Research Article

A Three-Year Prospective Study Assessing the Application of Chromosomal Microarray Analysis in 576 High-Risk Pregnant Women

Minmin Jiang,1 Shengwen Huang,1 Xingwei Ma,1 Ping Xie,2 Lingyan Ren,1 Qian Jin,1 and Keyan Linghu1

1Prenatal Diagnosis Center, Guizhou Provincial People’s Hospital, Guiyang 550004, China
2Department of Ultrasonography, Guizhou Provincial People’s Hospital, Guiyang 550004, China

Correspondence should be addressed to Minmin Jiang; drjiangminmin@outlook.com

Received 22 July 2022; Revised 21 September 2022; Accepted 22 September 2022; Published 15 October 2022

Academic Editor: Xueliang Wu

Copyright © 2022 Minmin Jiang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. The use of chromosomal microarray analysis (CMA) in prenatal diagnosis of chromosomal and genetic diseases has resulted in a significant improvement in the diagnosis of genetically caused congenital malformations, neurodevelopmental disorders, and congenital anomalies, with a high diagnostic yield in selected prenatal cases. Objective. The objective of this study was to evaluate the application of CMA in the prenatal diagnosis of high-risk pregnant women. Method. A total of 576 pregnancies were selected from May 2018 to October 2020 in our hospital, including amniotic fluid chromosome, karyotype analysis, and CMA detection. The study group was divided into two groups based on the indications for testing: group A has 88 patients at the age of 35 years or older, and group B patients were in high-risk pregnancies, which consisted of 33 cases of bad pregnancy history, 252 high-risk serological screenings, 70 high-risk non-invasive prenatal testing (NIPT), 65 cases of B-ultrasound indicated fetal development abnormalities or ultrasonic soft marker abnormalities, and 68 other cases of pregnant women or both who have genetic or chromosomal abnormalities. At last, we have an analysis of the detection rate from different testing methods.

Results. Based on the follow-up test, 576 high-risk pregnant women showed an amniotic fluid chromosome karyotype rate of 18.1% (104/576), and the remaining 472 of these cases suffered a CNV ratio of 14.2% (67/472). 16 people showed a clear cause ratio 3.39% (16/472), and 28 of the 472 (5.93%) cases showed polymorphism. Conclusions. In our study, CMA significantly improved the fetal detection rate and diagnosis rate in high-risk pregnant women, which proved to be a very useful method in the diagnosis of genetically caused neurodevelopmental disorders and congenital anomalies. The use of CMA in high-risk pregnant women is justified, and these women can detect an additional (3.40%, 16/472) of pathogenic microdeletions and microduplications in the cases.

1. Introduction

Chromosomal microarray analysis (CMA) is also called “molecular karyotype” technology, including array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphism microarrays (SNP arrays). With the advantages of high resolution (0.1–0.3 mb), high efficiency, and high sensitivity, CMA can accurately locate abnormal fragments, display affected genes, and help identify disease-related genes. The chromosome karyotype analysis uses “fetal cells” as the test specimen and is recognized as the gold standard for genetic prenatal diagnosis of abnormal chromosome number, large fragment deletion/duplication (>10 mb), rearrangement, and inversion. Some studies have reported that about 12.4% to 35% of fetal ultrasound abnormalities are caused by chromosomal aberrations, of which about 25% are abnormal in karyotype structure or number, and about 10% are abnormal in chromosomal microstructure [1]. In addition, chromosomal microarray analysis (CMA) is a useful technique used to detect clinically significant microdeffects or duplications with high sensitivity to submicroscopic aberrations [2].
2. Methods

2.1. Patients and Design. From May 2018 to October 2020, 576 high-risk pregnant women were selected, including 88 pregnant women located at the age of 35 years or older, 33 cases of bad pregnancy history, and 252 high-risk serological screenings, 70 high-risk NIPTs, 65 cases of B-ultrasound indicated fetal development abnormalities or ultrasonic soft markers abnormalities, and 68 other cases of pregnant women or both who have suffered genetic or chromosomal abnormalities. They all underwent amniotic fluid chromosome karyotype analysis and CMA testing in our hospital from May 2018 to October 2020 to explore the application of chromosomal microarray analysis (CMA) in prenatal diagnosis.

2.2. Interventional Prenatal Diagnosis. Before carrying out invasive prenatal diagnosis, we carried out necessary genetic counseling services for high-risk pregnant women, fully informed them of the technical advantages and possible risks, and had them sign an informed consent form. Amniocentesis under ultrasound guidance was used to quantify 30 ml of amniotic fluid, which was then sent to the genetic laboratory for chromosome karyotype analysis and CMA analysis.

