

Effectiveness of Chromosomal Microarray Analysis for Prenatal Diagnosis of Fetal Echogenic Intracardiac Focus: A Single-Center Experience

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Background: Echogenic intracardiac focus (EIF) is a common ultrasound finding during pregnancy. However, the correlation between fetal EIF and cardiac abnormality remains in dispute until now. The study aimed to examine the association of fetal EIF with chromosomal abnormality by means of chromosomal microarray analysis (CMA).

Materials and Methods: A total of 192 pregnant women with fetal EIF undergoing amniocentesis or umbilical cord blood puncture were recruited and assigned into groups A (8 cases with isolated EIF alone), B (75 cases with EIF and other cardiac malformations) and C (109 cases with EIF and extracardiac malformations). All fetuses underwent karyotyping analysis and CMA simultaneously. The detection of chromosomal abnormality and copy number variations (CNVs) were compared.

Results: Chromosomal karyotyping identified 5 fetuses with chromosomal abnormality, including 3 cases with trisomy 21, one fetus with Turner's syndrome, and one fetus with chromosome 8 mosaicism, while CMA detected 6 additional fetuses with CNVs, including 2 fetuses with pathogenic CNVs and 4 fetuses with variants of uncertain significance (VOUS). There was no significant difference among groups A (0), B (5.33%) and C (6.42%) in terms of the prevalence of chromosomal abnormality ($P > 0.05$). Among the 4 fetuses with VOUS, pregnancy continued in 2 fetuses, and pregnancy was terminated in other 2 fetuses.

Conclusion: An isolated EIF may not correlate with chromosomal abnormality. However, CMA is recommended in fetuses with CMA complicated by other abnormal cardiac ultrasound findings, which facilitates the prediction of fetal outcomes during the genetic counseling and precision assessment of prognosis.

Keywords: echogenic intracardiac focus, chromosomal microarray analysis, copy number variation, prenatal diagnosis, pregnant outcome

Introduction

Echogenic intracardiac focus (EIF), a common ultrasound finding during pregnancy, is described to be a local echogenic area appearing in single-sided or bilateral cardiac cavity.¹⁻³ It is estimated that EIF is detected in 5% of second-trimester ultrasound examinations.⁴⁻⁶ Previous studies have demonstrated that fetal EIF is associated with an increased risk of Down syndrome and trisomy 13 (T13),⁷⁻⁹ and fetuses with an EIF may have other structural cardiac malformations.¹⁰ The identification of an EIF by ultrasonography is an incentive for further prenatal diagnoses to evaluate the risk of fetal outcomes.¹¹ Chromosomal karyotyping analysis has been found to fail to detect copy number variations (CNVs) in small fragments, and chromosomal

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microarray analysis (CMA) may be used as a supplement to conventional karyotyping.^{12–14} This study aimed to examine the association between fetal EIF and the risk of chromosomal abnormality, and our data may provide insights into prenatal diagnosis and genetic counseling in fetuses with an EIF.

Subjects and Methods

Subjects

A total of 192 pregnant women with fetal EIF undergoing amniocentesis or umbilical cord blood puncture at the Center for Prenatal Diagnosis of Fujian Provincial Maternity and Children's Hospital (Fuzhou, China) from December 2015 to December 2018 were recruited. The pregnant women had a mean age of 29 years (range, 21 to 41 years) and gestational ages of 18+2 to 34+5 weeks. All pregnant women had no medical history of hypertension or diabetes, and all women had no viral infections or were exposed to known teratogens during pregnancy. All fetuses were assigned into three groups. Fetuses in Group A had isolated EIF alone ($n = 8$), and those in Group B had EIF and other cardiac malformations ($n = 75$), while fetuses with EIF and abnormal extracardiac ultrasound findings were assigned to Group C ($n = 109$).

Karyotype Analysis

Amniotic fluid (122 samples) and umbilical cord blood samples (70 samples) were collected from the 192 fetuses through B-mode ultrasound-guided abdominal puncture, amniocentesis and amniocentesis, respectively. All prenatal samples were routinely cultured, mounted on slides and subjected to G-banding karyotype analysis on a GSL-120 Streamlines Cytogenetic Analysis System (Leica Microsystems; Mannheim, Germany). At least 40 karyotypes were counted for each case, and 5 karyotypes were randomly selected for analysis.

