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Experience of Copy number variation sequencing applied in production of conception from first- and second- trimester miscarriage

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Abstract

Background

We evaluated the application value of copy number variation sequencing (CNV-seq) to analyze chromosomal abnormalities in products of conception (POCs) from first- and second- trimester miscarriages.

Methods

Approximately 650 POCs from spontaneous abortion were collected from April 2018 to May 2020. CNV-seq and QF-PCR were performed to determine the characteristics and frequencies of copy number variants (CNVs) with clinical significance. Clinical features were recorded.

Results

Clinically significant chromosomal abnormalities were identified in 355 (54.6%) POCs, including 217 (33.4%) autosomal trisomies, 42(6.5%) chromosomal monosomy and 40 (6.2%) pathogenic CNVs (pCNVs). Chromosomal trisomy mainly occurred on chromosomes 16, 22, 21, 18, and 15. Gestational week was a negative correlative factor for chromosome abnormality.Maternal age was the positive correlative factor of chromosome abnormality. However the occurrence of monosomy X was not related to maternal or gestational age. The frequency of chromosomal abnormalities in women with a normal live birth history was 55.3%, vs 54.4% in women without a normal live birth history ($P > 0.05$). There were no significant differences among women without, with 1, and ≥ 2 previous miscarriages history regarding the rate of chromosomal abnormalities ($P > 0.05$); CNVs were less frequently detected in women with advanced maternal age than in women aged ≤ 29 years and 30–34 years ($P < 0.05$).

Conclusion

Chromosomal abnormalities are the most common causes of pregnancy loss, maternal and gestational age are strongly associated with fetal autosomal trisomy aberrations. Embryo chromosomal examination is recommended regardless of gestational age, modes of conception or previous abortion status.

Background

Miscarriage is the spontaneous loss of a pregnancy less than 28 weeks, or the spontaneous loss of the fetus with a weight less than 1000g, when miscarriage occurs before 13 gestational weeks it is called first- trimester miscarriage or early abortion, and when it occurs from 13 to 28 gestational weeks it is called second-trimester miscarriage or late abortion [1]. Stillbirth is the death of a fetus in the uterus after 20 weeks of gestation [2]. The incidence of miscarriage is approximately 15–20%, with 25% of females

experiencing at least one spontaneous abortion [3–4]. Studies have shown that genetic factors play an important role in miscarriage, with 50% of cases being caused by chromosomal abnormalities [5], while the risk factors for stillbirth (≥ 28 gestational weeks) are mainly immune and environmental factors [6]. Researchers found that fetal chromosomal aneuploidy was the primary cause of miscarriage [7], with aneuploidy of chromosomes 13, 16, 18, 21, 22, and sex chromosomes being ubiquitous [8, 9]. Previous studies focused on populations with specific clinical factors, such as early spontaneous abortion or recurrent spontaneous abortion, and there are few cross-sectional comparative studies of populations with these different factors. In this study, we analyzed chromosomal abnormalities in spontaneous abortions under different clinical conditions to provide evidence for clinical advice and genetic counseling.

G-banding karyotype analysis is widely used in the genetic analysis of miscarriage samples; however, this method is limited by low resolution, culture failure, poor chromosome morphology, long turn-round time, and maternal cell contamination (MCC), which may lead to false-negative results. Other methods such as fluorescence in situ hybridization and multiplex ligation-dependent probe amplification have also been used to identify the genetic causes of miscarriages [10]. None of these methods are capable of detecting chromosomal abnormalities at the whole-genome level, whereas chromosomal microarray analysis (CMA) has proven to be a powerful technology for genetic diagnosis that can detect aneuploidy, polyploidy, microdeletion/microduplication at the genome-wide level. However, a major shortcoming of CMA is its high cost, which restricts its use as a routine detection method for spontaneous abortions [11, 12]

Next-generation sequencing (NGS) is a low-cost technique with a short turn-around time, unprecedented resolution, reliable high-throughput, and minimal DNA requirement, and it has been widely used in clinics [13]. Compared with CMA, NGS has significant advantages in terms of quality, speed, and affordability [14–16]. Copy number variation sequencing (CNV-seq), an NGS-based method, has been used in most pediatric and prenatal diagnostic applications as a viable alternative methodology to CMA owing to its ability to simultaneously detect aneuploidies and submicroscopic chromosomal imbalances [16–18]. Nevertheless, CNV-seq fails to detect MCC and polyploidy, limiting its application in abortion detection. Quantitative fluorescence polymerase chain reaction (QF-PCR) is a rapid chromosomal detection method commonly used in the clinical setting. It can identify MCC, some euploidies, and some common aneuploidies by amplification of selected short tandem repeat (STRs) sites and quantitative analysis of the allelic dosage ratios to evaluate the number of copies of specific chromosomes [19]. Therefore, we speculated that the combination of CNV-seq and QF-PCR would be a reliable approach for chromosome detection in POCs, as confirmed prenatally [18, 20]. In this study, we aimed to evaluate the combined application of CNV-seq and QF-PCR as a tool for the identification of chromosomal abnormalities, investigate the frequency and type of chromosomal aberrations in POCs of participants who had at least one miscarriage, and probe the influencing factors of chromosomal abnormalities related to miscarriages.

