Research Square

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Prenatal diagnosis of fetal chromosomal abnormalities among 2318 pregnant women with indications in southern China: a retrospective study

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Abstract

Background

To investigate the frequency of fetal chromosomal abnormalities among women with abnormal ultrasound, abnormal biochemical marker screening or noninvasive prenatal testing results, advanced maternal age, or history of miscarriage in southern China.

Methods

We retrospectively analyzed prenatal samples from pregnant women between 2015 and 2019. Conventional karyotyping was performed using GTG banding. Copy number variation sequencing was used when indicated to identify chromosomal abnormalities.

Results

A total of 2,318 prenatal samples (188 chorionic villus samples, 2,003 amniotic fluids, and 127 cord blood) were analyzed. The frequency of chromosomal abnormalities was 12.4% (288/2,318) in prenatal samples, and frequency in chorionic villus samples (23.9%) was higher than in amniotic fluids (13.5%) and in cord blood (5.0%; $P < 0.001$). Numerical anomalies were detected in 195 (8.4%) cases and the most common abnormality were trisomy 21 (103/2,318; 4.4%), trisomy 18 (31/2,318; 1.3%) and monosomy X (18/2,318; 0.8%). Sructural anomalies were found in 29 (1.3%) cases, rare anomalies such as deletions (4 cases), duplications (2 cases), and complex rearrangement (1 case), were detected. Two cases with common chromosomal polymorphisms, inv(9)(p11q12) and inv(9)(p11q13), associated with recurrent spontaneous abortion, were detected. Five fetuses had normal karyotypes and definite pathogenic copy number variations, with microdeletions at 16p13.11, 16p12.1 (2 cases), and 17p12 and a microduplication at 7q11.23; all had normal phenotypes after birth.

Conclusion

Our study indicated that fetal chromosomal anomalies can be detected in early gestation and provided valuable information for interpretation of chromosomal polymorphisms and copy number variations.

Introduction

China has a large population and a rising incidence of fetal birth defects that needs to be addressed. Chromosomal abnormalities are the main cause of fetal birth defects, and chromosomal abnormalities occur in up to 1 in 60 live births in China (1). The most frequent chromosomal abnormalities are aneuploidies of autosomes and sex chromosomes. In autosomal abnormalities, Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13) are prevalent; these are

associated with a high likelihood of miscarriage and life-threatening fetal abnormalities (2). Sex chromosome aneuploidies are present in 1 in 450 births in China (1); Turner syndrome (45,X), Klinefelter syndrome (47,XXY), and 47,XYY syndrome are common sex chromosome aneuploidies, and are associated with abnormal pregnancy, sterility, and language developmental disorders (3–5). Although the likelihood of point mutations is slightly elevated in sperm of older men, the incidence of trisomy is remarkably increased in women of advanced maternal age (AMA; age ≥ 35 years) (6, 7). The average age of women at childbearing has increased worldwide; at the same time, prenatal diagnosis technology has greatly improved and can often easily determine the fate of the fetus (8).

Among several prenatal genetic screening tests, ultrasound screening is the most convenient noninvasive procedure by which to identify fetal anomalies (e.g., increased nuchal translucency (NT), heart abnormality, choroid plexus cyst, neck lymphatic hydrocele, bone abnormality, multi-system malformations) (9). Maternal serological double-marker [free beta human chorionic gonadotropin (free β-hCG) and pregnancy-associated plasma protein A (PAPP-A)] or triple-marker [free β-hCG, alpha fetoprotein (AFP), and unconjugated estriol (uE3)] screening combined with ultrasound can be used to determine the risk of fetal aneuploidy of chromosome 21, 18, or 13 (10). However, these remain risk screening tests because the definitive chromosomal diagnosis can be only be made through analysis of fetal cells. With improved invasive prenatal techniques, it is safe to obtain fetal cells from chorionic villus samples (CVS), amniotic fluid (AF), or cordocentesis (cord blood, CB) for precise prenatal diagnosis (11).

