that the difference was not statistically significant [25–27]. We investigated the early miscarriage history among couples with balanced translocation. PGD is the most effective method to prevent recurrent miscarriage in couples with balanced translocation [28]. As indicated in our study, couples who opted for natural conception faced a significantly higher risk of recurrent pregnancy loss than those who chose PGD assisted conception. In both the natural and assisted conception groups, couples with female carrier were more likely to experience repeated miscarriages, particularly those exceeding two occurrences. Miscarriage, especially recurrent miscarriage, imposes significant psychological and physical harm on pregnant women. Therefore, in order to avoid the occurrence of recurrent miscarriage, couples with balanced

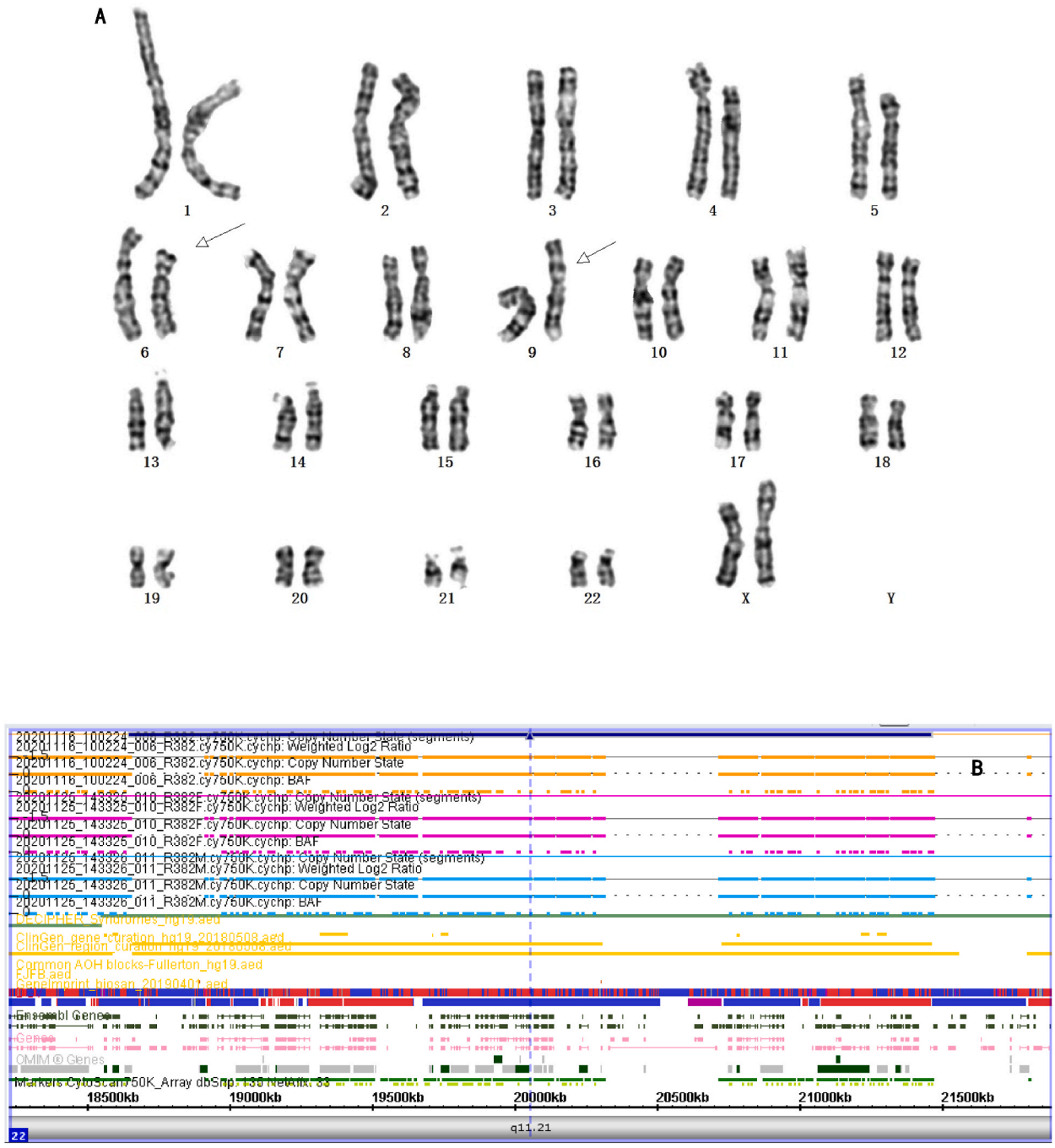

Fig. 1. The results of karyotyping and SNP array analysis for case 8. A: The karyotype revealed balanced t(6; 9) translocation. B: SNP array demonstrated a 2.8 Mb duplication on 22q11.2 region.