Materials And Methods

A total of 650 fetal specimens, of which 597 were chorionic villi and 53 were fetal muscle tissues, were obtained from female participants who had undergone spontaneous abortion between April 2018 and December 2020. The mean age of the patients was 31.29 years old, (19–46 years) and the mean gestational age was 9.1 weeks, (5–25 weeks). Clinical information including early miscarriage history, normal live birth history, and mode of conception were recorded. Maternal age was classified into the following four groups: ≤ 29, 30–34, 35–39, and ≥ 40 years. The number of previous early miscarriages was classified into four groups: 0, 1, 2 and ≥ 3. The normal live birth history was categorized as “0” and “≥ 1” groups. The mode of conception was categorized into assisted and natural conceptions.

The present study was approved by the Protection of Human Ethics Committee of Fujian Provincial Maternity and Children’s Hospital, affiliated Hospital of Fujian Medical University. Written informed consent was obtained from individual or guardian participants.

Copy number variation sequencing

The specimens were carefully rinsed with sterile physiological saline and dissected from blood, clot and maternal decidua base on our experience. Approximately 5–10 mg POC was selected for genomic DNA extraction using Qiagen Blood & Tissue Kit (Qiagen GmbH, Germany). 10 mg/L agarose gel electrophoresis was used to detect genomic DNA integrity. The library preparation was performed by the rapid and straightforward library construction method (EZ-GALO) (provided by Beijing Berry Health Biotechnology Co, Ltd.). The library quality was controlled by the real-time fluorescence quantitative PCR method and finally, sequencing was performed on NextSeq CN 500 high-throughput Platform at an approximately 1× depth. After the sequencing is completed, the obtained fastq data is filtered by bioinformatics software.

QFPCR

Maternal peripheral blood was obtained for the QF-PCR.DNA from maternal blood and POCs was extracted using a QIAGEN kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Multiple QF-PCR was performed using Chromosome (13/18/21/X/Y) multiplex STR Genotyping Kit (Guangzhou Darui Biotechnology Co., Ltd.) containing 20 STR markers (fourteen STR markers on autosomes 13, 18, and 21, four on the chromosome X-linked markers, one on amelogenin, and SRY on chromosome Y). PCR products were separated on an ABI 3500 (Applied Biosystems, Foster City, CA, USA) capillary genetic analyzer and results were analyzed by ABI genemapper 6.0. The informative markers presented in the POC DNA sample were compared to those in a maternal DNA sample to estimate the presence of maternal cell contamination.

Evaluation of CNVs

Databases (ISCA, DGV, Decipher, Ensemble, OMIM, ClinGen, UCSC and PubMed)were used to analyze the suspected pathogenic regions. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, the clinical significance of copy number variations (CNVs) in genetic diagnoses. CNVs is divided into five levels: pathogenic, likely pathogenic, variants of uncertain significance (VOUS), likely benign, and benign [21, 22].

2.3 Statistical analysis

SPSS 22.0 software was used for data analysis. Quantitative data were expressed by mean ± standard deviation (X ± S), and comparison between groups was performed by T test. Qualitative data were represented by the number of cases (percentage), and comparison between groups was performed by paired Chi-square test. Logistic regression analysis was used to analyze the factors related to chromosome abnormality. A value of $P < 0.05$ was considered as statistically significant.

Results

CNV-seq was used to analyze 650 samples of aborted tissues in early and middle pregnancy. The success rate of all tests was 100%.The rate of chromosomal abnormalities was 54.6% (355/650), of which 37.1% (241/650) were single aneuploidies, 2.6% (17/650) multiple aneuploidies, 5.2% (34/650) polyploidy, 3.5% (23/650) mosaicism, and 6.2% (40/650) pathogenic copy number variations (pCNVs). Variants of uncertain significance (VOUS) were identified in 60 cases (9.2%),and normal results were identified in 235 cases (36.2%). Most of the aneuploidies were autosomal trisomies (217/650, 33.4%), while the others were monosomies found on chromosome X (39/241, 16.2%) and chromosome 21(3/241, 1.2%). (Table 1).