2.3. Ultrasonography. Prenatal ultrasound grade III screening for selected high-risk pregnant women is referred to as “Prenatal Ultrasound Diagnosis of Fetal Malformations” [11]. Screen out fetuses with ultrasound soft indicators such as lateral ventricle normal high value, intestinal echo enhancement, renal pelvis separation, ventricular bright spot, choroid plexus cyst, single umbilical artery, and other ultrasound soft indicators, as well as cases of fetal structural malformations. Partial results were presented in supporting information (Figure S1).

2.4. Amniotic Fluid Cell Karyotype Analysis. First, 10 mL of amniotic fluid that centrifuged at 1500 r/min for 10 min, then discarded the supernatant. After adding amniotic fluid cell culture medium (PAN, Germany), we placed it in a 37°C, 5% volume fraction carbon dioxide cell incubator for about 5 to 7 days. Finally, we had to mix 10 μL of colchicine with the product for 2 hours, and routine G-banding chromosome analysis was performed. We were required to detect 20 karyotypes under the microscope (CytoVision®) and analyze 5 of them. Additionally, in cases of abnormal karyotype or chimera, no less than 60–100 cell division phases were analyzed [12]. Partial results were presented in supporting information (Figure S2).

2.5. Prenatal CMA Testing. The experimental reaction process refers to the experimental operation process provided by Illumina. Research and application of the whole genome HumanCyto SNP-12 BeadChip Kits chip provided by Illumina in the United States, which contains about 300,000 detection sites. It can detect abnormal chromosomal copy number changes and loss of heterozygosity, such as chromosome microdeletion/microduplication and chromosome subtelomere deletion syndrome, with clinical significance in the whole genome. After whole genome amplification, denaturation, and renaturation of fetal DNA, data collection is performed using the Iscan scanning system, and data analysis is performed using KaryoStudio software. The test results are compared and analyzed with the following public databases: http://omim.org/(OMIM database); http://genome.ucsc.edu/(UCSC database); http://ncbi.nlm.nih.gov/pubmed (NCBI database); and https://decipher.sanger.ac.uk/(DECIPHER database).

The nature of CNVs is determined through database comparison; pathological CNVs and benign CNVs are identified. For CNVs of unknown clinical significance, further family analysis is carried out to determine the source of abnormality and assist in evaluating the prognosis.

2.6. Statistical Methods. Data are collected using EXCEL forms, and counting data are expressed as the number of cases and percentages.

3. Results

3.1. Amniotic Fluid Chromosome Abnormalities. According to clear research objects and methods, 2719 samples were screened, which is the total number of pregnant women tested by related items in our hospital from May 2018 to October 2020, and 576 samples of high-risk pregnant women meeting the research conditions were
selected for analysis. There were 472 cases with normal karyotypes and 104 cases with abnormal karyotypes, including 29 cases of the 21-trisomy syndrome (27.9%, 29/104), 9 cases of the 18-trisomy syndrome (8.65%, 9/104), 2 cases of the 13-trisomy syndrome (1.92%, 2/104), supernumerary3 cases of the syndrome (2.88%, 3/104), 5 cases of the Klinefelter syndrome (4.80%, 5/104), 33 cases of polymorphism (31.7%, 33/104), and 23 cases of abnormal chromosome structure (22.1%, 23/104) (Table 1).

Among the 472 cases with normal karyotype analysis, 67 cases were abnormal CNV, 16 cases (23.9%, 16/67) had clear pathogenicity, 18 cases (26.9%, 18/67) had polymorphism, and 33 cases had no clinically meaningful significance (49.3%, 33/67). Through analysis, it is found that 16 additional cases (3.40%, 16/472) of pathogenic CNV abnormalities can be detected by CMA technology in high-risk pregnant women (Table 2).

### 4. Discussion

Academically, structural variations above 1 kb in the DNA genome, including microdeletions and microduplications, are collectively named genome copy number variation (CNV). Such submicroscopic structural changes are also abnormalities in CNV, which cannot be passed through conventional karyotype analysis technology to distinguish [13]. As a high-resolution, high-efficiency molecular biology detection method, CMA can detect small fragments <50 bp that cannot be detected by traditional chromosome karyotypes, and the mutation detection rate is significantly improved.

The results of this study showed that the incidence of chromosome abnormalities accounted for 18.1% (104/576), the incidence of normal chromosome karyotypes and genome copy number variation was 11.6% and 14.2% (67/472), among which the pathogenic chromosomal microdeletions and microduplications accounted for 3.39% (16/472), and the report rate of unclear clinical significance was 4.87% (23/472). Based on chromosome karyotype analysis combined with CMA, 16 cases of karyotype-negative fetuses were detected with a chromosomal microstructural variation. The detection rate of pathogenic CNV abnormalities increased by 3.40%. This result was consistent with previous reports that CMA testing can significantly increase the mutation rate of chromosomal diseases. HILLMAN et al. [14] reported that the CMA confirmed that fetal cases with a normal chromosomal karyotype but abnormal ultrasound structure had a pathogenic rate higher than 3% to 5%.

