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Original Article

Diagnostic Accuracy and Chromosomal Microarray and Karyotype Analysis with Different Clinical Biomarkers for Prenatal Diagnosis of Fetal Genetic Diseases

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Abstract

Background: We compared the diagnostic accuracy and application value of chromosome microarray (CMA) technique and karyotype analysis for prenatal diagnosis of fetal genetic diseases using different clinical markers.

Methods: This is a prospective clinical study involving 1587 pregnant women who underwent amniocentesis for prenatal diagnosis due to various abnormal clinical indications in China between May 2018 and Nov 2021. Both chromosome microarray and karyotype analysis were applied. Participants were categorized into six groups based on different indications for prenatal diagnosis. The detection rates of chromosome microarray and karyotype analysis were compared. The study utilized SPSS version 20 for data analysis, employing descriptive statistics for count data results and chi-square statistics for statistical associations between outcomes and predictors.

Results: Chromosome microarray and karyotype analysis detected more abnormal chromosomes in the group with abnormal NIPT, with positive detection rates of 59.68% and the group in other situation with positive detection rates of 39.22%. Overall, 343 chromosome abnormalities were detected among participants. Overall, 101 cases chose induced labor, 240 cases gave birth, 1 newborn died after delivery, 1 case of twin chose selective reduction, another fetus gave birth, and 1 case lost to follow-up. The detection rate of chromosome abnormality in high-risk population was more than 1/5, highlighting the importance of reducing the incidence of birth defects through interventional prenatal diagnosis.

Conclusion: Clinically, Down's screening, NIPT and prenatal ultrasound screening can be conducted initially, followed by karyotype analysis and CMA detection for those with abnormal findings.

Keywords: Chromosome abnormality; Chromosome microarray analysis; Karyotype analysis; Prenatal diagnosis; Noninvasive screening

Introduction

Chromosomal disorders are caused by numeric or structural chromosomal anomalies involving the missing or gaining, as well as the breakage and incorrect rejoining of a piece of a chromo-

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some or the entire chromosome (1,2). Fetal chromosomal anomalies are among the leading causes of birth defects worldwide (3,4). Chromosomal abnormalities and gene mutations constitute the primary causes of genetic disorders (4). Chromosomal abnormalities and gene mutations constitute the primary causes of genetic disorders and are also key drivers of cancer development, contributing to genomic instability and uncontrolled cell proliferation. Chromosomal abnormalities and gene mutations constitute the primary causes of genetic disorders and are also key drivers of cancer development, contributing to genomic instability and uncontrolled cell proliferation Chromosomal aneuploidies represent the most common anomalies of all fetal chromosomal anomalies (5-7).

In China, more than 36000 newborns are born with chromosome abnormalities every year, causing a significant burden to families and society (3). The annual birth defects rate was 5.6% with the annual growth rate of 900,000 cases (8). Increased advanced maternal age pregnancy and rising trend in environmental pollution results in increased incidence rate of chromosomal abnormalities (7). Currently, there is no effective therapy for chromosomal disorders in China (9).

Today, prenatal diagnostic technology applies combinations of procedures in the first- and second-trimester, using concentrations of serum analysis, maternal age, genetic history, and ultrasound imaging data (10). Cytogenetic fetal karyotyping offers a reliable detection rate for aneuploidy and large rearrangements of greater than 7–10 megabases (7,11). However, it was not able to detect unbalanced structural abnormalities caused by submicroscopic chromosomal anomalies (7,11). Chromosomal microarray analysis (CMA) is a cytogenetic molecular technique that has a high detection rate for microscopic and submicroscopic chromosomal abnormalities in patients with neurodevelopment disorders (12).