Table 1
Baseline characteristics and details of 650 cases of chromosomal abnormalities

Characteristis	Number	Proportion (%)
Age of mother who had miscarriages (31.29 ± 4.55years)		
≤ 29	255	39.2
30–34	234	36.0
35–39	128	19.7
≥ 40	33	5.1
Gestational week of fetuses(9.1 ± 2.42w)		
Early pregnancy(≤ 12w)	597	91.8
Middle pregnancy(13-28w)	53	8.2
Aneuploidy	259	39.9
Autosomal trisomy	217	33.4
Monosomy X	39	6.0
Autosomal monosomy	3	0.45
Sex chromosome trisomy	2	0.3
Chimera	27	4.2
Polyploidy	34	5.2
pCNV	40	6.2
VOUS CNV	60	9.2
Normal	235	36.2

Different distribution of chromosomal abnormalities were detected between first-trimester and second-trimester abortion.In the first-trimester pregnancy loss, all chromosomes were involved in trisomies except for chromosome 1, with T16 being the most common finding, followed by T22, T21,T18,and T15. Monosomy X was the most frequently encountered sex chromosomal abnormality and the incidence was 6.0% (Fig. 1). 17 abnormalities occurred in second-trimester miscarriage, and these abnormalities mainly involved T18, T21, 45,X and pCNVs. The most frequent karyotype was trisomy 18 (29.4%, 5/17), followed by monosomy X (23.5%, 4/17), trisomy 21 (23.5%, 4/17), and pCNVs (17.6%, 3/17) (Fig. 2)

The associations between chromosomal abnormalities and gestational age, maternal age, previous miscarriages, live birth history, and mode of conception are presented in Table 2. The rate of chromosomal abnormalities in first-trimester pregnancy loss (56.6%) was significantly higher than that in second-trimester pregnancy loss (32.1%) ($P < 0.05$). Autosomal trisomy was less common in second-trimester pregnancy loss than in first-trimester pregnancy loss ($P < 0.05$), but no statistical difference was found for the frequency of 45, X (Table 4). Similar incidences of chromosomal abnormalities were found among women aged ≤ 29, 30–34, and 35–39 years ($P > 0.05$), and they were all significantly lower than those in women ≥ 40 years old ($P < 0.05$). The incidence of autosomal trisomy also increased with maternal age ($P < 0.05$). The frequency of 45, X decreased with maternal age, but the difference was not statistically significant ($P > 0.05$). No significant differences were observed among women with different previous miscarriages, between women with different modes of conception, or between cases with and without a live birth history ($P > 0.05$).

Table 2
Association between clinical information and the frequency of chromosomal abnormalities

	Normal n(%)	Abnormal n(%)	X2	P
Maternal age (years) (N = 650)			9.366	0.025
≤ 29 (N = 255)	105(41.2)	150(58.8)		
30–34 (N = 234)	83(35.5)	151(64.5)		
35–39 (N = 125)	41(32.8)	84(67.2)		
≥ 40 (N = 36)	6(16.7)	30(83.3)		
Previous miscarriage (N = 650)			2.051	0.562
0 time(N = 198)	137(46.0)	161(54.0)		
1 times(N = 206)	91(44.2)	115(55.8)		
2 times(N = 104)	44(42.3)	60(57.7)		
≥ 3 times(N = 42)	23(54.8)	19(45.2)		
Gestational age			11.828	0.001
first- trimester	259(43.4)	338(56.6)		
second- trimester	36(67.9)	17(32.1)		
Normal live birth history (N = 650)			0.045	0.831
No(N = 491)	224(45.6)	267(54.4)		
Yes(N = 159)	71(44.7)	88(55.3)		
Mode of conception (N = 650)			0.437	0.509
Natural conception (N = 568)	255(44.9)	313(55.1)		
Assisted conception (N = 82)	40(48.8)	42(51.2)		

Table 3
Logistic regression analysis of chromosomal abnormalities in miscarriage samples

Variables	Regression coefficient	Standard error	WaldX2 value	*P* value	OR value	95%CI
Maternal age	0.594	0.203	8.579	0.003	1.810	1.217–2.693
Gestational age	-1.018	0.311	10.705	0.001	0.361	0.196–0.665

Table 4
Distribution profile and frequency of chromosomal abnormalities in different maternal age and gestational age.

	CNVs (n,%)	Monosomy X (n,%)	Autosomal trisomy (n,%)
Gestational age			
13w (N = 597)	91(15.2)	35(5.9)	204(34.2)
≥ 13w (N = 53)	9(17.0)	4(7.5)	10(18.9)
X^2	0.113	0.245	5.162
P	0.737	0.621	0.023
Maternal age			
≤ 29 (N = 255)	45(17.6)	20(7.8)	59(23.1)
30–34(N = 234)	43(18.4)	12(5.1)	75(32.1)
35–39(N = 125)	8(6.4)	6(4.8)	58(46.4)
≥ 40(N = 36)	4(11.1)	1(2.8)	22(61.1)
X^2	10.868	2.833	34.371
P	0.012	0.418	0.000