translocation, especially those where the female partner is a carrier, should be advised to undergo PGD assisted conception as soon as possible.

As indicated in the present study, a significant proportion of balanced translocation carriers opt for natural conception other than PGD, even after experiencing recurrent miscarriages. Consequently, invasive prenatal diagnosis remains crucial. However, is cytogenetic testing alone sufficient? It has been suggested that apparently balanced translocations inherited from phenotypically normal parents typically result in a normal phenotype. However, for patients carrying de novo balanced translocations, approximately 6 % have shown an abnormal phenotype [29]. Several factors might contribute to this. Firstly, the breakpoints may interrupt or modulate the expression of genes [30–32]. Additionally, rather than being truly balanced, there could be a cryptic duplication or deletion related

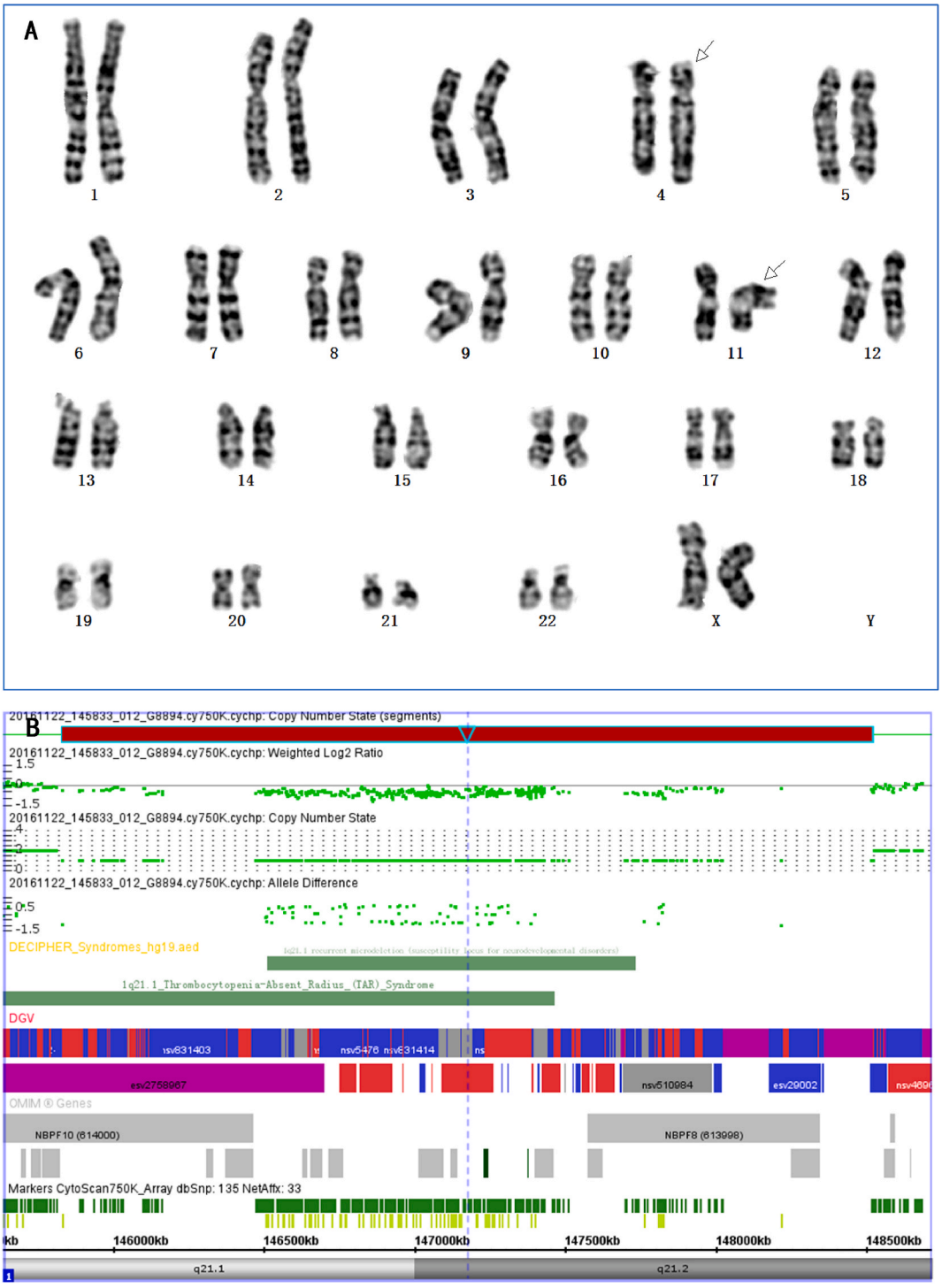

Fig. 2. The results of karyotyping and SNP array analysis for case 12. A: The karyotype showed balanced t(4; 11) translocation. B: SNP array demonstrated a 2.7 Mb deletion on 1q21.1q21.2 region.

or unrelated to the breakpoints [30,31,33]. Therefore, providing prenatal genetic counseling for fetuses with apparently balanced translocations identified by karyotyping can be challenging. SNP array analysis has provided new perspectives for genetic counseling for apparently balanced translocations. In a study by Baptista et al. [34], no genomic imbalances at the breakpoints or elsewhere in the genome, nor cryptic chromosome rearrangements, were found in phenotypically normal carriers. However, carriers of balanced translocations with abnormal phenotypes may harbor breakpoints-related or -unrelated deletions. Gribble et al. [31] reported on a