In the second trimester, AF studies are carried out at approximately 16 weeks of gestation without major technical difficulties. However, other options include chorionic villus sampling at 11 to 13 weeks of gestation and early percutaneous ultrasound-monitored amniocentesis (12). Recently, it has been demonstrated that fetal DNA can be isolated from maternal circulation, although this technique is reliable only for identification of trisomies 21, 18, and 13 (13). In addition, copy number variation sequencing (CNV-seq) analysis can be implemented to detect clinically significant fetal abnormalities but problems remain in interpretation of CNV (14)

Until now, few studies have focused on the incidence of chromosomal abnormalities in prenatal samples among pregnant women in southern China. The main purpose of the present study was to evaluate the frequency and type of fetal chromosomal abnormalities among pregnant women following an abnormal ultrasound, positive serological screen, or noninvasive prenatal testing (NIPT) result or those with AMA or a history of miscarriage, to provide valuable information for prenatal counseling. Therefore, we present the results of karyotyping CVS, AF, and CB samples over a five-year period.

Results

In total, 2,318 prenatal samples were included in our study, including 188 CVS, 2,003 AF, and 127 CB. Of these 2,318, 1,060 cases had abnormal ultrasound results, 922 were positive by serological marker screen test, 146 were positive for NIPT, 103 presented with AMA, and 87 had a history of miscarriage. The average age of all pregnant women was 29.7 ± 5.9 years (range 16 to 47 years).

The frequency of chromosomal abnormalities was 12.4% (288/2,318) in prenatal samples [CVS (n = 43), AF (n = 239), and CB (n = 6); Fig. 1], and the frequency in CVS (23.9%) was higher than in AF (13.5%) and in CB (5.0%; $P < 0.001$). Chromosomal abnormalities of clinical significance were detected in 236 (10.2%) cases and heterotypic variants were found in 52 (2.2%) cases (Table 1). The chromosomal aberrations associated with various risk factors are shown in Table 1. Of the 288 cases with chromosomal abnormalities, 132 (45.8%) had abnormal ultrasound results, 56 (19.4%) had positive serological marker screen tests, 82 (28.5%) were positive for NIPT, 4 (1.4%) were associated with AMA, and 14 (4.9%) had a history of miscarriage.

Table 1

Distribution of chromosomal abnormality with risk indicators (n = 288)

	Numerical	Structural abnormalities						Variant	CNV
		Reciprocal	Rob	Del	Dup	Inv	Complex[&]		
CVS (n = 43)									
Abnormal ultrasound	31	1	-	-	-	-	-	3	2
Positive serological	3	-	-	-	-	-	-	-	-
History of miscarrage	3	-	-	-	-	-	-	-	-
AF (n = 239)									
Abnormal ultrasound	48	2	3	4	1	3	-	21	7*
Positive serological	27	-	1	-	1	2	-	19	3
Positive NIPT	78	-	-	-	-	-	1	3	-
AMA	1	-	-	-	-	-	-	3	-
History of miscarrage	-	6	1	-	-	2	-	2	-
CB (n = 6)							-		
Abnormal ultrasound	4	-	-	-	-	1	-	1	-

CVS: chorionic villus samples; AF: amniotic fluids; CB: cord blood. Reciprocal: Reciprocal translocation; Rob: Roberrsonian translocation; Del: Deletions; Dup: Duplications; Inv: Inversion; Complex[&]: complex, unbalanced rearrangement; Variant: chromosomal polymorphism, included satellite and heterochromatic polymorphism; CNV: Definite pathogenic copy number variation (CNV) detected by next-generation sequencing. Abnormal ultrasound:included increased nuchal translucency (≥ 2.5 mm); heart abnormality; choroid plexus cyst; neck lymphatic hydrocele; bone abnormality; brain abnormality; increased nuchal fold thickness (≥ 6.0 mm); kidney abnormality, and bowel abnormality. Positive serological: cut-off value ≥ 1/270 for trisomy 21 or ≥ 1/350 in trisomy 18. Positive NIPT: positive non-invasive prenatal testing results, included absolute value of z-score ≥ 3 for chromosome 21, 18 and 13, and definite pathogenic copy number variant detected in chromosome. AMA: advanced maternal age, age ≥ 35 years. *Two samples with definite pathogenic CNV were twins, and one of them was shown normal ultrasound result.

Numerical chromosomal anomalies were detected in 195 of 2,318 (8.4%) cases. The most common aneuploidies were trisomy 21 (103/2,318; 4.4%), followed by 47,XXY (Klinefelter syndrome, 16/2,318; 0.7%), 47,XXX (6/2,318; 0.3%), 47,XYY (4/2,318; 0.2%), and 69,XXX (triploidy, 3/2,318; 0.1%; Table 2).