For reports with unclear clinical significance, prenatal genetic counseling for high-risk pregnant women will generally confuse clinicians and pregnant women. Therefore, this study hopes to reduce the rate of reports with unclear clinical significance. In 2012, Wapner et al. [15] published a research article showing that the incidence of CNV with unclear clinical significance was about 2.5%.
2018, the research article changed the reporting rate of CNV with unclear prenatal clinical significance from a 2.5% decline to 0.9% [16]. In the results of this study, the report rate of unclear clinical significance is 4.87%, which is much higher than the report of Wapner et al. [16]. This will be our next task. We will continue to accumulate a large number of clinical case studies in order to provide more reliable data for prenatal genetics, counseling, and clinical screening.

Application of CMA in prenatal consultation CMA technology can be described as a “double-edged sword” for prenatal diagnosis. The sensitivity to small fragments increases the positive rate of test results, reduces the birth of children with chromosomal defects, and can be used as a means of genetic evaluation and used for assisted reproduction. However, the clinical significance is unknown, and the results of complex mutations can cause anxiety in pregnant and lying-in women, and even terminate the pregnancy by mistake. In this study, a total of 16 copy variant sites were detected. After database comparison and analysis and parental chromosome comparison, the CMA test results were classified into pathogenic, possibly pathogenic, unknown significance, and possibly benign classification [3].

Table 2: 16 cases of abnormal detection of CNV with normal karyotype.

| Case | Chromosome location | Microdeletions, microduplications | Karyotype |
|------|---------------------|----------------------------------|-----------|
| 1    | Chromosome2         | 2q31.2518 kb duplicate (verified from father) | 46, XN    |
| 2    | Chromosome2         | 2P 509 kb missing                | 46, XN    |
| 3    | Chromosome22        | 22q11.21 1.3 mb repeat           | 46, XN    |
| 4    | Chromosome20        | 20q13.13 209 kb duplicate        | 46, XN    |
| 5    | Chromosome11        | 11q14.3 371 kb missing           | 46, XN    |
| 6    | Chromosome15        | 15q11.2 1.04 mb repeat           | 46, XN    |
| 7    | Chromosome4         | 4p15.1p14 913 kb microdupliclate | 46, XN    |
| 8    | Chromosome5         | 5p 854 kb repeat; 16 to 718 kb deletion | 46, XN    |
| 9    | Chromosome8         | 8p2.25M repeat                   | 46, XN    |
| 10   | Chromosome12        | 12P11.22 224 kb duplicate        | 46, XN    |
| 11   | Chromosome18        | 18p11.3 repeat 497 kb           | 46, XN    |
| 12   | Chromosome5         | arr5q21.1 (315 kb repeat), arr5q33.3 (228 kb repeat), arr5q32.1 (519 kb repeat) | 46, XN    |
| 13   | Chromosome21        | Trisomy 21 repeats               | 46, XN    |
| 14   | Chromosome11        | 11P11.12 repeat 878 kb          | 46, XN    |
| 15   | Chromosome13        | 13q33.1 missing 323 kb          | 46, XN    |
| 16   | Chromosome18        | 18P11.21 repeat 509 kb          | 46, XN    |

**Abbreviations**

NIPT: Non-invasive prenatal testing
CNVs: Copy number variations
CMA: Chromosomal microarray analysis
aCGH: Array-based comparative genomic hybridization
SNP: Single nucleotide polymorphism microarray arrays
ACOG: American Society of Obstetricians and Gynecologists.
SMFM: Society of Maternal and Fetal Medicine.

**Data Availability**

Raw data are archived at the Prenatal Diagnosis Center, Guizhou Provincial People’s Hospital, Guiyang, China. Data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Ethical Approval**

Ethical approval for this study was obtained from the Guizhou Provincial People’s Hospital Ethical Review Authority. Participants gave their informed consent for study participation by submitting the survey. The study was performed in accordance with the Declaration of Helsinki, and all methods were performed in accordance with the relevant guidelines and regulations.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Minmin Jiang, Shengwen Huang, and Xingwei Ma conceptualized the study design. Ping Xie, Lingyan Ren, Qian Jin, and Keyan Linghu collected the data. Minmin Jiang performed the analysis and wrote the manuscript. Drafts were critically discussed and revised by all authors. All
authors approved the submission. All authors read and approved the final manuscript.