This study used the 2003 Ministry of Health guideline to assess investigations for fetal abnormalities in China. The indications are excessive amniotic fluid, abnormal fetal development, exposure to congenital defects, family history of diseases, and age over 35. Prenatal genetic testing is essential for detecting chromosomal abnormalities, with karyotype analysis being the most common diagnostic method. However, it is limited in detecting chromosomal abnormalities smaller than 5 Mb, which can result in missed diagnoses. To overcome this limitation, CMA is recommended as a high-resolution molecular technique that can identify submicroscopic copy number variations (CNVs) associated with developmental and genetic disorders (14-16). CMA also offers faster results by eliminating the need for cell culture, making it a more sensitive and comprehensive tool for prenatal genetic screening. Given its superior sensitivity, CMA is increasingly recognized as a valuable tool in prenatal diagnostics.

We compared karyotype analysis and CMA, highlighting their diagnostic capabilities and the need for a more precise approach in prenatal genetic screening. Moreover, the study assessed the diagnostic accuracy and value of CMA and karyotype analysis for prenatal diagnosis of chromosomal abnormalities in different clinical indications.

Methods

Study subjects and setting

Pregnant women who underwent prenatal consultation and amniocentesis tests in First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China between May 2018 and Nov 2021 were selected as the study subjects. The inclusion criteria were pregnant women aged 35 or over, abnormal results of noninvasive fetal chromosome aneuploidy testing, high level of maternal blood Down's screening, ultrasound abnormalities (thickening of nuchal transparent layer (greater than 2.5 mm), and fetal dysplasia or structural deformity. Participant who had spousal history of adverse pregnancy or reproduction, consanguineous marriage, chromosome abnormality or family genetic disease, or mental retardation or mental illness were excluded.

Overall, 1587 subjects were participated. All participants were consulted by a qualified genetic

consultant to fully understand the risks of amniocentesis as well as the advantages, limitations and potential results of CMA and karyotype analysis techniques. Amniocentesis, CMA and karyotype analysis were performed from 17 and 24 weeks of gestation.

Reagents and instruments

For the Karyotype analysis: The culture medium, colchicine, plant hemagglutinin, KCl solution, trypsin solution, glacial acetic acid and methanol, Gi-emsa staining solution, NaOH solution, normal saline, cell incubator, and GenetixGSL-120 automatic chromosome analyzer were employed. For the CMA analysis: Affymetrix GeneChip System 3000Dx v.2 chip system and CytoScan HD chip were employed.

Grouping and experiments

Sample collection: pregnant women underwent amniocentesis at 17 ~ 24 wk, and 25-30 ml amniotic fluid was extracted under strict aseptic operation.

CMA: The amniotic fluid was transported to Hangzhou Jinyu Medical Laboratory, where Affmetrix Company's kit was employed along with optimized standard operating procedures. Genome-wide scanning was conducted using CytoScanHD/CytoScan750 k. Before detection, the amniotic fluid DNA was analyzed by linkage with maternal blood DNA to exclude maternal cell contamination. The entire process was executed in strict accordance with quality control standards, encompassing DNA extraction, enzyme digestion, ligation, PCR product purification, fragmentation, labeling, hybridization, scanning and result analysis. Affymetrix Chromosome Analysis Suite Software was utilized for analysis, along with querying information in clingen, decipher and HGMD databases.

Karyotype analysis: After the fetal cells in amniotic fluid undergo culture, digested, hypotonic, fixed, dropped, baked, trypsin banding and stained in the laboratory, the chromosome karyotype is analyzed following scanning.

Statistical analyses

Data were analyzed using SPSS ver. 20 (IBM Corp., Armonk, NY, USA) statistical software**.** Descriptive statistics were used to summarize count data as frequencies and percentages. To assess statistical associations between outcome variables and predictors, the Chi-square test was applied, with a significance threshold set at $P<0.05$. Additionally, confidence intervals (CIs) were reported where applicable to enhance the reliability of the findings.

Follow-up

Follow up and record the pregnancy status, pregnancy outcome and postnatal health status of newborns by telephone and outpatient visiting.