Table 2
Summary of numerical chromosomal anomalies (n = 195)

	Chromosomal abnormalities	Number
numerical anomalies	47,XN,+21	103
	47,XN,+18	31
	47,XN,+13	8
	45,X	18
	47,XN,+9	2
	47,XN,+10	1
	47,XNN	26
	48,XNNN	2
	69,XNN	3

Structural anomalies were found in 29/2,318 (1.3%) cases (Table 3). We detected nine reciprocal translocations, five Robertsonian translocations, eight inversions (excluding those in chromosome 9), four deletions, two duplications, and one complex, unbalanced rearrangement. Deletions, duplications, and complex rearrangements detected in our study are shown in Fig. 2. Chromosomal polymorphisms were observed in 52/2,318 (2.2%) cases (Table 4). We detected 11 cases with heterochromatic polymorphisms in chromosomes 1, 9, and Y; 10 cases of satellite polymorphisms in chromosomes 13, 14, 15, 21, and 22 (D and G groups); and 31 cases of inversion in chromosome 9.

Table 3
Summary of structural chromosomal anomalies (n = 29)

	Chromosomal abnormalities	Number
reciprocal translocation (n = 9)	46,XN,t(1;3)(p32;q29)	1
	46,XN,t(1;14)(q42;q13)	1
	46,XN,t(1;19)(p10;q10)	1
	46,XN,t(3;8)(q21;q13)	1
	46,XN,t(4;21)(q13.2;p11.2)	1
	46,XN,t(5;9)(q22;q21.2)	1
	46,XN,t(6;7)(q15;q23)	1
	46,XN,t(6;13)(q23;q32)	1
	46,XN,t(11;14)(q21;q24)	1
Robertsonian translocation (n = 5)	45,XN,der(13;14)(q10;q10)	1
	45,XN,der(14;15)(q10;q10)	1
	46,XN,der(14;21)(q10;q10),+21	2
	46,XN,der(21;21)(q10.q10)	1
inversion (n = 8)	46,XN,1qh+,inv(5)(p15.1q13.1)	1
	46,XN,inv(7)(q22q32),inv(9)(p12q13)	1
	46,XN,inv(8)(p21.3q22.3)	1
	46,XN,inv(8)(p23.1q13)	1
	46,XN,inv(11)(p13q13)	1
	46,XN,inv(18)(p11.32q11.2)	1
	46,X,inv(Y)(p11.2q11.23)	2
deletion (n = 4)	46,XN,del(4)(pter→p15.2)	2
	46,XN,del(5)(p15.1→qter)	1
	46,XN,del(15)(q22.1q22.2)	1
duplication (n = 2)	46,XN,dup(4)(q32.2→qter)	1
	46,XN,dup(14)(q21.1q22.3)	1

	Chromosomal abnormalities	Number
unbalanced rearrangement (n = 1)	46,XN,ins(4)t(4;11)(q35;q14.2→qter)dup(11)(q14.2→qter)	1

Table 4

Summary of chromosomal polymorphism (n = 52)

	Chromosomal abnormalities	Number
variant	46,XN,1qh+	5
	46,XN,9qh+	2
	46,XN,9qh-	2
	46,XN,13pstk+	1
	46,XN,14pstk+	1
	46,XN,14pstk-	1
	46,XN,15pstk+	5
	46,XN,21pstk+	1
	46,XN,22pstk+	1
	46,X,Yqh+	2
	46,XN,inv(9)(p13q13)	28
	46,XN,inv(9)(p11q12)	1
	46,XN,inv(9)(p11q13)	1
	46,XN,inv(9)(p12q13)	1
qh+/-: heterochromatic polymorphism. stk+/-: satellite polymorphism. inv: inversion.		