To identify significant CNVs related to miscarriage, cases with numerical chromosomal abnormalities were excluded from CNV analysis. As a result, a total of 60 pCNVs in 40 cases were subjected to further analysis, including 29 with duplications in 25cases and 31 with deletions in 15cases. The pCNVs of deletions and duplications ranged in size from 450Kb-35.6Mb and 0.38Mb-217.86Mb, respectively. The distribution of all detected pCNVs in all chromosomes is shown in Table 5. The deletions occurred mostly in chromosome8, followed by chromosomes 4 and X, The duplications occurred mostly in

chromosome16. CNVs were less frequently detected in women with advanced maternal age than in women aged ≤ 29 years and 30–34 years ($P < 0.05$). However, no statistical difference was found in the frequency of CNVs at different gestational ages($P > 0.05$).

Table 5 Details of 40 cases with pCNVs

Case	Chr	Location of the fragments	Size range	Cytoband
1	17	1100001 -1480000	+0.38 Mb	p13.3
2	16	5810001-7460000	+1.65Mb	p13.3
3	X	17460001-18800000	-1.34 Mb	p22.13
4	21	14300000-30300000	-16.00 Mb	q11.2-q21.3
	20	15500001-62920000	+47.42 Mb	p12.1-q13.33
5	1	243460001-249220000	-5.76 Mb	q43-q44
	2	219140001 -243020000	+23.88 Mb	q35q37.3
6	2	1-121920000	+121.92 Mb	p25.3-q14.2
	16	80001-17380000	+17.30 Mb	p13.3-p12.3
7	4	128040001-190940000	+62.90 Mb	q28.1-q35.2
	8	160001-26340000	-26.18 Mb	p23.3-p21.2
8	3	126280001-129240000	-2.96 Mb	q21.3-q22.1
9	7	146760000-159138663	-12.38	q35-q36.3
	7	131700000-144160000	+12.46	q32.3-q35
10	4	177920001-190940000	-13.02 Mb	q34.3-q35.2
	9	200001-38780000	+38.58 Mb	p24.3-p13.1
11	4	40001-6020000	-5.98 Mb	p16.3-p16.1
12	22	49680001-51180000	-1.50 Mb	q13.33
13	2	1-217860000	+217.86 Mb	p25.3-q35
	8	118120001-146300000	+28.18 Mb	q24.11-q24.3
14	16	5810001-7450000	-1.64Mb	p13.3
15	18	2440001-4980000	+2.54 Mb	p11.32-p11.31
16	18	2120001-78020000	+75.90 Mb	p11.32-q23
	18	120001-2120000	-2.00 Mb	p11.32
17	15	95520000-100140000	-4.62Mb	q26.2-q26.3
18	8	10001-5060000	-5.05Mb	p23.3-p23.2
19	X	2700000-3580000	-0.88Mb	P22.33
20	4	10001-3015501	-3.005Mb	p16.3

	4	3065500-28284500	-25.22Mb	p16.3-p15.1
21	Y	13120000-28800000	-15.68Mb	q11.1-q11.23
22	7	126350000-141575001	+15.225Mb	q31.33-q34
	7	107700001-126300001	+18.6Mb	q31.1-q31.33
	7	141625000-159125000	-17.5Mb	q34-q36.3
23	5	10001-22810000	-22.8 Mb	p15.33-p14.3
24	X	6526735-8101017	+1.80Mb	P22.31
25	8	10001-46860001	-46.85Mb	p23.3-q11.1
	8	46910000-146265100	+99.355Mb	q11.1-q24.3
26	22	44300004-51200004	-6.9 Mb	q13.31-q13.33
27	8	10001-6810000	-6.8Mb	p23.3-p23.1
	8	8110001-30360001	-22.25Mb	p23.1-p12
	8	30410000-31860000	+1.45Mb	p12
	8	.43810001-146265100	+102.455Mb	p11.1-q24.3
28	11	647170016-6517000	+1.8Mb	q13.1-q13.2
29	12	109460002-133810501	+24.35Mb	q24.11-q24.33
30	6	410001-75360000	+74.95 Mb	p25.3-q13
31	16	61810001-90060000	+28.25 Mb	q21-q24.3
	10	60001-4160000	-4.1 Mb	p15.3-p15.1
32	4	10001-9134500	-9.124 Mb	p16.3-p16.1
	4	49657701-191032600	+141.37 Mb	p11- q35.2
33	X	31760001-32210000	-450Kb	p21.1-p21.1
34	7	92450001-142225001	+49.775Mb	q21.2-q34
	7	142275000-159125000	-16.85Mb	q34-q36.3
35	16	29510001-30360000	+850Kb	P11.2
36	8	160001-35760000	-35.6Mb	p23.3-p12
37	11	122067001-134917000	-12.85Mb	q24.1-q25
	5	10001-14960000	+14.95Mb	p15.33-p15.2
38	4	10001-17584500	-17.57Mb	p16.3-p15.32