Results

Overall, 1587 subjects were participated. Participants were categorized to advanced maternal age, Down's screening high-risk, NIPT abnormality, prenatal screening ultrasound abnormality, poor pregnancy and childbirth history, and group with other situations. Overall, 343 abnormalities (using CMA plus karyotype) were identified, resulting in a positive detection rate of 21.61%. Furthermore, 125 cases exhibited pathogenic chromosomal abnormalities, yielding a detection rate of 7.88%. Specifically, within the advanced maternal age group, 119 cases were abnormal, with a positive rate of 16.83% and a pathogenic chromosome abnormality rate of 5.52%. In the Down's screening high-risk group, 66 cases were abnormal, with a positive detection rate of 19.41% and a pathogenic chromosomal abnormality detection rate of 5.59%. Among those in the abnormal NIPT group, 37 cases were identified, resulting in a positive detection rate of 59.68% and a pathogenic chromosome abnormality detection rate of 48.39%.

In the abnormal prenatal ultrasound screening group, 68 cases were abnormal, with a positive detection rate of 24.03% and a pathogenic chromosomal abnormality detection rate of 9.19%. Within the poor pregnancy and childbirth history

group, 33 cases were abnormal, with a positive detection rate of 22.92% and a pathogenic chromosomal abnormality rate of 4.86%. Finally, in the group with other situations, 20 cases were abnormal, resulting in a positive rate of 39.22% and a pathogenic chromosome abnormality rate of 11.76%. The group with the highest detection rate of chromosome abnormality was the NIPT abnormal group, with a positive detection rate of 59.68%. The second-highest rate of chromosome abnormality was observed in the group with other situations (positive rate: 39.22%). The third-highest rate of chromosome abnormality was found in the abnormal prenatal ultrasound screening group (positive detection rate: 24.03%) (Table 1 and Fig. 1).

Table 1: The detection of chromosome abnormalities in different clinical indications by CMA and karyotype analysis

Group	Abnormal	Normal	Total	Detection rate (%)
Group 1	119	588	707	16.83
Group 2	66	274	340	19.41
Group 3	37	25	62	59.68
Group 4	68	215	283	24.03
Group 5	33	111	144	22.92
Group 6	20	31	51	39.22
Total	343	1244	1587	21.61
Notes: 1:advanced maternal age; 2:Down's screening high-risk; 3:abnormal NIPT; 4:abnormal prenatal ultrasound screening; 5:poor pregnancy and childbirth history; and 6:other situations				

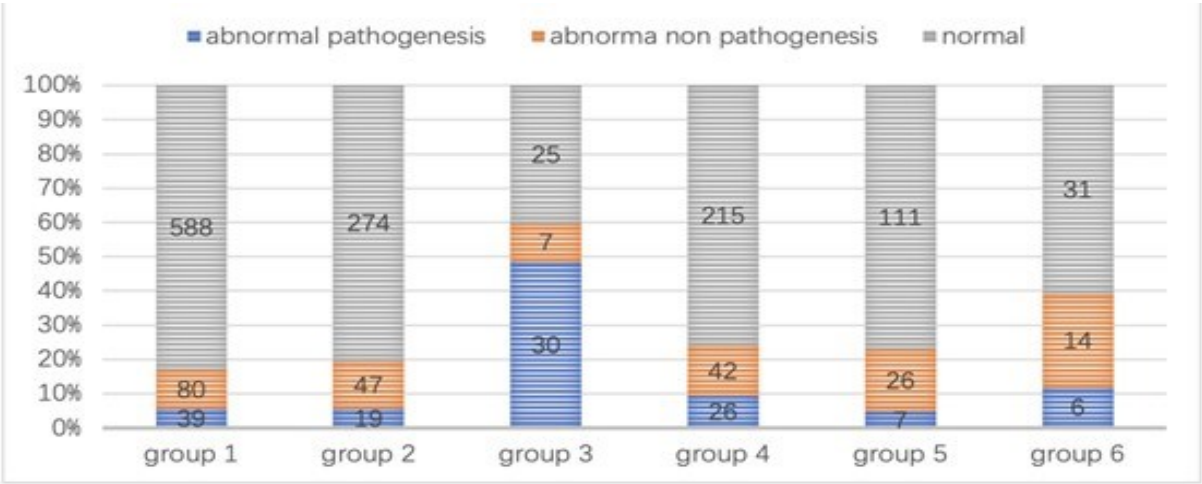

Fig. 1: Abnormal chromosomes and pathogenicity in amniocentesis with different indication.