Definite pathogenic CNVs with normal karyotypes were detected in 12 of 2,318 (0.5%) cases (Table 5). Fetal CNVs (one with 7q11.23 duplication; three with 16p12.1 deletion, one with 16p13.11 deletion, and one with 17p12 deletion) inherited from phenotypically normal fathers were detected in six cases in our study. After counseling, five of the patients continued their pregnancies and gave birth to five normal children (follow-up period ranged from 3 to 10 months; cases 1 to 5 in Table 5). In one case, selective reduction was performed because of multiple fetal abnormalities detected by ultrasonography (case 6; Case 5 and Case 5 were twins; Table 5). Of the other six cases, five fetal CNVs (included 1 with 1q21.1 duplication, 1 with 22q11.21 duplication, 1 with 15q11.2 deletion, and 2 with 22q11.21 deletion) were confirmed to have arisen de novo and the patients chose to terminate the pregnancies (cases 7 to 11; Table 5). In the sixth case, the fetus died 2 week after chorionic villus sampling (case 12; Table 5).

Table 5
Summary of definite pathogenic copy number variation (n = 12)

	Case No.	Chromosomal abnormalities	Known syndrome
CNV	1	17p12(14.06 Mb-15.7 Mb) × 1	HNPP
	2	7q11.23(72.72 Mb-74.12 Mb) × 3	7q11.23 duplication syndrome
	3	16p13.11p12.3(14.8 Mb-16.84 Mb) × 1	16p13.11 microdeletion loci
	4	16p12.2(21.94 Mb-22.42 Mb) × 1	16p12.1 microdeletion syndrome
	5*	16p12.2(21.96 Mb-22.44 Mb) × 1	16p12.1 microdeletion syndrome
	6*	16p12.2(21.96 Mb-22.44 Mb) × 1	16p12.1 microdeletion syndrome
	7	1q21.1q21.2(146.50 Mb-147.84 Mb) × 3	1q21.2 microduplication syndrome
	8	22q11.21(18.92 Mb-21.46 Mb) × 1	22q11.2 microdeletion syndrome
	9	15q11.2(22.76 Mb-23.1 Mb) × 1	15q11.2 deletion syndrome
	10	15q11.2(22.76 Mb-23.1 Mb) × 1	15q11.2 deletion syndrome
	11	22q11.21q11.23(21.46 Mb-23.64 Mb) × 1	22q11.2 microdeletion syndrome
	12	22q11.21(18.96 Mb-21.46 Mb) × 3	22q11.2 microduplication syndrome

CNV: copy number variation (CNV). *Case 5 and Case 6 were twins and both detected with the same CNV. ×1: Deletions. ×3: Duplications. HNPP: Hereditary compression predisposition to neuropathy. 16p13.11 microdeletion loci: locations of susceptibility genes to neurocognitive impairment.

Discussion

The present study showed a relatively high frequency of chromosomal anomalies (12.4%) in prenatal samples Hakka pregnant women in southern China; fetal chromosomal anomalies can be detected in early gestation. Further, our findings indicated that many fetal intrauterine deaths are caused by chromosomal abnormalities, and our results provide valuable information about interpreting chromosomal polymorphisms and CNVs.

The incidence of chromosomal abnormalities in our study was higher than that previously described by Yuning et al. (1.5%; n = 46,258) (15), Yanmei et al. (2.0%; n = 3,387) (16), Ye et al. (3.9%; n = 4,224) (17), Rulin et al. (4.1%; n = 4,953) (7), Frenny et al. (7.4%; n = 1,728) (6), and Huafeng et al. (8.5%; n = 4,206) (18), but was lower than reported by Hongyan et al. (15.5%; n = 3,608) (19). Several reasons explain the variable incidence across studies. First, criteria for case inclusion, sample types, and samples sizes varied across studies; and these may draw different conclusions. Second, socioeconomic conditions in certain regions might limit the use of molecular cytogenetic technologies. Previous studies showed that CNV-seq

analysis (20) and array comparative genomic hybridization (aCGH) increase the detection rate of chromosomal abnormalities but at high cost (21, 22).

In our study, the most common indicator among cases with chromosomal abnormalities was abnormal ultrasound findings. However, previous studies found that a positive biochemical marker screening test was the most frequent indicator (6, 7). These differences may be explained as follows: (1) ultrasonography is a safe imaging technique and is readily accepted by pregnant women in southern China; the proportion (45.7%) of patients presenting abnormal ultrasonography was high in our study; (2) patients, especially with AMA, who have a positive biochemical double-marker screening test are more likely to undergo invasive tests (23); only 11 of 920 cases had positive biochemical triple-marker screening tests in our study.

