

OBSTETRICS

Prenatal chromosomal microarray analysis in fetuses with congenital heart disease: a prospective cohort study

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BACKGROUND: Currently, chromosomal microarray analysis is considered the first-tier test in pediatric care and prenatal diagnosis. However, the diagnostic yield of chromosomal microarray analysis for prenatal diagnosis of congenital heart disease has not been evaluated based on a large cohort.

OBJECTIVE: Our aim was to evaluate the clinical utility of chromosomal microarray as the first-tier test for chromosomal abnormalities in fetuses with congenital heart disease.

STUDY DESIGN: In this prospective study, 602 prenatal cases of congenital heart disease were investigated using single nucleotide polymorphism array over a 5-year period.

RESULTS: Overall, pathogenic chromosomal abnormalities were identified in 125 (20.8%) of 602 prenatal cases of congenital heart disease, with 52.0% of them being numerical chromosomal abnormalities. The detection rates of likely pathogenic copy number variations and variants of uncertain significance were 1.3% and 6.0%, respectively. The detection rate of pathogenic chromosomal abnormalities in congenital heart disease plus additional structural anomalies (48.9% vs 14.3%, $P < .0001$) or intrauterine growth retardation group (50.0% vs 14.3%, $P = .044$) was significantly higher than that in isolated congenital heart disease group.

Additionally, the detection rate in congenital heart disease with additional structural anomalies group was significantly higher than that in congenital heart disease with soft markers group (48.9% vs 19.8%, $P < .0001$). No significant difference was observed in the detection rates between congenital heart disease with additional structural anomalies and congenital heart disease with intrauterine growth retardation groups (48.9% vs 50.0%), congenital heart disease with soft markers and congenital heart disease with intrauterine growth retardation groups (19.8% vs 50.0%), or congenital heart disease with soft markers and isolated congenital heart disease groups (19.8% vs 14.3%). The detection rate in fetuses with congenital heart disease plus mild ventriculomegaly was significantly higher than in those with other types of soft markers (50.0% vs 15.6%, $P < .05$).

CONCLUSION: Our study suggests chromosomal microarray analysis is a reliable and high-resolution technology and should be used as the first-tier test for prenatal diagnosis of congenital heart disease in clinical practice.

Key words: chromosomal abnormalities, chromosomal deletion, chromosomal duplication, chromosomal microarray analysis, congenital heart disease, copy number variation, microdeletion, microduplication, prenatal diagnosis

Introduction

Congenital heart disease (CHD) is the most common birth defect, occurring in about 4-13 per 1000 live births¹ and in 10% of stillbirths.² Chromosomal abnormalities, single gene disorders, environmental teratogens, maternal exposures, and infectious agents are all considered to be the potential causes of CHD.³⁻⁶ Among these, chromosomal abnormalities account for approximately 20% of CHDs in prenatal diagnosis.^{7,8} With the development of medical and surgical treatments after birth, most types of CHD can be repaired to achieve normal heart function. However, when

combined with chromosomal abnormalities, the prognosis of fetuses with CHD would be poor due to severe extracardiac structural anomalies and/or postnatal neurodevelopmental disorders. Therefore, prenatal diagnosis of chromosomal abnormalities in fetuses with CHD is highly recommended.

G-banding karyotyping coupled with fluorescence in situ hybridization (FISH) is the predominant strategy applied for detecting chromosomal abnormalities in fetuses with CHD in clinical practice over the past few decades. However, G-banding karyotyping is time-consuming and limited by low-resolution while FISH is hampered by limited coverage on the whole genome.⁹ Chromosomal microarray analysis (CMA), which is capable of simultaneously detecting numerical chromosomal abnormalities and submicroscopic chromosomal imbalances at the whole-genome level, has been applied to identify chromosomal abnormalities in postnatal and prenatal

subjects with CHD.^{8,10-18} In 2013, the American Congress of Obstetricians and Gynecologists recommended CMA to pregnant women with fetal structural abnormalities and that this technique could replace karyotyping in prenatal diagnosis.¹⁹ However, most published reports include small cohorts and describe only the incremental yield of CMA. Prospective large-scale research focusing on CHD that applied CMA as the first-tier test in prenatal setting has scarcely been reported.

In this study, we conducted a prospective study to evaluate the clinical value of CMA as a prenatal diagnostic tool for a cohort of 602 fetuses with CHD. We also stratified the data on 602 cases to better understand the detection rates of chromosomal abnormalities for different types of CHDs. Furthermore, we compared the frequency of chromosomal abnormalities among fetuses with isolated CHD, CHD with soft markers, CHD with intrauterine growth

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retardation (IUGR), and CHD with additional structural anomalies.