Prenatal diagnosis results of the advanced maternal age group

Overall, 707 cases of amniocentesis were performed for "advanced maternal age," resulting in the detection of 119 cases of abnormal chromosomes. Based on the age of pregnant women, they were divided into two groups at the age of 40 (Table 2). There was no statistical difference in the positive detection rate of chromosome abnormalities between the two groups (17.87%, 15.22%) (Table 3). The detection rates of pathogenic chromosomal abnormalities in the groups were 6.50% and 3.99%, respectively. The pregnant women who performed amniocentesis due

to "advanced age" were found to have 119 cases of chromosomal abnormalities. Among them, 27 cases opted for mid-term induced labor, while 92 cases proceeded to give birth. Thirty-nine cases exhibited pathogenic or potentially pathogenic chromosomal abnormalities, and 16 chose to continue pregnancy until delivery. In some instances, chromosome deletion or duplication originated from either the father or mother, and the parents' phenotype was not significantly abnormal. Alternatively, the prognosis of newborns with chromosome abnormalities was deemed acceptable after genetic counseling, prompting pregnant women to choose to continue pregnancy to term delivery. In such cases, newborns generally exhibited good health at birth (Table 4).

Table 2: The chromosome abnormalities in advanced age group

Group	Abnormal	Normal	Total	Rate (%)
< 40	77	354	431	17.87
≥40	42	234	276	15.22
Total	119	588	707	16.83

Table 3: The detection of pathogenic chromosomal abnormalities in advanced age group

Group	Abnormal		Normal	Total	Rate (%)
	Pathogenic	Nonpathogenic			
< 40	28	49	354	431	6.50
≥ 40	11	31	234	276	3.99
Total	39	668		707	5.52

Table 4: Follow up of continued pregnancy with pathogenic abnormal chromosomes detected in advanced age group

Case	Age	karyotype	CMA	clinical significance	Outcome
1	35		46,XX,del(22)(q11.21)	Pathogenic	Term delivery
2	36		46,XX,del(16)(p11.2)	Pathogenic From mother	Term delivery
3	36		46,XX,del(15)(q11.2)	Possibly pathogenic	Term delivery
4	36	47,xxx	47,xxx	Superfemale syndrome	Full term cesarean section
5	36		46,XX,del(17)(p12)	Pathogenic	Term delivery
6	36		SBDS heterozygous mutation	Pathogenic	Term delivery
7	36		46,XX,del(22)(q11.21)	Possibly pathogenic	Term delivery
8	37		46,XX,del(15)(q11.2)	Possibly pathogenic	Cesarean section at 36W+5D Breech presentation
9	37	47,XXY	47,XXY	Klinefelter syndrome	Full term cesarean section
10	38	47,XXY	47,XXY	Klinefelter syndrome	Term delivery
11	38		46,XX,dup(16)(p13.11)	Possibly pathogenic From father	Full term cesarean section
12	38		46,XX,del(17)(p12)	Pathogenic	Term delivery
13	40	46,XN,t(2;5)(p25;q35) From father	46,XX,dup(22)(q11.21q11.22)	Pathogenic	Full term cesarean section Shoulder presentation (8-10)
14	40		46,XX,dup(X)(p21.1)	X-linked recessive disease	Term delivery
15	40		46,XX,del(12)(q21.2q21.31)	Pathogenic	Term delivery 2190g（8-10）
16	42		46,XX,del(1)(q21.1q21.2)	Pathogenic From father	Term delivery