Karyotype analysis is generally considered the gold standard for prenatal diagnosis of chromosomal anomalies because of its accuracy and reliability. In our study, 266 of 288 cases with chromosomal anomalies were detected by conventional karyotyping. We observed 45 of 288 cases with chromosomal anomalies in CVS, this indicated that CVS used for genetic analysis has a higher priority among pregnant women with high risk prenatal diagnosis indications, as to provide a good care for them. Similar to recent studies reported in several regions in China (7, 17, 18), the most frequent numerical chromosomal abnormalities were trisomy 21, trisomy 18, and monosomy X in the present study. However, the incidence of trisomy 21 was relatively higher (4.4%) in our study because of full implementation of the two-child policy in China beginning in 2016. Several studies have shown that triploidy in prenatal samples can be detected in first-trimester screening programs (24, 25); 3 cases of triploidy were detected during the second trimester in this study. To provide good care for women, early prenatal screening is recommended.

Translocations were found in 14 cases in our study (9 reciprocal and 5 Robertsonian). Consistent with previous studies (18, 26), the 9 cases of balanced, reciprocal translocations were associated with normal phenotypes after birth. Structural imbalances are related to fetal loss, increased risk of developmental delay, and cancer (26, 27). After prenatal counseling, the patients carrying fetuses with Robertsonian translocations chose to end the pregnancies. Chromosome inversions in which the inverted segment is less than one-third of the total chromosome length are thought to be associated with a low chance of clinical abnormalities (28). In one case in our study, the fetus inherited an inv(8)(p21.3q22.3) and was healthy at birth; in other seven cases, the parents opted to terminate the pregnancy in which the fetuses carried de novo inversions due to other risk indicators. The EUROCAT database of congenital abnormalities in Europe (29) and a multicentric study of prenatal samples (30) show a low frequency of visible chromosomal deletions detected by microscope. Likewise, we found only four chromosomal deletions (two cases in chromosome 4 related to Wolf-Hirschhorn syndrome, one in chromosome 5 associated with Cri-du-chat syndrome, and one case in chromosome 15). Chromosomal duplications were also rare (30), with one chromosome 4 duplication related to 4p-distal trisomy syndrome and one chromosome 14 duplication in our study. Although the number of cases (< 300) reported in the literature is small (31), fetuses with complex chromosomal rearrangements have low survival. Interestingly, one complex rearrangement in our study was caused by CNV in chromosomes 4 and 11 and was detected by

NIPT, indicating that definite pathogenic CNV detected by NIPT should be considered in prenatal counseling.

Chromosomal polymorphisms are generally considered normal mutations without significant clinical phenotypic effects (32); 33 of 52 cases with identified polymorphisms in our study had no pathogenic clinical features and resulted in the delivery of normal newborns. The frequency of inversion in chromosome 9 was 1.3% in our study, higher than the reported incidence of 0.4% (18), and most common inversion was inv(9)(p13q13) in our study. One case with inv(9)(p11q12) and one case with inv(9)(p11q13) were detected in our study. These two inversions were inherited from the mothers, and both women had histories of recurrent spontaneous abortion. Our finding indicated that the inversion breakpoints, at chromosome 9p11q12 and 9p11q13 may related to pregnancy loss, but are different from the finding reported in the literature (26). It remains the observation of the clinical features for more cases of these two inversions in our further study.

Definite pathogenic CNVs with normal karyotypes were detected in 12 cases in our study; five of these were inherited from the parents and resulted in five healthy children. Our results suggested that fetuses had normal clinical features and inherited CNVs such as 16p13.11 microdeletion, 16p12.1 microdeletion, 17p12 microdeletion, or 7q11.23 microduplication are likely to result in normal after birth, although previous studies have recognized these 4 CNVs as pathogenic (33–36). Whether pathogenic CNVs are associated with increased NT varies in different studies (37, 38), which may due to a limitation of detection by aCGH used in these studies. In the current study, four cases with CNVs presented only with increased NT, and one fetus with a 22q11.21 microduplication died 2 weeks after chorionic villus sampling. Thus, pathogenic CNVs may provide information to explain a history of miscarriages and help in prenatal counseling. A limitation of our study was that the small samples size, single center retrospective study could not provide more information to confirm the association between the clinical symptom and chromosomal abnormalities.