39	6	131260001-171010000	+39.75Mb	q23.2-q27
40	1	910001-2560000	+1.65Mb	p36.33-p36.32
	15	94226624-102476623	-8.25Mb	q26.1-q26.3

The results of the logistic regression analysis identified a trend suggesting that the percentage of fetal chromosomal abnormalities was significantly higher in advanced maternal age (OR = 1.810, 95% CI 1.217–2.693), and in lesser gestational age (OR = 0.361, 95% CI 0.196–0.665).(Table 3)

Discussion

The overall detection rate of clinically significant chromosomal abnormalities was 54.6%, and the rate of VOUS was 9.2%,which is in accordance with previous studies [11, 23]. We found that the largest proportion of chromosomal abnormalities was autosomal trisomies 33.4%, followed by CNVs 15.4% and monosomy X 6.0%. The frequencies of aneuploidy and polyploidy (39.9% and 5.2%) in our study were similar to the frequencies obtained in a large-scale study (42.5% and 7.5% respectively) conducted by Sahoo et al. [11]. T16 and T22 are the most common trisomy, followed by T21, T15, T18 and T13. Trisomy can be detected on all chromosomes except for T1.

The rate of chromosomal abnormalities in second-trimester miscarriages was as high as 32.1% in this study but was lower than that in early miscarriages(56.6%). The lower frequency of other chromosomal trisomies maybe because most trisomic embryos end in embryo implantation failure, and not all embryos have the opportunity to manifest as abortion after implantation. In contrast, fetuses withT16, T22, and T15 routinely have no opportunity to survive; therefore, these fetuses are almost always miscarried in early pregnancy, implying that T16, T22, and T15 may impact on embryo development than embryo implantation. The risk of chromosomal abnormalities was significantly lower in the mid-trimester group than in the early pregnancy group (26.4% vs. 50.4%, $P < 0.05$); however, it still has a high risk of occurrence in this period and is the leading cause of embryonic abortion in the mid-trimester. Therefore, chromosomal testing is necessary to identify the cause of miscarriage even in the second trimester. According to previous studies, most 45,X, T21,and T18 will be miscarried in early pregnancy, while some will continue to develop and survive in mid and late pregnancy. Further scientific studies are needed to reveal the underlying mechanisms[24],which also supports self-repair mechanisms during further embryo development, including apoptosis and selective differentiation [25], resulting in a substantial decrease in the proportion of abnormal chromosome chimerism at mid-pregnancy. In our study, the incidence of polyploidy in the early pregnancy was as high as 10.0% (34/338), no polyploidy was detected in the second-trimester, and 97.1% (33/34) were triploid.

Advanced maternal age (≥ 35 years) is a well-known independent factor associated with the frequencies of chromosomal abnormalities in miscarriages [26–28]. In this study, the frequencies of chromosomal abnormalities in women aged up to 30 years and 30–34 years were similar, but lower than those in

woman aged 35–39 years; and all of them were significantly lower than those in women aged ≥ 40 years. This tendency was consistent with that of autosomal trisomy, which confirmed the close association between maternal age and viable autosomal trisomy. In recent years, some studies have proposed that the incidence of post-meiotic abnormalities such as structural abnormalities is not directly related to maternal age [29, 30]. In our study, a higher frequency of aneuploidy and lower frequency of CNVs were identified in the advanced maternal age group. We provide more support for the theory that the incidence of embryonic aneuploidy increases with maternal age, while the incidence of CNVs seems irrelevant to maternal age. Monosomy X is the most commonly encountered viable sex chromosome abnormality. Unlike viable autosomal trisomy, the frequency of monosomy X did not increase with maternal age, which agrees with previous reports [5, 26, 31]. Hassold et al. [31, 32] found that paternal sex chromosome loss was the most common error leading to 45,X. They speculated that monosomy X was more likely to be derived from the meiotic error of the father than the mother. Two possible reasons have been raised: an increase in the frequency of monosomy X conceptions related to events in meiosis, fertilization, or early zygotic division, or an increase in the rate of survival of monosomy x conceptions to the stage of recognizable pregnancies.

Sub-microscopic genomic imbalances or CNVs have been shown to play an important role in prenatal ultrasound anomalies and neuron-developmental disorders such as intellectual disability, autism, and epilepsy [33, 34]. Attempts are being made to identify lethal human CNVs all the time. Analysis the functions of the genes contained in the CNVs, showed that the percentage of pathogenic CNVs in miscarriage tissues ranged from 6 to 15% [30, 35, 36]. The detection rate of CNVs in our study was 15.4%, including 6.2%pathogenic CNVs. Among these cases, 4p16.3 microdeletion, 8p23.3 microdeletion, 16p13.3 microdeletion, 16p13.3 duplications and 16q24.3 duplications were found,some of which were also reported in other studies concerning miscarriage [37, 38], these microdeletions/micro-duplications might be related to pregnancy loss by comparing the CNVs prevalence in miscarriage products and the general population, there is still no definite conclusion due to the lack of more powerful evidence. More large-scale studies are required to confirm whether these CNVs are causative of miscarriage.

