

Performance of Chromosomal Microarray Analysis for Detection of Copy Number Variations in Fetal Echogenic Bowel

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Background: Fetal echogenic bowel (FEB) is associated with an increased risk of poor pregnant outcomes; however, karyotyping fails to detect copy number variations (CNVs) in FEB. This study aimed to evaluate the performance of chromosomal microarray analysis (CMA) for detection of FEB.

Methods: The medical records of 147 pregnant women with FEB recruited during December 2015 to December 2018 were retrospectively reviewed, and prenatal samples were collected for karyotyping and CMA. The detection of chromosomal abnormality was compared between karyotyping and CMA.

Results: Karyotyping identified eight cases with abnormal karyotypes (5.44% prevalence), including four fetuses with pathogenic aneuploidy, three with chromosome polymorphism and one with balanced chromosome translocation. CMA identified 13 abnormal CNVs (8.84% prevalence), including 4 fetuses with pathogenic aneuploidy as detected by karyotyping and 9 additional CNVs with normal karyotypes; however, CMA failed to detect chromosome polymorphism and balanced chromosome translocation. In fetuses with isolated FEB, no cases presented pathogenic findings and CMA detected two cases with variants of uncertain significance (VOUS). In cases presenting FEB along with other ultrasound abnormalities, CMA detected three cases with pathogenic CNVs and four cases with VOUS in addition to four cases with aneuploidy. There was no significant difference in the detection of abnormal CNVs between the fetuses with echogenic bowel alone and along with other ultrasound abnormalities (10% vs 8.67%, $P > 0.05$). Except 9 fetuses lost to the follow-up, the other 138 fetuses with echogenic bowel were successfully followed up. Pregnancy was terminated in 5 fetuses with chromosomal abnormality, 2 with pathogenic CNVs and 1 with VOUS, and other 16 with normal karyotypes and CMA findings but showing ultrasound abnormalities or multiple malformations.

Conclusion: Isolated FEB is associated with a good prognosis, and a satisfactory pregnant outcome is expected for fetuses with echogenic bowel that are negative for chromosomal anomalies and other severe structure abnormalities. CMA shows an important value in the genetic diagnosis of FEB. As a supplement to karyotyping, CMA may improve the accuracy of prenatal diagnosis of fetal intestinal malformations in pregnant women with FEB.

Keywords: fetal echogenic bowel, chromosomal microarray analysis, karyotype analysis, copy number variation, prenatal diagnosis

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Introduction

Fetal echogenic bowel (FEB) refers to an abnormality of the fetal bowel on ultrasound scans during the pregnancy, in which the bowel has brightness similar to that seen in bone.¹ This disorder is estimated to occur in approximately 1% of total pregnancies.² The majority of FEB is considered as non-specific ultrasound features, which may attribute to intestinal hypoperistalsis.³ However, this disorder is also reported to be linked with an increased risk of chromosomal abnormality, intrauterine infections, cystic fibrosis, congenital bowel abnormality, intrauterine growth restriction, and fetal mortality.⁴ In addition, the clinical significance of FEB is reported to vary in the echogenic intensity,⁵ and higher echogenic intensity predicts poorer pregnant outcomes.⁶

Currently, it is difficult to identify FEB as pathogenic or benign by ultrasound alone, and the identification requires the addition of medical history and laboratory examinations.⁷ In addition, early detection of severe fetal abnormality may facilitate the prenatal counseling and the decision to terminate the pregnancy, and a test with a higher diagnostic efficiency is required in fetuses diagnosed with abnormal ultrasound findings and a normal karyotype.⁸

Recently, chromosomal microarray analysis (CMA), a high-throughput assay that is effective to detect chromosomal deletions and duplications, has been widely employed in prenatal diagnosis.^{9–11} Unlike karyotyping that detects the abnormality of chromosomal fragments with a size of >10 Mb, CMA is sensitive to identify chromosomal microdeletion and microduplication syndromes.¹² However, the detection of variants of uncertain significance (VOUS) by CMA challenges the prenatal counseling.¹³ The purpose of the present study was to evaluate the performance of CMA for detection of copy number variations (CNVs) in FEB.