CMA Detection

CMA detection was performed as described previously.¹⁵ Briefly, approximately 10 mL of amniotic fluid was sampled from each fetus and centrifuged, and the sediment was collected. Genomic DNA was extracted from amniotic fluid cells using the QIAampDNA Blood Mini Kit (Qiagen; Hilden, Germany), digested, amplified, purified, fragmented, labeled and hybridized to the array on the Affymetrix SNP Array 6.0 (Affymetrix; Santa Clara, CA, USA). The CytoScan HD array, which includes the copy

number variation (CNV) probe and SNP probe, may detect CNV, mosaic (mosaic proportion >10%) and loss of heterozygosity (LOH). All data analyses were performed using the software Chromosome Analysis Suite (ChAS) version 3.2 (Affymetrix; Santa Clara, CA, USA), and the interpretation of CNVs was identified using online public databases, including the database of genomic variants (DGV, <http://projects.tcag.ca/variation>), the DECIPHER database (<https://decipher.sanger.ac.uk/>), the OMIM database (<http://www.omim.org>), the International Standards for Cytogenomic Arrays (ISCA) Consortium and Public Database (<https://www.iscaconsortium.org/>), the CAGdb database (<http://www.cagdb.org/>), the CHDWiki database and the NCBI database. CNV was classified as pathogenic, variants of uncertain significance (VOUS) and benign, and pathogenic CNVs detected by CMA were further validated using fluorescence in situ hybridization (FISH) assay. Peripheral blood was sampled from the parents of the fetus with VOUS for CMA detection, and the type of CNV was identified by means of CMA and pedigree analysis.

Ethical Statement

This study was approved by the Ethics Review Committee of Fujian Provincial Maternity and Children's Hospital. All procedures were performed following the Declaration of Helsinki, as well as international and national laws, guidelines and regulations. All pregnant women and their spouses received prenatal genetic counseling and signed informed consent with a detailed description of invasive diagnosis and CMA.

Statistical Analysis

All statistical analyses were conducted using the statistical software SPSS version 20.0 (SPSS, Inc.; Chicago, IL, USA). The detection of CNVs was compared among groups using chi-square test or Fisher's exact test, with a P value of <0.05 indicative of statistical significance.

Results

Karyotype Analysis

Among the 192 fetuses with EIF, chromosomal karyotyping identified 5 cases with chromosomal abnormality (2.6% prevalence), including 3 fetuses with T21, one fetus with Turner's syndrome, and one fetus with chromosome 8 mosaicism.

Fetal Chromosomal Abnormality by CMA

CMA was successfully performed in all 192 samples, and the overall prevalence of chromosomal abnormality was 5.73%, which included chromosomal abnormality (5 fetuses) and CNVs (6 fetuses). Inconsistent ratio of chromosome 8 mosaicism was detected between karyotyping (47,XY,+8[5]/46,XY[140]) and CMA (8p23.3q24.3(158,-048-146,295,771)×2-3mosaicism), and the six fetuses with CNVs included 2 fetuses with pathogenic CNVs and 4 fetuses with VOUS (Table 1).

Detection of CNVs in Different Groups

The rate of CNVs detected by CMA in group A, B and C was 0/8 (0%), 4/75 (5.33%), 7/109 (6.42%), respectively. In addition, there was no significant difference in the detection of CNVs among groups A, B and C ($P > 0.05$) (Table 2).

Fetal Follow-Up Outcomes

The pregnancy was terminated in 5 fetuses with abnormal chromosome numbers. Of the 4 fetuses with VOUS, pregnancy termination was done in two fetuses (case numbers 4 and 6), and pregnancy continued in the other two fetuses (case numbers 2 and 5), both having good development after birth. Of the remaining 2 fetuses with pathogenic CNVs, one was terminated for pregnancy and one had no abnormal phenotypes 7 months after birth (Table 2).