Materials and Methods

Subjects

From January 2012 through February 2017, 602 pregnant women with fetal CHD detected by echocardiogram were referred to the Prenatal Diagnostic Center in Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University in China for CMA testing by invasive procedure. Pretest genetic counseling was carried out by trained clinical geneticists regarding the advantages and potential risks of CMA, including the possibility of findings of uncertain significance, nonpaternity, consanguinity, and adult-onset diseases. Written informed consent was supplied by all patients. Mean gestational age of mothers was 28.4 years (range, 22-42 years) and the mean age of fetuses at invasive procedure was 25.2 weeks (range, 21-31 weeks). In the 602 fetuses with CHD, 421 had isolated CHD, the other 181 had CHD plus other ultrasound anomalies, including structural anomalies ($n = 94$), soft markers ($n = 81$), and IUGR ($n = 6$). Mild ventriculomegaly (10-15 mm) was categorized as soft markers in this study. CHD was classified using a method described by Botto et al.²⁰ All fetal samples were collected by amniocentesis. Clear amniotic fluid samples were used for CMA testing directly, while blood-stained amniotic fluid samples were cultured before CMA testing. This work was approved by the Medicine Ethics Committee of Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University.

Chromosomal microarray analysis

Genomic DNA was extracted from 10-mL amniotic fluid samples using a QIAamp DNA mini kit (Qiagen, Hilden, Germany). Human cyto12 single nucleotide polymorphism (SNP) array (Illumina, San Diego, CA) comprising about 300,000 SNP probes with average marker spacing of roughly 1 probe every 10 kilobase (kb) was applied for the whole-genome scan. SNP array experiments were carried out as previously described²¹ and molecular karyotype analysis was

performed using software (KaryoStudio, Version 1.4.3.0; Illumina). Copy number variations (CNVs) were called at an effective minimal resolution of 100 kb involving at least 10 contiguous probes. Regions of allelic homozygosity (ROHs) were displayed at a threshold of 5 Mb.

Chromosomal mosaicism was determined by a combination of log R ratio and B-allele frequency²² and was reported when the detection threshold of 30% was exceeded. Regarding uniparental disomy, we reported only uniparental isodisomy in this study. Maternal cell contamination (MCC) was evaluated by B-allele frequency.²³ Significant MCC was defined as levels of MCC >30%. The CNVs were further classified based on their size. CNVs >10 Mb were defined as partial aneuploidy and CNVs <10 Mb were defined as chromosomal microdeletions/microduplications.

Detected CNVs were evaluated based on a scientific literature review and the following public databases: Database of Genomic Variants (<http://projects.tcag.ca/variation/>); Database of Genomic Variation and Phenotype in Humans Using Ensembl Resources (<http://decipher.sanger.ac.uk/>); University of California—Santa Cruz (<http://genome.ucsc.edu/>); and Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/omim>). Following the American College of Medical Genetics and Genomics standards and guideline for interpretation of copy number variants, chromosomal microdeletions/microduplications were classified into 5 categories: pathogenic CNVs, likely pathogenic CNVs, variants of uncertain significance (VOUS), likely benign CNVs, and benign CNVs. CNVs were defined as pathogenic if: (1) the CNV was documented as clinically significant in multiple peer-reviewed publications, regardless of its penetrance and expressivity; or (2) the CNV overlapped a smaller interval with clearly established clinical significance. CNVs were defined as likely pathogenic if: (1) the CNV was described in a single case report but with well-defined breakpoints and phenotype, both specific and relevant to the patient findings; or (2) a gene within the CNV interval had a very compelling gene

function that was relevant and specific to the reason for patient referral. CNVs with no genes in interval or described in a small number of cases in databases of variation in the general population were considered likely benign. CNVs coinciding with known polymorphic CNVs or reported in multiple peer-reviewed publications or databases as benign variants were considered benign. CNVs that did not fit any of the above criteria were considered as VOUS. In this study, we reported only pathogenic CNVs, likely pathogenic CNVs, and VOUS.

Parental study

If CNVs were detected in the fetus, parental samples were subsequently analyzed by karyotyping, FISH, or CMA according to the CMA result. Routine G-banding karyotyping was performed according to standard methods. FISH analysis was performed according to the manufacturer's protocols (VYSIS Inc, Downers Grove, IL) using commercially available subtelomeric specific probes.

Statistical analyses

Comparisons between groups were conducted using χ^2 test or Fisher exact test. A P value <.05 was defined as statistically significant in all the tests.

Results

Diagnostic yield of CMA testing for fetuses with CHD

We analyzed a total of 602 prenatal cases of CHD by SNP array. Overall, SNP array was performed on 595 uncultured amniotic fluid samples and 7 cultured amniotic fluid samples due to maternal blood contamination. None of these cases was identified with significant MCC. The overall diagnostic yield of CMA testing for fetuses with CHD was 20.8% (125/602). When taking likely pathogenic CNVs into account, the detection rate was 22.1% (133/602). VOUS were obtained in 36 cases (6.0%) (Supplementary Table 1). The types of CHD and the detection rates of pathogenic findings for fetuses with CHD in different groups are summarized in the Figure 1, Table 1, and Supplementary Table 2.

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