Table 5
Details of the 6 cases with ultrasound abnormalities and genetic abnormalities.

Case number	Parental karyotype	Ultrasound findings	Fetal karyotype	CMA result	Size/Clinical significance
20	46,XX,t(11; 22)(q23; q11)	Ventricular septal defects	47,XY,+der(22)t(11; 22)(q23; q11)	arr[GRCh37] 11q23.3q25(116,683,754–134,937,416) × 3,22q11.1q11.21(16,888,899–20,312,661) × 3	18.2Mb/pathogenic, 3.4Mb/pathogenic
21	46,XY,t(2; 22)(q37.3; q13.2)	Nuchal translucency thickening	46,XY,der(2)t(2; 22)(q37.3; q13.2)	arr[GRCh37] 2q37.3(241008898–242782258) × 1, 22q13.2q13.33(41132074–51197766) × 3	1.7Mb/VOUS,10.0Mb/ pathogenic
22	46,XX,t(2; 9)(q32.2; p24)	Nuchal translucency thickening	46,XX,der(9)t(2; 9)(q32.2; p24)	arr[GRCh37] 2q32.2q37.3(191220533–242771400) × 3, 9p24.3p24.2(208455–4529860) × 1	51.5Mb/pathogenic, 4.3Mb/likely pathogenic
23	46,XY,t(4; 11)(q35; q22.3)	Nuchal fold thickening	46,XX,t(4; 11)(q35; q22.3)	arr[GRCh37] 1q21.1q21.2(145,829,473–148,520,164) × 1	2.7Mb/pathogenic
24	46,XX,t(6; 9)(p21.3; p22)	Transient mitral regurgitation	46,XX,t(6; 9)(p21.3p23)	arr[GRCh37] 22q11.21(18,648,855–21,461,017) × 3	2.8Mb/pathogenic
25	46,XX,t(7; 14)(p14.3q32.3)	Bilateral choroid plexus cysts	46,XY,der(14)t(7; 14)(p14.3q32.3)	arr[GRCh37] 7p22.3p14.3(43,376–31,039,092) × 3, 14q32.33(105,090,669–106,257,269) × 1, 14q32.33(106,705,895–107,284,437) × 1	30.9Mb/pathogenic, 1.1Mb/pathogenic, 579Kb/likely benign