The present study has some limitations. First,the overall sample size was small,particularly during the mid-trimester. More cases, especially for mid-trimester miscarriage, should be collected in future studies,and further functional studies should be performed on CNVs and genes associated with miscarriage.Second, parental karyotyping was offered to the couples whose POC revealed pCNVs abnormalities.

Conclusions

Our results confirmed that chromosomal abnormalities are the most common cause of pregnancy loss.In addition, maternal and gestational age are strongly associated with fetal chromosome aberrations. Embryo chromosomal examination is recommended regardless of gestational age, modes of conception or previous abortion status. Some useful and accurate genetic etiology information has been obtained, which provides useful genetic guidance for high-risk pregnanies.

Abbreviations

CNV-seq	copy number variation sequencing
POCs	products of conception
pCNVs	pathogenic copy number variants
MCC	maternal cell contamination
CMA	chromosomal microarray analysis
NGS	Next-generation sequencing
QF-PCR	Quantitative fluorescence polymerase chain reaction
STRs	short tandem repeat
VOUS	variants of uncertain significance

Declarations

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Contributors Dr Xu LP,Lin N had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dai YF, Wu XQ , Xu LP and Lin N contributed equally to this work.

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Ethics approval The study was approved by the Protection of Human Ethics Committee of Fujian Provincial Maternity and Children's Hospital, affiliated Hospital of Fujian Medical University. Informed

consent was obtained from the patients. **All methods were carried out in accordance with relevant guidelines and regulations.**

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References

1. Van den Berg MM, van Maarle MC, van Wely M, et al. Genetics of early miscarriage. Biochim Biophys Acta. 2012;12:1951–9.
2. Smith LK, Dickens J, Bender Atik R, et al. Parents' experiences of care following the loss of a baby at the margins between miscarriage, stillbirth and neonatal death: a UK qualitative study. BJOG. 2020;127(7):868–74.
3. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368:601–11.
4. Dai R, Li L, Zhu H, et al. Effect of maternal age on spontaneous abortion during thefirst trimester in Northeast China. J Matern Fetal Neonatal Med. 2018;31:1824–9.
5. Ozawa N, Ogawa K, Sasaki A, et al. Maternal Age, History of miscarriage, and embryonic/fetal size are associated with cytogenetic results of spontaneous early miscarriages. J Assist Reprod Genet. 2019;36:749–57.
6. Meng L, Wang Z, Reilly M, et al. Amniotic immune biomarkers as risk factors in women with different symptoms of threatened late miscarriage. Am J Reprod Immunol. 2020;83(5):e13232.
7. Petracchi F, Colaci DS, Igarzabal L, et al. Cytogenetic analysis of first trimester pregnancy loss. Int J Gynaecol Obstet. 2009;104:243–4.
8. Russo R, Sessa AM, Fumo R, et al. Chromosomal anomalies in early spontaneous abortions: interphase FISH analysis on 855 FFPE first trimester abortions. Prenat Diagn. 2016;36:186–91.
9. An N, Li LL, Zhang XY, et al. Result and pedigree analysis of spontaneously abortion villus chromosome detecting by FISH. Genet Mol Res. 2015;14:16662–6.
10. Zimowski JG, Massalska D, Pawelec M, et al. First-trimester spontaneous pregnancy loss - molecular analysis using multiplex ligation-dependent probe amplification. Clin Genet. 2016;89:620–4.
11. Sahoo T, Dzidic N, Strecker MN, et al. Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. Genet Med. 2017;19:83–9.
12. Petracchi F, Paez C, Igarzabal L. Cost-effectiveness of cytogenetic evaluation of products of conception by chorionic villus sampling in recurrent miscarriage. Prenat Diagn. 2017;37:282–8.
13. Hardwick SA, Deveson IW, Mercer TR. Reference standards for next generation sequencing. Nat Rev Genet. 2017;18:473–84.
14. Hawan D, Padh H. Pharmacogenetics: technologies to detect copy number variations. Curr Opin Mol Ther. 2009;11:670–80.