Discussion

As an ultrasound soft marker, the prevalence of EIF is estimated to be 2% to 6% on fetal echocardiography.^{7,16} Previous studies have shown a high rate of fetal cardiac abnormality if EIF occurs in the left or right ventricle alone, or in bilateral ventricles.^{10,17-19} However, fetal EIF was also found to be not associated with a significant increase in the risk of structural cardiac abnormality.²⁰ Precision diagnosis of EIF by echocardiography is therefore of great importance for the decision of pregnancy.¹¹

EIF is most likely to gradually weaken, shrink and even disappear with the increase in the gestational age.¹ In this study, all fetuses with an isolated EIF were followed up, and no severe diseases or symptoms were seen. This is not in agreement with previous studies showing a correlation between fetal EIF and congenital heart diseases.^{10,21,22} Currently, karyotyping, FISH, multiplex ligation-dependent probe amplification (MLPA) and

CMA are major tools used to detect the chromosomal abnormality in fetuses with ultrasound soft markers.^{23,24}

Chromosomal karyotype analysis is limited by the success rate of culture and the resolution, while CMA is effective to detect abnormality of chromosomal fragments with >1 kb in size and microduplication and microdeletion syndromes.²⁵ The introduction and developments of CMA allow the detection of chromosomal abnormality which is not identified by karyotyping, and such an assay has been extensively employed in the diagnosis of fetal growth restriction and retardation, mental disorder, autism and multi-organ malformations.²⁶⁻²⁸

In the current study, we employed CMA to detect 192 fetuses with EIF, and no chromosomal abnormality was found in fetuses with EIF alone, which may be associated with the small sample size of the isolated EIF. In addition, CMA detected a 5.33% prevalence rate of chromosomal abnormality in Group B and 6.42% in Group C, with no significant difference seen between these two groups ($P > 0.05$). Our data demonstrate that EIF is not associated with an increase in the risk of fetal cardiac malformations and shows no significant correlations with fetal structural cardiac abnormality. In this study, CMA showed a 5.73% overall detection of chromosomal abnormality, which increased by 3.13% as compared to karyotype analysis, and among fetuses with normal karyotypes, CMA still identified chromosomal microdeletions/microduplications, and pathogenic CNVs. Previous studies have proved that CMA is feasible to improve the detection of chromosomal abnormality and may serve as a supplement for conventional karyotype analysis during the prenatal diagnosis.¹²⁻¹⁴ Taken together, CMA is therefore strongly recommended for prenatal diagnosis of EIF, which may provide more accurate genetic proof for prenatal counseling and prediction of fetal outcomes.¹²

In the present study, CMA detected chromosomal mosaicism in a fetus and CNVs in 6 fetuses with EIF, in addition to aneuploidy. CMA detected a mosaicism of the 8p23.3q24.3 in one case, which is reported to manifest mental retardation, cardiac malformation and dysgenesis of the corpus callosum;²⁹ however, there are also fetuses with a mosaicism of the 8p23.3q24.3 presenting normal phenotypes.³⁰ Mosaicism remains a major concern during the prenatal genetic counseling.³¹ Since mosaic cells are present in different embryonic layers of diverse proportions, and fetuses with a mosaicism present a wide range of clinical phenotypes, it is very difficult to predict the pregnant outcomes of fetuses with a low-level

Table 1 Demographic, Clinical Features and Pregnant Outcomes of 7 Fetuses with Chromosomal Abnormalities

Group	Patient Number	Age (Years)	Gestational Age (Weeks)	Ultrasound Findings	Karyotype	CMA Findings	Clinical Significance	Pregnant Outcome
B	1	26	28	Suspected fetal ventricular septal defect, and left-ventricular echogenic focus	Normal	arr[hg19]16p13.11 (14,897,401–16,534,031)×1	16p13.11 microdeletion, and presence of incomplete penetrance. The clinical phenotype may include cognitive dysfunction and developmental retardation	Normal delivery (7 months)
	2	36	24	Smaller fetal biparietal diameter and head circumference for the gestational age, increased heart rate, left-ventricular echogenic focus, tricuspid regurgitation and local echogenic bowel	Normal	arr[hg19]18q21.33q22.1 (60,147,532–65,974,912)×1	VOUS	Normal delivery
	3	34	24	Fetal left- and right-ventricular echogenic focus, mitral regurgitation and tricuspid regurgitation	Normal	arr[hg19]Xp22.33 or Yp11.32 (168,551–629,999) or 118,551–579,999)×1	Definite pathogenicity, and development of microsomia at difference degrees, short forearm and arched radius	No delivery
C	4	25	24	Maternal mental retardation, fetal left- and right-ventricular echogenic focus and separation of right renal sinus	Normal	arr[hg19]6p21.32p21.31 (32,965,747–35,234,269)×3	VOUS	Induction of labor
	5	28	24	Fetal nasal bone hypoplasia, biparietal diameter and head circumference shorter for less than 2 standard deviation of the gestational age, left-ventricular echogenic focus, normal non-invasive prenatal testing, and risk ratio of 1:378 by serological screening	Normal	arr[hg19]Xq21.33 (95,227,256–95,972,695)×3	VOUS	Normal delivery (16 months)
	6	32	25	Fetal growth restriction, and left-ventricular echogenic focus	Normal	arr[hg19]22q11.21 (18,512,066–19,004,772)×3	VOUS	Induction of labor