study of ten phenotypically abnormal carriers of apparently balanced translocations using SNP array, revealing that three carriers had imbalances in regions unrelated to translocation breakpoints. In our present study, SNP array analysis identified two clinically significant submicroscopic aberrations in fetuses with inherited balanced translocations. Notably, these aberrations were de novo and unrelated to the breakpoints. Interestingly, we observed two breakpoints-related CNVs in fetuses with a normal karyotype, classified as likely benign CNVs due to their inheritance from phenotypically normal parents. Our findings from SNP array analysis suggested that parental balanced translocations might not significantly increase the fetal risk of pathogenic CNVs associated with translocation breakpoints, although a larger sample size is required to validate this claim. Meanwhile, our study emphasizes the importance of SNP array in uncovering the nature of imbalances in some carriers of inherited balanced translocations.

In general, for reciprocal translocations, the most common disjunction leading to an imbalance is adjacent-1 malsegregation. This often results in both copy number gains and copy number losses, as evidenced in cases 1 and 2. Carriers with reciprocal translocations involving a small acrocentric chromosome are more likely to have unbalanced gametes caused by a 3:1 segregation [26,35,36], which occurs more frequently in cases of maternal translocations. In our study, we identified 3:1 segregations in two cases of reciprocal translocation involving chromosomes 21 and 22 (Cases 4 and 5). Case 5 [47,XX,t(9; 21)(q22; q21)mat,+21] represents a unique type of Down syndrome resulting from the involvement of chromosome 21 in the parental reciprocal translocation. Moreover, when the translocation breakpoint is locating in the p-arm of an acrocentric chromosome, SNP array often fails to indicate the copy number changes in the p-arm region, as the probe does not cover this area. For instance, in case 2 [46,XY,der(13)t(11; 13)(q23; p11)], theoretical assessments by karyotyping suggested 11q partial trisomy and 13p partial monosomy, whereas SNP array only revealed 11q partial duplication. These findings collectively illustrate the limitations of applying SNP array in the context of chromosomal rearrangements.

Previous publication have investigated the impact of the translocations on genome stability on embryos at the blastocyst stage. Quadrivalent structures may disrupt the proper pairing and disjunction of other chromosomes during meiosis I, potentially contributing to additional translocation-unrelated aneuploidies [37–39]. Despite these possibilities, our study did not identify any translocation-unrelated aneuploidies. This could be attributed to the limited sample size and the fact that our analysis focused on later stages of pregnancy.

Our study underscores the importance of ultrasonography in estimating clinically significant abnormalities for pregnancies from balanced translocation carriers. The risk of visible karyotype aberrations and submicroscopic aberrations of clinical significance increased significantly with the presence of ultrasound abnormalities. In pregnancies with ultrasound anomalies, the incidence of clinically significant CNVs was 12.5 %, a rate much higher than that reported in pregnancies from couples with normal chromosomal profiles [40,41]. Notably, among the 179 cases without ultrasound anomalies, no submicroscopic CNVs of clinical significance were detected.

The study was limited by the small sample size, which may impact the generalizability of the findings. The limited number of cases could influence the statistical power and robustness of the conclusions drawn from the analysis. Thus future research with larger sample sizes would contribute to a more comprehensive understanding of the subject.

In conclusion, our study indicates that repeated pregnancy loss was more often observed in couples with a female balanced-translocation carrier than with male carrier. Fetuses from balanced translocation carriers are more likely to have normal karyotypes or inherited balanced translocations if they survive until the second trimester. The presence of breakpoints-related and -unrelated submicroscopic aberrations in fetuses with inherited balanced translocations is a possibility. However, there is currently insufficient evidence to firmly establish a correlation between balanced translocations and an increased likelihood of clinically significant CNVs related to the translocation breakpoint.

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Ethics approval and consent to participate

The study was approved by the Protection of Human Ethics Committee of Fujian Provincial Maternity and Children's Hospital (Approval number: 2017039). Written informed consent was obtained from participants.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Xiaoqing Wu: Writing – original draft, Resources. **Shengrong Du:** Methodology, Investigation. **Bin Liang:** Software, Data curation. **Linjuan Su:** Data curation. **Ying Li:** Data curation. **Yuqin Chen:** Visualization, Data curation. **Lin Zheng:** Formal analysis. **Na Lin:** Writing – review & editing. **Hailong Huang:** Writing – review & editing. **Liangpu Xu:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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