Subjects and Methods

Study Subjects

A total of 147 pregnant women with FEB detected by ultrasound at Fujian Maternity and Child Health Hospital (Fuzhou, China) during the period from December 2015 through December 2018 were recruited. The pregnant women had a mean age of 29 years (range, 20 to 42 years) and a mean gestational age of 25.7 weeks (range, 18 to 34 weeks). According to ultrasound findings, the study subjects were classified into cases with FEB alone

(n = 20) and cases presenting FEB along with other ultrasound abnormalities (n = 127) ([Supplementary File 1](#)). Amniotic fluid and umbilical cord blood samples were collected from the study subjects for karyotyping and CMA.

Karyotype Analysis

Chorionic villus, amniotic fluid and umbilical cord blood samples were collected 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 (additional C-banding and N-banding if required). Karyotype analysis was performed 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

Approximately 10 mL of amniotic fluid was sampled 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, includes the 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 CNV, which was classified as pathogenic, VOUS and benign, 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. The pathogenic CNVs detected by the SNP array were validated using fluorescence in situ hybridization (FISH) assay. Peripheral blood was sampled from the parents of the fetus with VOUS for the SNP array, and the type of

CNV was identified by means of the SNP array and pedigree analysis.

Statistical Analysis

All statistical analyses were performed using the statistical software SPSS version 22.0 (SPSS, Inc.; Chicago, IL, USA). Differences of proportions were tested for statistical significance with a chi-square test, and a *P* value of <0.05 was considered statistically significant.

Ethical Statement

This study was approved by the Ethics Review Committee of Fujian Maternity and Child Health Hospital. All procedures were performed following the Declaration of Helsinki, as well as international and national laws, guidelines and regulations. Signed informed consent was obtained from all subjects with a detailed description of the purpose of the study.

Results

Chromosomal Karyotypes

Karyotype analysis was successfully performed in 147 prenatal samples. Karyotype analysis identified eight cases with abnormal karyotypes (5.44% prevalence), including four fetuses with aneuploidy (one case with T21, two cases with 47,XXY and one case with small supernumerary marker chromosome (47,XY,+mar)), three fetuses with chromosome polymorphism (two fetuses with 46,XX,inv(9)(p12q13) and one fetus with 46,inv(Y)(p12q13)), and one fetus with balanced chromosome translocation ((46,XY,t(10;17)(q26;p11.2)).

CMA Findings

CMA was successfully performed in 147 prenatal samples, and 13 abnormal CNVs were identified (8.84% prevalence), including 4 fetuses with pathogenic aneuploidy as detected by karyotyping. Of the fetus detected with the small supernumerary marker chromosome (47,XY,+mar) by karyotyping, CMA identified a 4.6 Mb duplication at the p12.1p11.1 region of the chromosome 3 and a 1.6 Mb duplication at the 11.1q11.2 region of the chromosome 3, which may be a novel pathogenic mutation. Moreover, CMA detected nine additional CNVs with normal karyotypes; however, CMA failed to detect chromosome polymorphism and balanced chromosome translocation due to no loss of chromosomal materials. The size of the detected abnormal CNVs was 0.708 to

19.2 Mb in fetuses with echogenic bowel, and three fetuses with pathogenic CNVs (a case with 10q11.1q11.22 microdeletion, a case with 16p13.11 microdeletion and a case with Xq28 microduplication) and six fetuses with VOUS (a case with 16p13.11 microduplication, a case with 16p13.11 microdeletion, a case with 18q21.33q22.1 microdeletion, a case with 9p21.1 microdeletion, a case with 5q33.2q33.3 microduplication, and a case with LOH at the q23.2q24.3 region) were detected (Table 1).