15. Zhu X, Li J, Ru T, et al. Identification of copy number variations associated with congenital heart disease by chromosomal microarray analysis and next-generation sequencing. Prenat Diagn. 2016;36:321–7.
16. Wang H, Dong Z, Zhang R, et al. Low-pass genome sequencing versus chromosomal microarray analysis: implementation in prenatal diagnosis. Genet Med. 2020;22:500–10.
17. Dong Z, Zhang J, Hu P, et al. Low-pass whole-genome sequencing in clinical cytogenetics: a validated approach. Genet Med. 2016;18:940–8.
18. Wang J, Chen L, Zhou C, et al. Prospective chromosome analysis of 3429 amniocentesis samples in China using copy number variation sequencing. Am J Obstet Gynecol. 2018;219:287.e1-.e18.
19. Nicolini U, Lalatta F, Natacci F, et al. The introduction of QF-PCR in prenatal diagnosis of fetal aneuploidies: time for reconsideration. Hum Reprod Update. 2004;10:541–8.
20. Wang J, Chen L, Zhou C, et al. Identification of copy number variations among fetuses with ultrasound soft markers using nextgeneration sequencing. Sci Rep. 2018;8:8134.
21. Brandt T, Sack LM, Arjona D, et al. Adapting ACMG/AMP Sequence Variant Classification Guidelines for Single-Gene Copy Number Variants. Genet Med. 2020;22:336–44.
22. Riggs ER, Andersen EF, Cherry AM. **et al**. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020;22:245–57.
23. Shen JD, Wu W, Gao C. **et al**.Humphrey Ochin Chromosomal copy number analysis on chorionic villus samples from early spontaneous miscarriages by high throughput genetic technology. Mol Cytogenet. 2016;9:7.
24. Vanneste E, Voet T, Le Caignec C, et al. Chromosome instability is common in human cleavage stage embryos. Nat Med. 2009;15:577–83.
25. Cram DS, Leigh D, Handyside A, et al. PGDIS Position Statement on the transfer of mosaic embryos 2019. Reprod Biomed Online. 2019;39(Suppl 1):e1–1e4.
26. Menasha J, Levy B, Hirschhorn K, Kardon N. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med. 2005;7:251–63.
27. Tamura Y, Santo M, Araki Y, et al. Chromosomal copy number analysis of products of conception by conventional karyotyping and next-generation sequencing. Reprod Med Biol. 2021;20:71–5.
28. Gomez R, Hafezi N, Amrani M, et al. Genetic findings in miscarriages and their relation to the number of previous miscarriages. Arch Gynecol Obstet. 2021;303:1425–32.
29. Simpson JL, Rechitsky S, Kuliev A. Before the beginning: the genetic risk of a couple aiming to conceive. Fertil Steril. 2019;112:622–30.
30. Chen L, Wang L, Tang F, et al. Copy number variation sequencing combined with quantitative fluorescence polymerase chain reaction in clinical application of pregnancy loss. J Assist Reprod Gene. 2021;38:2397–404.

31. Hassold T, Arnovitz K, Jacobs PA, May K, Robinson D. The parental origin of the missing or additional chromosome in 45, X and 47, XXX females. Birth Defects Orig Artic Ser. 1990;26:297–304.
32. Hassold T, Benham F, Leppert M. Cytogenetic and molecular analysis of sex-chromosome monosomy. Am J Hum Genet. 1988;42:534–41.
33. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018;109:201–12.
34. Deshpande A, Weiss LA. Recurrent reciprocal copy number variants: roles and rules in neurodevelopmental disorders. *DevNeurobiol.*2018;78:519–30.
35. Viaggi CD, Cavani S, Malacarne M, et al. First-trimester euploid miscarriages analysed by array-CGH. J Appl Genet. 2013;54:353–9.
36. Rajcan-Separovic E, Qiao Y, Tyson C, et al. Genomic changes detected by array CGH in human embryos with developmental defects. Mol Hum Reprod. 2010;16:125–34.
37. Liu S, Song L, Cram DS, et al. Traditional karyotyping vs copy number variation sequencing for detection of chromosomal abnormalities associated with spontaneous miscarriage. Ultrasound Obstet Gynecol. 2015;46:472–7.
38. Zhu X, Li J, Zhu Y, et al. Application of chromosomal microarray analysis in products of miscarriage. Mol Cytogenet. 2018;11:44.