Table 2 Detection of CNVs in Fetuses with Diverse Ultrasound Findings

Group	Total Number	No. of Fetus with CNVs	Pathogenic CNVs	VOUS
A	8	0	–	–
B	75	4	3	1
C	109	7	4	3
Total	192	11	7	4

Abbreviations: CNV, copy number variation; VOUS, variant of uncertain significance.

T8mosaicism in amniotic fluid or umbilical cord blood samples.³² Previous studies have reported that a chromosome 8 mosaicism has a primary ultrasound feature of intra-uterine growth retardation or shows no remarkable phenotype.³⁰ The fetus with a chromosome 8 mosaicism detected by CMA in this study presented with multiple cardiac abnormalities, which is in agreement with the clinical phenotype caused by a mosaicism, suggesting that cardiac abnormality may be indicative of a chromosome 8 mosaicism during the prenatal diagnosis. In addition, we detected an Xp22.33 or Yp11.32 microdeletion in Case 3, and such a microdeletion is likely to be associated with the *SHOX* gene.³³ Loss of the *SHOX* gene function may cause microsomia, Madelung's deformity of the wrist, joint movement restriction and Leri-Weill dyschondrosteosis.³⁴

Although CMA has shown great values in prenatal diagnosis, this assay may detect VOUS, pathogenic CNVs in adulthood, and disease carriers.³⁵ Results from the NICHD trial showed that CMA identified 1.8% of the study fetuses with possible benign CNVs and 1.6% with possible pathogenic CNVs.³⁶ This VOUS may be attributed to rare or new CNVs, or due to variable penetrance.³⁷ In this study, CMA identified a 1.6 Mb deletion on the 16p13.11 region. This region is overlapped with the 16p13.11 recurrent region (including the *MYH11* gene), which contains susceptible foci of neurocognitive disorders.³⁸ Patients with a chromosome 16p13.11 microdeletion syndrome mainly manifest bradykinesia, facial deformity, microcephaly, gastroesophageal reflux disease (GERD) and congenital heart defects, and the carriage of chromosome 16p13.11 microdeletions is less than 1% in normal populations.³⁹ Similar cases have been reported in DGV, DECIPHER and ISCA databases, which show the definite pathogenicity of 16p13.11 microdeletions; however,

16p13.11 microdeletion is also considered as VOUS or benign and incomplete clinical penetrance. Our follow-up results showed no obvious abnormality in this fetus 7 months after birth, which may be attributed to the benign CNVs or limitation of the follow-up period that is insufficient to identify the development of potential neurocognitive disorders. In the current study, we detected that Case 6 carried 22q11.21 microduplications, which is partially overlapped with the chromosome 22q11.2 recurrent (DGS/VCFS) region (proximal, A-B); however, this region did not contain key pathogenic genes. The frequency of 22q11.21 microduplication is very likely to be higher than that of 22q11.2 microdeletion in normal populations, and this microduplication is approximately 3 Mb in size, which has clinical phenotypes of cardiac defects, velopharyngeal insufficiency, hearing impairment, growth retardation, cognitive deficits, behavioral abnormality, epilepsy and facial deformity.⁴⁰ In this study, Case 6 had the ultrasound findings associated with the phenotype of CNVs, and we found no insufficient pathogenic proof; however, the likelihood of pathogenicity cannot be excluded. Due to the presence of VOUS, many microduplications and microdeletions of unknown clinical significance require further pedigree analyses to validate their associations with pathogenicity. However, many patients refuse to undergo further pedigree analyses due to economic factors, which is a concern during the prenatal diagnosis.

Conclusions

In summary, CMA is effective to improve the detection of chromosomal abnormality, and shows a high sensitivity for detection of chromosomal microdeletions or microduplications. The results of the present study demonstrate a low proportion of chromosomal abnormality in fetuses with an isolated EIF. However, CMA is recommended in fetuses with CMA complicated by other abnormal cardiac ultrasound findings, which facilitates the prediction of fetal outcomes during the genetic counseling and precision assessment of prognosis.

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Disclosure

The authors declare no competing interests.

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