In cases with isolated FEB, no case presented pathogenic findings, and two cases were detected with VOUS. In cases presenting FEB along with other ultrasound abnormalities, there were seven cases with pathogenic CNV and four cases with VOUS. However, there was no significant difference in the prevalence of abnormal CNVs between the fetuses with isolated echogenic bowel and along with other ultrasound abnormalities (10% vs 8.67%, *P* > 0.05) (Table 2).

Follow-Up Outcomes

Except 9 fetuses lost to the follow-up, the other 138 fetuses with echogenic bowel were successfully followed up. Pregnancy was terminated in 5 fetuses with chromosomal abnormality, 2 fetuses with pathogenic CNVs, 1 fetus with VOUS and 16 fetuses with normal karyotypes and CMA findings but showing abnormal ultrasound findings or multiple malformations. In addition, the parents decided to continue the pregnancy in four fetuses with VOUS, and the postnatal follow-up showed well growth and development of the newborn (Table 3). In second trimesters, 96% cases had normal deliveries (52/54), which higher than third trimester (87%, 73/84).

Discussion

To date, the correlation between isolated FEB and chromosomal abnormality remains controversial.^{14,15} The incidence of gastrointestinal abnormalities was reported to be 4.76% in fetuses with antenatal echogenic bowel.¹⁶ A retrospective review of 682 cases of hyperechogenic fetal bowel collected from 22 molecular biology laboratories in France showed a 3.5% incidence rate of chromosomal anomaly in the case series (including 2.5% incidence of Down's syndrome and 1% incidence of other severe chromosomal anomaly), 6.9% prevalence of multiple malformations, 3% prevalence of cystic fibrosis, and 2.8% prevalence of viral infections.¹⁷ Ultrasound screening of FEB is therefore of great clinical significance

Table 1 Abnormal CMA Detection Results Among Fetuses Presenting Echogenic Bowel on Ultrasound with Normal Karyotypes

Group	Case Number	CMA Detection Results	B-Mode Ultrasound Findings	Clinical Significance	Fragment Size (Mb)	Pregnant Outcomes
Fetal echogenic bowel alone	1	16p13.11 (151,771,146–16,309,046)×3 mat	Fetal echogenic bowel	VOUS	1.1	Normal
	2	5q33.2q33.3 (154,435,034–156,727,811)×3 pat	Fetal echogenic bowel	VOUS	2.29	Normal
Fetal echogenic bowel along with other ultrasound abnormality	3	10q11.1q11.22 (39,058,630–48,006,310)×1 dn	Fetal growth restriction, fetal echogenic bowel	Pathogenic	8.9	Induction of labor
	4	16p13.11 (14,897,401–16,534,031)×1	Fetal echogenic bowel and tricuspid regurgitation	VOUS	1.6	Normal
	5	16p13.11 (15,422,960–16,508,123)×1 dn	Fetal bilateral ventricle broadening and fetal echogenic bowel	Pathogenic	1.0	Induction of labor
	6	18q21.33q22.1 (60,147,532–65,974,912)×1 dn	Fetal biparietal diameter and head circumference smaller for gestational age, high heart rate, tricuspid regurgitation and fetal echogenic bowel	VOUS	5.8	Normal
	7	9p21.1 (28,552,246–30,820,392)×1	Fetal nuchal translucency of 2.7 mm, peritoneal effusion, small gastric bubble and echogenic bowel	VOUS	2.2	Induction of labor
	8	Xq28 (152,713,658–153,421,838)×3dn	Fetal ventricular septal defects, tricuspid regurgitation and echogenic bowel	Pathogenic	0.708	Induction of labor
	9	16q23.2q24.3 (79,800,878–90,146,366) hmz16p13.3p12.3 (94,807–19,302,326)hmz	Fetal growth restriction, fetal ventricular septal defects, aortic stenosis, dysplasia or absence of the left kidney and echogenic bowel	VOUS	10.3Mbfragment at q23.2q24.3 and 19.2 Mb fragment at p13.3p12.3	Induction of labor