Conclusions

This retrospective study indicated that cases with common chromosomal polymorphisms, such as inv(9)(p11q12) and inv(9)(p11q13), may cause recurrent spontaneous abortion. We also showed that fetuses with inherited definite pathogenic CNVs, including 16p13.11 microdeletion, 16p12.1 microdeletion, 17p12 microdeletion, and 7q11.23 microduplication, can be healthy after birth. Overall, our findings indicate that pregnant women in southern China should undergo early prenatal screening to identify chromosomal abnormalities and facilitate prenatal genetic counseling.

Material And Methods

Patients

Our retrospective study evaluated prenatal samples from Hakka women undergoing invasive testing at the prenatal diagnosis department of Meizhou People's Hospital, Guangdong Province, in southern China, between January 1, 2015, and December 31, 2019. Native Hakka was defined as people lived in Meizhou for more than three generations. Prenatal samples consisted of CVS, AF, and CB. This study was approved by the Ethics Committee of Meizhou People's Hospital (No. MPH-HEC 2015-A-22). Informed consent was obtained by phone from all participants. Follow-up data on pregnancy outcomes and fetal health were collected by phone from all pregnant women (3-month follow-up).

Invasive testing was conducted for the following indications: abnormal ultrasound result, positive serological markers (cut-off value ≥ 1/270 for trisomy 21 or ≥ 1/350 in trisomy 18), positive NIPT result (absolute value of z-score ≥ 3 for chromosomes 21, 18, and 13, and definite pathogenic CNV detected in chromosome), AMA (age ≥35 years), and history of miscarriage. Abnormal ultrasound results included increased NT (≥ 2.5 mm), heart abnormality, choroid plexus cyst, neck lymphatic hydrocele, bone abnormality, brain abnormality, kidney abnormality, and bowel abnormality.

Cytogenetic Analysis

Chorionic villus samples were cultured in Amniotic Cell Medium (DahuiBio, Guangzhou, China) for 11 to 13 days at 37 ℃ with 5% CO_2. Amniotic fluid cells were cultured in Amniotic Cell Medium for 8 to 10 days at 37 ℃ with 5% CO_2. Some CVS and AF samples were cultured for an additional 7 to 10 days if the cells divided slowly. Cord blood was cultured in Cell Culture Medium (DahuiBio) for 3 days at 37 ℃. Three hours before the endpoint of culture, CVS, AF, and CB cultures were treated with colcemid solution (DahuiBio) and then harvested through conventional techniques. Karyotype analysis was performed following GTG banding (39). A Zeiss Axio Imager.Z2 system (Zeiss, Wetzlar, Germany) was used to capture microscopic images of metaphase cells for karyotype preparation. For each sample, at least 20 GTG-banded metaphases were counted and at least five metaphases were analyzed. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature 2013 (39).

CNV-seq analysis was performed for cases with abnormal ultrasound, AMA, or history of miscarriage combined with a normal fetal karyotype. Genetic DNA isolated from CVS, AF and CB was performed using a nucleic acid extraction kit (CapitalBio Genomics, Beijing, China) following the manufacturer's protocol. Briefly, DNA was used for library construction and semiconductor sequencing on a BioelectronSeq 4000 sequencer (CapitalBio Genomics, Beijing, China). Bioinformatics results were obtained by comparing the read-sequence information with the human reference group (GRCh37, hg19) of the Burrows-Wheeler Aligner (version 0.7.15; https://sourceforge.net/p/bio-bwa/activity/) software. The pathogenicity of CNV (≥100 kb) was evaluated through the database of International Standards for Cytogenomic Arrays Consortium (ISCA; http://dbsearch.clinicalgenome.org/search/), the database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER; https://decipher.sanger.ac.uk/) and other databases.