Prenatal diagnosis results of ultrasound abnormalities in prenatal screening

The prenatal diagnosis using "prenatal screening ultrasound abnormalities", resulted in the detection of 68 cases of abnormal chromosomes (Table 5). The group exhibiting ultrasound structural abnormalities had a similar detection rate of chromosome abnormalities with soft indicators (28.41% vs 22.05%). However, the detection rates of pathogenic abnormal chromosomes in the two groups were higher in ultrasonic structural abnormality (14.77% and 6.67%) (Table 6). Of 283 cases of amniocentesis performed using "prenatal screening ultrasound abnormalities", 68 cases of chromosomal abnormalities had been detected. Of these cases, 26 exhibited pathogenic or potentially pathogenic chromosomal abnormalities, and 6 cases choose to continue pregnancy until delivery (Supplementary Table 1). In Table 8, 42 cases of chromosomal abnormalities were non-pathogenic. Among them, six cases opted for induced labor in the second trimester due to "abnormal fetal structure indicated by ultrasound". The Karyotype analysis results showed chromosome polymorphism. However, CMA results were normal, indicating no chromosomal abnormalities related to phenotype (Supplementary Table 2).

Table 5: The detection of chromosome abnormalities in groups using abnormal prenatal ultrasound screening

Group	Abnormal	Normal	Total	Rate (%)
Structural abnormality	25	63	88	28.41
Soft indicator	43	152	195	22.05
Total	68	215	283	24.03

Table 6: Detection of pathogenic chromosomal abnormalities in group with abnormal prenatal ultrasound screening

Group	Abnormal		Normal	Total	Rate (%)
	Pathogenic	Nonpathogenic			
Structural abnormality	13	12	63	88	14.77
Soft indicator	13	30	152	195	6.67
Total	26	257		283	9.19

Prenatal diagnosis results of serum marker abnormalities in prenatal screening

The positive detection rates of abnormal chromosomes and pathogenic chromosomal abnormalities were lower in the group with Down's high-risk than abnormal NIPT (Table 7 and 8). Overall, 340 cases of amniocentesis were performed due to the "high risk of Down's screening", resulting in the detection of 66 cases of abnormal chromosomes. Among them, 13 cases opted for mid-term induced labor while 53 cases continued to birth. Nineteen cases exhibited pathogenic or possibly pathogenic chromosomal abnormalities, and 7 cases continued pregnancy until delivery (Supplementary Table 3 and Table 9). Amniocentesis tests on 62 cases with "NIPT abnormality" resulted in 37 cases showing chromosomal abnormalities. Among them, 27 cases chose mid-term induced labor, 1 case underwent selective reduction because one of the twins had trisomy 21 syndrome, and 9 cases proceeded to final delivery. Of those showing chromosomal abnormalities, 30 exhibited pathogenic or possibly pathogenic chromosomal abnormality, and 4 chose to continue pregnancy until delivery. All of them delivered at term, with the newborns being

in good conditions after birth (Supplementary Table 4). As described in Supplementary Table 5, seven cases of chromosomal abnormalities were non-pathogenic. The CMA result showed about 12.3 Mb homozygous phenomenons in the region of 10p15.3p13, with unknown clinical significance, leading the parents to opt for induced labor. Another case showed no abnormality in karyotype analysis, but CMA revealed a 29.3 Mb homozygous phenomenon in 15q13.1q21.3, with unclear clinical significance, resulting in induced labor. The other 5 cases were delivered, with the newborns being in good condition after birth.

Table 7: Detection of chromosome abnormalities in groups with serum marker abnormalities in prenatal screening

Group	Abnormal	Normal	Total	Rate（%）
Down's	66	274	340	19.41
NIPT	37	25	62	59.68
Total	103	299	402	25.62

Table 8: Detection of pathogenic chromosomal abnormalities in groups with serum marker abnormalities in prenatal screening

Group	Abnormal		Normal	Total	Rate (%)
	Pathogenic	Nonpathogenic			
Down's	19	47	274	340	5.59
NIPT	30	7	25	62	48.39
Total	49	353		402	12.19

Table 9: Adverse pregnancy outcome with non-pathogenic abnormal chromosomes in group with Down's high-risk

Case	Indications	karyotype	CMA	clinical significance	Outcome
1	Down's screening high-risk	46,xn,t(4;20)(q22;p12)		balanced translocation	Term delivery Congenital ventricular septal defect
2	Down's screening high-risk		46,XX,dup(6)(q26)	Unknown	Mid-term induced labor

Prenatal diagnosis results of the poor pregnancy and childbirth history group

A study of 144 cases of amniocentesis was performed due to poor pregnancy and childbirth history, mainly due to repeated abortions and multiple embryo terminations. About one-third of these individuals had a history of birth defects. Most abnormal chromosomes were found in this subset, with 33 exhibiting chromosomal abnormalities. Out of these, 7 were pathogenic or possibly pathogenic, and 3 continued pregnancy. The remaining 25 cases were non-pathogenic (Table 10).