Figures

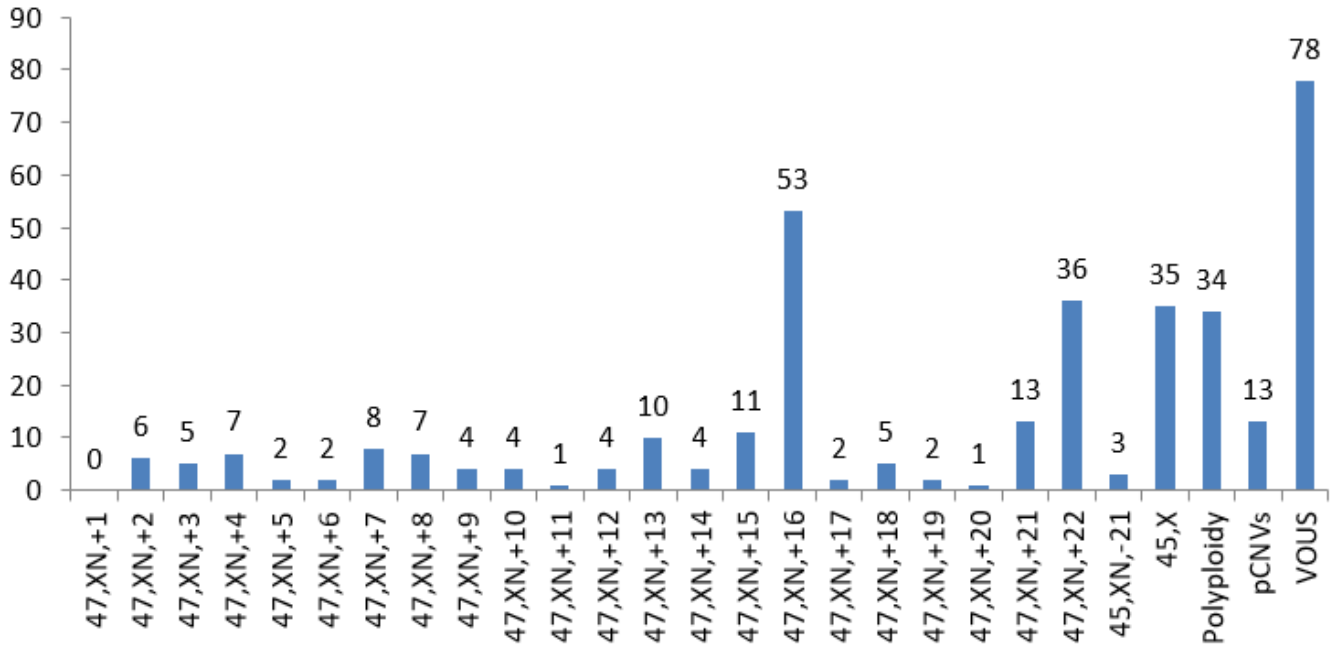

Figure 1

Distribution of chromosomal abnormalities in early abortion

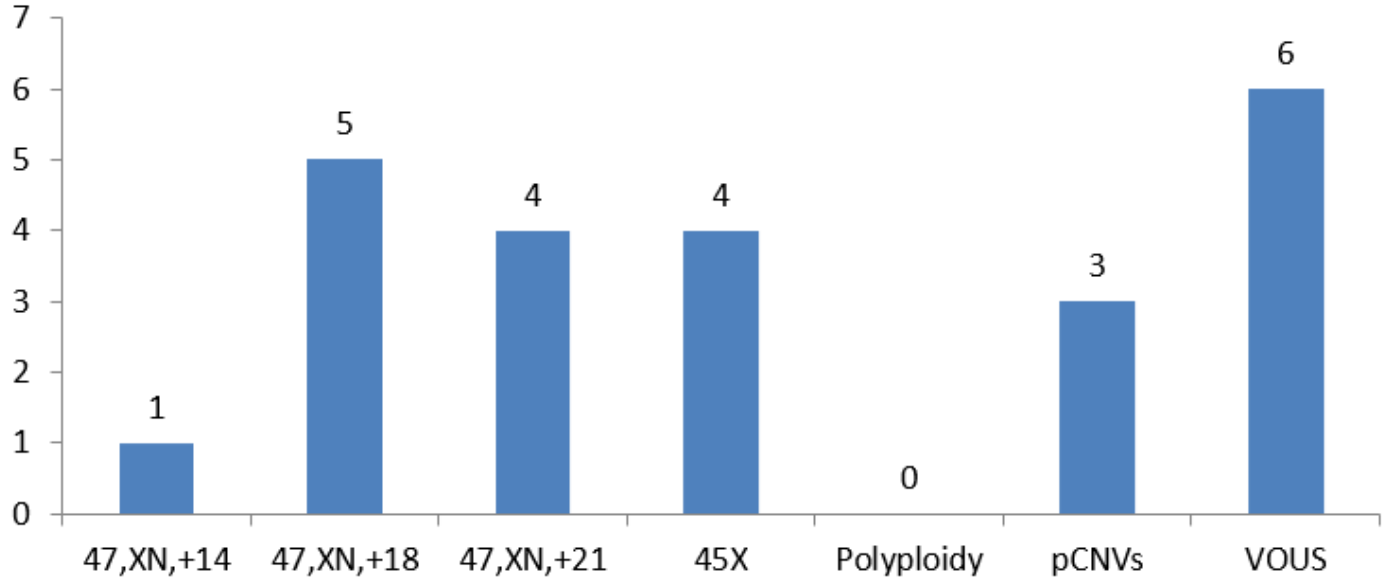

Figure 2

Distribution of chromosomal abnormalities in late abortion