Prenatal Serological Analysis

At 11 to 13^{+6} weeks of gestation, risk calculation for first-trimester combined screening (FTS) was performed using maternal age, fetal NT thickness, and maternal serum levels of free β-hCG and PAPP-A. At 15 to 20^{+6} weeks of gestation, risk calculation for second-trimester triple screening was performed using maternal age and maternal serum levels of AFP, free β-hCG, and uE3. Gestational week was determined by crown-rump length or biparietal diameter. Ultrasound testing and blood collection of pregnant women for FTS were performed on the same day. The levels of FTS serum markers were determined by Cobas e601 analyzer (Roche, Basel, Switzerland). The multiples of the median were derived from marker levels and NT thickness and used to calculate the risk of chromosomal abnormalities according to gestational age. Maternal weight, maternal age, and history of smoking were also considered in calculating Down syndrome risk on pregnancy. A risk cut-off value ≥ 1/270 was recognized as positive for trisomy 21 (Down syndrome) and a risk rate ≥ 1/350 was considered positive for trisomy 18 (Edwards syndrome).

NIPT Analysis

Five to ten milliliters of maternal peripheral blood was collected in EDTA-containing tubes (BD Biosciences, Franklin Lakes, NJ, USA). The blood sample was centrifuged at 1600 × g for 10 minutes at 4 ℃ to separated plasma from the peripheral blood cells. Followed by carefully transferred the plasma into a polypropylene tube and centrifuged at 16000 × g for 10 minutes at 4 ℃ to deposit the remaining cells. Briefly, cell-free DNA was extracted from 600 μL plasma using a nucleic acid extraction kit (CapitalBio Genomics, Beijing, China) according to the manufacturer's protocol. DNA was used for library construction and semiconductor sequencing, using a fetal aneuploidies (trisomies 21, 18, and 13) detection kit following the manufacturer's instructions (CapitalBio Genomics). An absolute value of z-score ≥ 3 in target chromosome and definite pathogenic CNV detected in chromosome were considered positive (40).

Statistical analysis

Data are analyzed using chi-squared test in SPSS 20.0 software (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Abbreviations

AMA: advanced maternal age; NT: nuchal translucency; free β-hCG: free beta human chorionic gonadotropin; PAPP-A: pregnancy-associated plasma protein A; uE3: unconjugated estriol; CVS: chorionic villus samples; AF: amniotic fluid; CB: cordocentesis, cord blood; CNV-seq: copy number variation sequencing; NIPT: noninvasive prenatal testing; ISCA: International Standards for Cytogenomic Arrays Consortium; DECIPHER: the database of Chromosomal Imbalance and Phenotype in Humans using

Ensembl Resources; FTS: first-trimester combined screening; aCGH: array comparative genomic hybridization.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Meizhou People's Hospital (No. MPH-HEC 2015-A-22). Informed consent was obtained by phone from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are included within the article.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

ZZ conceived and designed the experiments; XG contributed to the data collection and the manuscript draft. SL, HW, RW and XG helped to collect clinical data, conducted the clinical performances and researches; XG analyzed the data and wrote the paper.