Table 10: Follow up of continued pregnancy with pathogenic abnormal chromosomes detected in group with poor pregnancy and childbirth history

Case	Indications	karyotype	CMA	clinical significance	Outcome
1	history of neonatal death with Hirschsprung's disease		46,XX,del(20)(p12.3p12.2)	Pathogenic	Full term cesarean section
2	History of fetal malformation		46,XX,del(15)(q11.2)	Pathogenic low penetrance	Term delivery
3	History of intrauterine fetal death at 40 W	46,XX(70)/46,XY,(10)	46,XX/47,XXY, 46,XY/45,X	Pathogenic May have abnormal sexual development	Term delivery Neonatal pathological jaundice

Prenatal diagnosis results of other situations

The study analyzed 51 prenatal diagnoses, categorized into three groups: close relatives' marriage, one parent with chromosomal abnormalities, and one parent with mental retardation. All newborns were delivered normal at birth. Some cases had pathogenic deletions, leading to induced labor. Three out of six cases with abnormal chromosomes continued pregnancy, while 14 cases were non-pathogenic and all delivered. All newborns were in good condition after birth (Table 11).

Table 11: Follow up of continued pregnancy with pathogenic abnormal chromosomes detected in group with 'other' situations

Case	Indications	karyotype	CMA	clinical significance	Outcome
1	Maternal chromosome abnormalities		46,X,del(X)(q28)	X-linked recessive disease	Term delivery
2	Maternal chromosome abnormalities		46,XX,del(16)(p13.11p12.3)	Pathogenic	Term delivery
3	Maternal mental illness		46,XX,dup(20)(q13.33) 46,XX,del(7)(q36.3)	Pathogenic Unknown	Cesarean section at 35W+2D

Discussion

Evidence suggests, NIPT is commonly recommended for women aged 35-40 years, while amniocentesis is usually recommended for women over 40 yr old (17). This study, however, observed no difference between the positive detection rate of abnormal chromosomes and pathogenic abnormal chromosomes among study participants. This similarity could be related to small sample size.

Ultrasound revealed no difference in the detection rate of abnormal chromosomes between the groups with structural abnormalities or soft indi-

cators. However, there was a higher detection rate of pathogenic abnormal chromosome in group with structural abnormalities, and the difference was statistically significant. In this study, 88 cases of fetal structural malformations were diagnosed prenatally using ultrasound, and 25 cases of abnormal chromosomes were found. Interestingly, the phenotype observed via ultrasound did not consistently align with the pathogenic effects of abnormal chromosomes found in the database. Amniocentesis was performed due to the ultrasound indication of "fetal bilateral cleft lip with cleft upper alveolar process". However, no chromosomal abnormality related to this condition was detected by chromosome karyotype analysis or chromosomal copy number variation detection. Instead, a mutation of the COL2A1 gene was identified through WES.

Down's screening, as a traditional prenatal hematologic screening method, holds certain screening value, yet it also entails a proportion of false positives and false negatives. NIPT utilized free fetal DNA in maternal peripheral blood to screen chromosomal aneuploidy of the fetus. This approach significantly enhances the sensitivity and specificity of prenatal screening for chromosomal diseases (18,19). Several studies had underscored NIPT's superior screening efficacy for fetal aneuploidy compared to traditional serological screening methods (20-23).