Acknowledgments

This study was supported by the Guizhou Provincial Health and Family Planning Commission Science and Technology Fund Project (gzwjki2017-1-065) and Guizhou Provincial People’s Hospital Youth Fund (GZSYQN [2016] No. 12).

Supplementary Materials

Figure S1: Images of ultrasonography about an omphalocele in trisomy. Figure S2: Images of amniotic fluid cell karyotype analysis (Left 47, XN+21, Right 47, XYY). (Supplementary Materials)

References

[1] M. Badeau, C. Lindsay, J. Blais, L. Nshimyumukiza, Y. Takwoingi, and F. Rousseau, “Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women,” *Cochrane Database of Systematic Reviews*, vol. 1, no. 11, pp. 374–383, 2019.
[2] N. A. Batzir, M. Shohat, and I. Maya, “Chromosomal microarray analysis (CMA) a clinical diagnostic tool in the prenatal and postnatal settings,” *Pediatric Endocrinology Reviews*, vol. 13, no. 1, pp. 448–454, 2015.
[3] Clinical Genetics Group of the Medical Genetics Branch of the Chinese Medical Association, “Expert consensus on the application of low-depth whole-genome sequencing technology in prenatal diagnosis,” *Chinese Journal of Medical Genetics*, vol. 29, no. 4, 2019.
[4] S. B. Hay, T. Sahoo, M. K. Travis et al., “ACOG and SMFM guidelines for prenatal diagnosis: is karyotyping really sufficient?” *Prenatal Diagnosis*, vol. 38, no. 3, pp. 184–189, 2018 Feb.
[5] Y. Zhang, M. Zhong, and D. Zheng, “Chromosomal mosaicism detected by karyotyping and chromosomal microarray analysis in prenatal diagnosis,” *Journal of Cellular and Molecular Medicine*, vol. 25, no. 1, pp. 358–366, 2021.
[6] T. Hu, T. Tian, Z. Zhang et al., “Prenatal chromosomal microarray analysis in 2466 fetuses with ultrasonographic soft markers: a prospective cohort study,” *American Journal of Obstetrics and Gynecology*, vol. 224, no. 5, pp. S16.e1–16, 2021.
[7] N. Thakur, M. Kupani, R. Mannan, A. Pruthi, and S. Mehrotra, “Genetic association between CDKN2B/CDKN2B-AS1 gene polymorphisms with primary glaucoma in a North Indian cohort: an original study and an updated meta-analysis,” *BMC Medical Genomics*, vol. 14, no. 1, pp. 1–11, 2021.
[8] Y. Jin, *Microarrays in Prenatal Diagnosis*, Inner Mongolia Medical University, Hohhot, China.
[9] R. G. Sinkey and A. O. Odibo, “Cost-effectiveness of old and new technologies for aneuploidy screening,” *Clinics in Laboratory Medicine*, vol. 36, no. 2, pp. 237–248, 2016.
[10] “Application of chromosome microarray analysis technology in prenatal diagnosis collaborative group. Expert consensus on the application of chromosome microarray analysis technology in prenatal diagnosis,” *Chinese Journal of Obstetrics and Gynecology*, vol. 49, no. 8, pp. 570–573, 2014.
[11] S. Li, *Prenatal Ultrasound Diagnosis of Fetal Malformations*, pp. 123–589, People’s Military Medical Press, Beijing, China, 2004.
[12] J. Zhou, T. Jiang, and L. I. Li, “Utilizing amniotic fluid cell karyotype analysis and array-CGH to confirm NIPT results and discuss the clinical significance,” *Journal of Practical Obstetrics and Gynecology*, vol. 314 pages, 2015.
[13] F. Fiorentino, S. Napoletano, F. Caiazzo et al., “Chromosomal microarray analysis as a first-line test in pregnancies with a priori low risk for the detection of submicroscopic chromosomal abnormalities,” *European Journal of Human Genetics*, vol. 21, no. 7, pp. 725–730, 2013.
[14] T. Miyamoto, G. Minase, T. Shin, H. Ueda, H. Okada, and K. Sengoku, “Human male infertility and its genetic causes,” *Reproductive Medicine and Biology*, vol. 16, pp. 81–88, 2017.
[15] R. J. Wapner, C. L. Martin, B. Levy et al., “Chromosomal microarray versus karyotyping for prenatal diagnosis,” *New England Journal of Medicine*, vol. 367, no. 23, pp. 2175–2184, 2012.
[16] M. Stosic, B. Levy, and R. Wapner, “The use of chromosomal microarray analysis in prenatal diagnosis,” *Obstetrics and Gynecology Clinics of North America*, vol. 45, no. 1, pp. 55–68, 2018.