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Figures

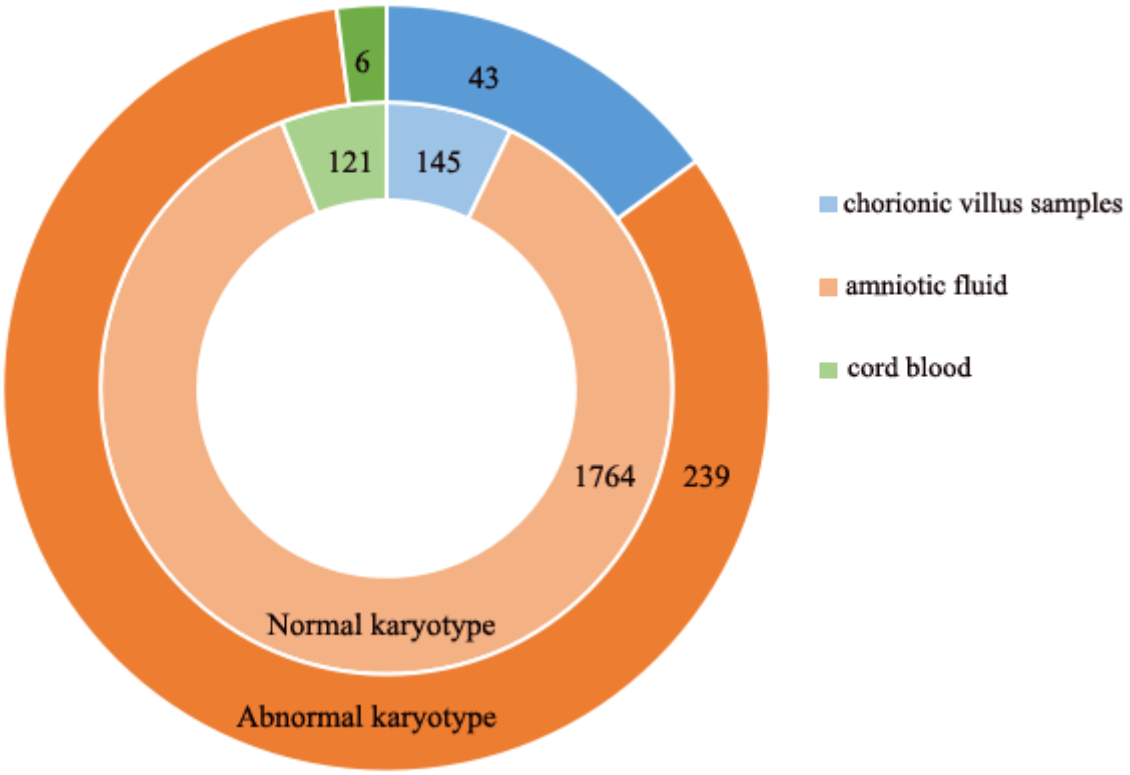

Figure 1

Number of normal and abnormal karyotype in different prenatal samples.

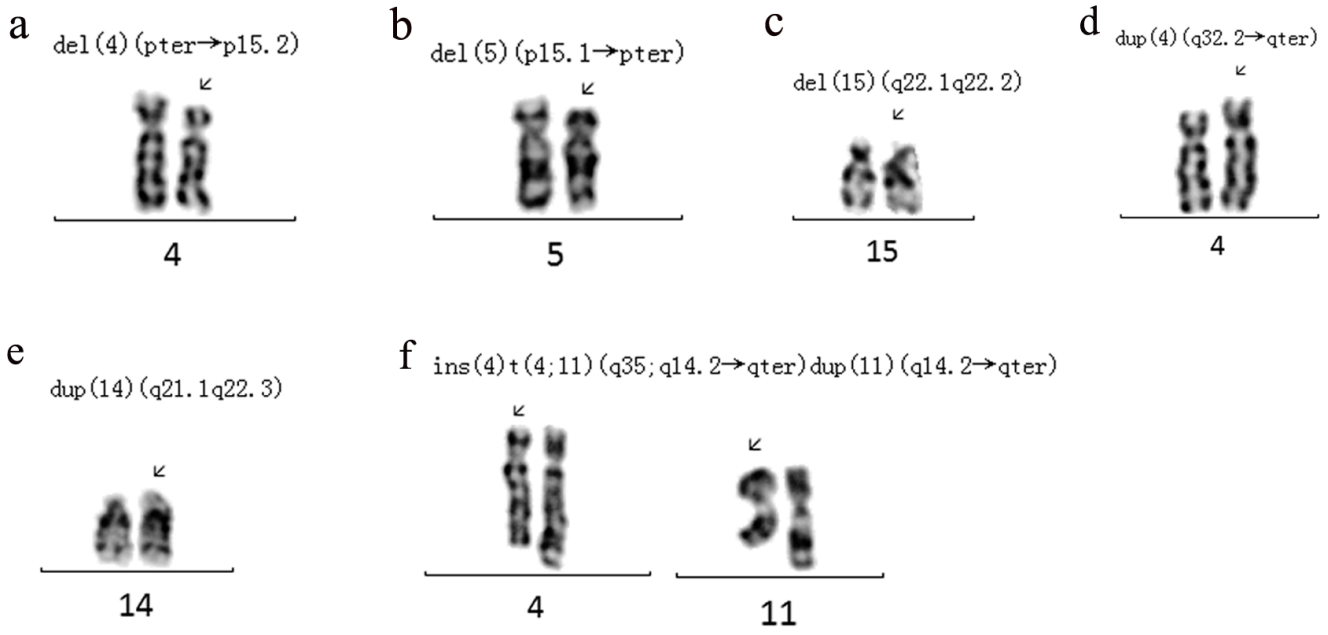

Figure 2

Deletions, duplications, and complex rearrangements in prenatal samples.