This was consistent with the current findings. This study also corroborated NIPT's specificity through cases, where sex chromosome abnormalities or chromosome deletions suggested by NIPT were confirmed via chromosome karyotype analysis or CMA. Moreover, NIPT provides broader application in pregnancy due to its safety, efficiency, and ease of promotion. It can be contemplated as a comprehensive substitute for traditional Down's screening if economic and medical constraints are absent, thereby mitigating unnecessary interventional prenatal diagnostic procedures. Nevertheless, given that abnormal chromosome fragments may originate from the pregnant mother or may be present in small numbers, NIPT still incurs some false positives and false negatives. Thus, NIPT can only serve as a prenatal screening method and cannot entirely supplant prenatal diagnosis.

In the group with adverse pregnancy and childbirth history, the positive detection rate of abnormal chromosome is not high. This could be attributed to the fact that many adverse histories do not specifically pertain to fetal malformations or chromosomal abnormalities. Abortions may occur due to unknown causes. Often, pregnant women do not investigate the genetic causes of abortion, and sometimes cannot even recall the circumstances of the event. Clinicians often struggle to ascertain the cause of abortion based on the pregnant women's medical history. Fetuses with congenital genetic material abnormalities frequently miscarry in early pregnancy. While there is a possibility of genetic coding errors during the formation of reproductive cells, recurrent miscarriages or a history of malformed fetuses warrant suspicion of abnormalities in the pregnant woman's own genetic material. In such cases, prenatal diagnosis in the second trimester of pregnancy should be recommended.

Although social development has increased awareness of the adverse effects of consanguineous marriage, it still occurs. Among the three cases of consanguineous marriage in this study, two cases exhibited chromosome abnormalities, characterized by large segments of homozygous phenomena. Homozygotes, also known as homozygotes are individuals with the same allele at a particular locus on homologous chromosomes in a diploid organism, categorized as dominant homozygotes or recessive homozygotes (24). Chromosome copy number variations are widely distributed in the human genome, constituting approximately 12% of the genome sequence (25). Despite detecting chromosomal homozygosity in two consanguineous marriages in this study, no abnormalities were found during the follow-up of newborns. However, long-term monitoring of newborn growth and development is warranted.

Future research in prenatal diagnostics should focus on integrating CMA with emerging technologies like next-generation sequencing (NGS) to enhance diagnostic accuracy and detect a broader range of genetic abnormalities. Addition-

ally, further studies are needed to assess the cost-effectiveness and clinical utility of CMA across diverse healthcare settings, ensuring its accessibility and feasibility for routine prenatal screening. Advancements in bioinformatics and machine learning may also improve data interpretation and risk prediction**,** further refining prenatal genetic assessments.

Ethic approval

Protocols and procedures of this study were approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (KY2023-R091), and in-line with the ethical standards and regulations of the studies on human subjects set by the Helsinki Declarations.

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Disclosure of interests

The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

Consent for participation

The objectives, the benefits and risks of the study were explained to the participants by enumerators. Subjects consented by written form.

Consent for publication

Participants consented for publication of the study findings.

Data availability

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

References

1. Montazerinezhad S, Emamjomeh A, Hajieghrari B (2020). Chromosomal abnormality, laboratory techniques, tools and databases in molecular cytogenetics. *Mol Biol Rep*, 47(11): 9055–9073.
2. Chen CP (2007). Chromosomal abnormalities associated with neural tube defects (I): Full aneuploidy. *Taiwan J Obstet Gynecol*, 46: 325–335.
3. Shan-shan L, Yan-fang Z, Feng-hua X, Dong-xiu L, Xiang H (2018). Clinical value of CMA and karyotype analysis in diagnosis of fetal chromosomal abnormalities. *J Modern Lab Med*, 126–129.
4. Lo YMD (2020). Screening of fetal chromosomal aneuploidy by noninvasive prenatal testing: From innovation to setting public health agendas to potential impact on other fields. *Clin Chem*, 66(1): 25–28.
5. Sun X, Lu J, Ma X (2019). An efficient method for noninvasive prenatal diagnosis of fetal trisomy 13, trisomy 18, and trisomy 21. *PLoS ONE*, 14(4): e0215368.
6. Singh RK, Prasad DN, Bhardwaj TR (2015). Synthesis, physicochemical and kinetic studies of redox derivative of bis (2-chloroethylamine) as alkylating cytotoxic agent for brain delivery. *Arab J Chemistry*, 8(3): 380–387.
7. Lu S, Kakongoma N, Hu WS, et al. (2023). Detection rates of abnormalities in over 10,000 amniotic fluid samples at a single laboratory. *BMC Pregnancy Childbirth*, 23(1): 102.
8. Li Z, Di J (2021). Prevention and control of birth defects in China: Achievements and challenges. *China CDC Weekly*, 3: 771.
9. Howell KB, Eggers S, Dalziel K, et al (2018). A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. *Epilepsia*, 59(6): 1177–1187.
10. Vermeesch JR, Voet T, Devriendt K (2016). Prenatal and pre-implantation genetic diagnosis. *Nat Rev Genet*, 17(10): 643-56.
11. Huang H, Wang Y, Zhang M, et al (2021). Diagnostic accuracy and value of chromosomal microarray analysis for chromosomal abnormalities in prenatal detection: A prospective clinical study. *Medicine (Baltimore),* 100(20): e25999.