Abbreviations: CMA, chromosomal microarray analysis; CNVs, copy number variations; VOUS, variants of uncertain significance.

during the first trimester, which is considered to correlate with fetal chromosomal abnormality.⁵

Previous studies have shown that isolated FEB is associated with a low risk of chromosomal abnormality, while FEB along with other ultrasound soft markers is associated with a remarkable increase in the risk of chromosomal anomaly.^{18,19} In this study, we detected 10% and 8.67% prevalence of abnormal CNVs in fetuses with echogenic bowel alone and along with other ultrasound abnormalities ($P > 0.05$), which was inconsistent with previous studies.^{18–20} This may be attributed to the addition of

CMA in our study, while karyotyping was performed in previous reports, or the small sample size of isolated fetal echogenic bowel. Further studies recruiting more fetuses with isolated echogenic bowel to compare the prevalence of chromosomal anomaly between fetuses with echogenic bowel alone and along with other ultrasound abnormalities seem justified.

In the current study, we detected three fetuses with chromosomal aneuploidy, including a case with T21 and two cases with the 47,XXY. The 47,XXY, also termed Klinefelter syndrome, is a common sex chromosomal aneuploidy and its

Table 2 Comparison of the Detection of the Prevalence of Abnormal CNVs Between Fetuses with Isolated Echogenic Bowel and Along with Other Ultrasound Abnormalities

Fetus Grouping	No. of Cases	Chromosome Aneuploidy	Abnormal CNVs	
			Pathogenic	VOUS
Isolated fetal echogenic bowel	20	0	0	2
Echogenic bowel along with other ultrasound abnormalities	127	4	3	4
Total	147	4	3	6

Abbreviations: CNVs, copy number variations; VOUS, variants of uncertain significance.

Table 3 Prenatal Outcomes in Cases with Fetal Echogenic Bowel Diagnosed at Different Trimesters

Gestational Age	No. of Cases	No. of Cases with Chromosome Aneuploidy	Abnormal CNV		No. of Cases with Normal Pregnant Outcomes
			Pathogenic	VOUS	
Second trimesters	54	1	0	1	52
Third trimester	84	3	3	5	73
Total	138	4	3	6	125

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

incidence is estimated to be 1 per 1000 live male births.²¹ Patients with the 47,XXY are reported to present behavioral disorders, testicular abnormalities, reduced intelligence quotient relative to brothers and sisters but remaining within the normal range.²² Previous studies have shown a strong association between T21 and FEB.^{23,24} In this study, CMA detected additional chromosomal abnormalities as compared to karyotyping in fetuses with echogenic bowel, and we identified a 10q11.1q11.22 microdeletion with a fragment of 8.9 Mb that contained 20 *OMIM* genes. Loss-of-function mutations in the *Ret* gene (MIM# 164761) may cause autosomal dominant familial and sporadic Hirschsprung's disease (MIM# 142623),²⁵ and the *Ret* gene mutation is detected in more than 50% of patients with familial Hirschsprung's disease.²⁶ Infants with Hirschsprung's disease may frequently develop symptoms of impaired bowel peristalsis within 2 months after birth, including failure of meconium passage within 48 hours of birth, constipation, vomiting, abnormal pain or distension, and diarrhea, and patients may manifest mental retardation and hypotonia.²⁷ Besides, the *Ret* gene was found to show a haploinsufficiency (score of 3).²⁸ In our study, CMA identified a pathogenic CNV in this fetus with the 10q11.1q11.22 microdeletion, and the pregnancy was terminated.