12. Miller DT, Adam MP, Aradhya S, et al (2010). Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*, 86(5): 749–764.
13. Li L, Zhang H, Yang Y, et al (2020). High frequency of Y chromosome microdeletions in male infertility patients with 45,X/46,XY mosaicism. *Braz J Med Biol Res*, 53(3): e9021.
14. Li W, Lu-Sha D, Xiao-Dan Z (2018). Application of chromosomal microarray analysis in prenatal diagnosis. *Matern Child Health Care China*, 139–141.
15. Levy B, Wapner R (2018). Prenatal diagnosis by chromosomal microarray analysis. *Fertil Steril*, 109(2): 201–212.
16. Subspecialty Group of Clinical Genetics et al (2016). Expert consensus on the clinical application of chromosome genome chips in pediatric genetic diseases. *Zhonghua Er Ke Za Zhi,* 54(6):410-3.
17. Haddow JE, Palomaki GE, Knight GJ, Cunningham GC, Lustig LS, Boyd PA (1994). Reducing the need for amniocentesis in women 35 years of age or older with serum markers for screening. *N Engl J Med*, 330(16): 1114–1118.
18. Dharajiya N, Zwiefelhofer T, Guan X, Angkachatchai V, Saldivar JS (2015). Noninvasive prenatal testing using cell-free fetal DNA in maternal plasma. *Curr Protoc Hum Genet*, 84:8.15.1–8.15.20.
19. Sahota DS, Leung WC, Chan WP, To WWK, Lau ET, Leung TY (2013). Prospective assessment of the Hong Kong Hospital Authority universal Down syndrome screening programme. *Hong Kong Med J*, 19(2): 101–108.
20. Iwarsson E, Jacobsson B, Dagerhamn J, et al (2017). Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high-risk population – a systematic review and meta-analysis. *Acta Obstet Gynecol Scand*, 96(1): 7–18.
21. Liu Y, Liu H, He Y, et al (2020). Clinical performance of non-invasive prenatal testing as a first-tier screening test for trisomy 21, 18, 13 and sex chromosome aneuploidy in a pilot city in China. *Hum Genomics*, 14(1): 21.
22. Jayashankar SS, Nasaruddin ML, Hassan MF, et al (2023). Non-invasive prenatal testing (NIPT): Reliability, challenges, and future directions. *Diagnostics (Basel)*, 13(15): 2570.
23. van der Meij KR, Sistermans EA, Macville MV, et al. (2019). TRIDENT-2: National implementation of genome-wide non-invasive prenatal testing as a first-tier screening test in the Netherlands. *Am J Hum Genet*, 105(6): 1091–1101.
24. Uzman A, Lodish H, Berk A, Zipursky L, Baltimore D (2000). *Molecular Cell Biology*. 4th ed. W. H. Freeman and Company, New York, NY, pp.: Section 1.2 – The Molecules of Life.
25. Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE (2010). Is the incidence of rheumatoid arthritis rising?: Results from Olmsted County, Minnesota, 1955–2007. *Arthritis Rheum*, 62(6): 1576–1582.