In this study, CMA detected 16p13.11 microdeletions in prenatal samples 4 and 5, and this microdeletion

contained 16p13.11 recurrent deletion/duplication regions, including *MYH11*. A previous case-control study reported that microdeletions at the 16p13.11 region strongly correlated with epilepsy, and recurrent microdeletions at 16p13.11 conferred a pleiotropic susceptibility effect to a broad range of neuropsychiatric disorders.²⁹ Infants with the 16p13.11 microdeletion may present a wide range of clinical manifestations, and the common clinical symptoms include mental retardation, epilepsy and microcephaly.³⁰ This microdeletion may be inherited from parents with normal phenotypes, and a 13.1% penetrance was reported.³¹ These two prenatal samples were finally detected with pathogenic CNVs by CMA. In our study, microduplication was detected at 16p13.11 in prenatal sample 1. Mutation of the *MYH11* gene is associated with the development of aortic aneurysm and dissection,³² and overexpression of the *MYH11* gene correlates with the increased risk of aortic dissection and schizophrenia.³³ In addition, the *NED1* gene is involved in mental and behavioral abnormalities.³⁴ It has been demonstrated that duplication at 16p13.11 exhibits triploinsufficiency and clinical heterogeneity.³⁵ Furthermore, two fetuses were identified with VOUS in prenatal samples 2, 6 and 7, which contained unclear pathogenic *OMIM* genes. The labor was induced in fetus 7 that presented additional ultrasound

abnormalities, while normal pregnant outcomes were observed in fetuses 2 and 6.

In this study, maternal uniparental disomy of chromosome 16 [UPD (16) mat] was identified in prenatal sample 9. Uniparental disomy of chromosome 16 has been reported to cause fetal developmental retardation, intrauterine growth restriction, reduced fetal movements, cardiac malformations and dysplasia of the urinary system.³⁶ However, there is also a report showing that uniparental disomy of chromosome 16 alone does not result in intrauterine growth restriction.³⁷ Since there is no pathogenic imprinting in chromosome 16, the exact pathogenicity of uniparental disomy of chromosome 16 remains to be investigated.³⁸

In addition to the increase in the detection of pathogenic CNVs, CMA may detect VOUS.³⁹ Because of penetrance and environmental factors, the individuals with the same pathogenic CNVs or VOUS may present diverse manifestations among family members, and there are also individuals with completely normal phenotypes. In addition, the detection of fetal parental samples with CMA may remain failure to identify the exact clinical significance of the VOUS.⁴⁰ In this study, we detected a case with novel Xq28 microduplication. The repetitive fragment contained *LICAM* and *MECP2* genes that are strongly associated with mental and intelligent developments,^{41,42} and the major clinical manifestations of the Xq28 microduplication syndrome include autism, mental retardation, communication dysfunctions, convulsion, hypotonia, repeated infections and abnormal bladder functions.⁴³

In addition to abnormal chromosome numbers and structure, chromosomal microdeletions or microduplications may contribute to the pathogenesis of fetal echogenic bowel.³ Our data showed that both karyotyping and CMA are effective to detect chromosomal aneuploidy; however, CMA may identify the origin, fragment size and pathogenicity of the supernumerary marker chromosome, which facilitates the precision evaluation of the prognosis in fetuses with echogenic bowel during the genetic counseling.¹¹ Previous studies have shown that CNV, a risk factor for complicated developmental malformations,^{44,45} is a major contributor to fetal echogenic bowel.¹

In summary, the results of the present study demonstrate that prenatal ultrasound is difficult to detect benign or pathogenic echogenic bowel, and a satisfactory pregnant outcome may be expected for fetuses with echogenic bowel that are negative for chromosomal anomalies and

other severe anatomic abnormalities. Isolated FEB is associated with a good prognosis compared with those presenting multiple ultrasound abnormalities. In addition, CMA shows an important value in the genetic diagnosis of FEB, and such a tool, as a supplement to karyotyping, may improve the accuracy of prenatal diagnosis of fetal intestinal malformations in pregnant women with FEB.

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Disclosure

The authors declare no conflicts of interest.

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