

## ORIGINAL ARTICLE

# Influence of validating the parental origin on the clinical interpretation of fetal copy number variations in 141 core family cases

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**Funding information**

This study was supported by National Key R&D Program of China (grant number: 2018YFC1002206).

**Abstract**

**Background:** The sources and variants types of the copy number variations (CNVs) in prenatal fetal, and the critical role of parental origin on the interpretation of fetal CNVs are unclear.

**Methods:** One hundred and forty-one prenatal core families with abnormal CNVs were selected and performed by low-coverage massively parallel CNV sequencing (CNV-seq).

**Results:** The data showed that 72.3% of fetal CNVs were derived from parents, and 27.7% were new variations. Sixty-three cases were heterozygous deletion, 70 cases were threefold duplication, six cases were complex deletion and duplication, and two cases were fourfold repeats. That means the rate of heterozygous deletion and duplication was approximate one. In addition, in parental-derived fetal abnormal CNVs reports, before validating parental origin, 62 CNVs were variants of uncertain significance (VUS), 15 CNVs were likely benign, 20 CNVs were likely pathogenic, and 5 CNVs were pathogenic. However, after validating parental origin, the total clinical significance changed into 12 VUS, 89 likely benign, 1 likely pathogenic, and 0 pathogenic. The clinical interpretation of 78.4% fetal CNVs was changed and tended to be benign after parental CNVs were detected. Besides, we followed up all families. 93.3% parental-derived fetal and 30.3% fetus in new mutation group were born healthy.

**Conclusion:** Parental origin verification has an important significance for interpretation on the clinical significance of fetal CNVs.

**KEYWORDS**

clinical interpretation, CNV-seq, copy number variations (CNVs), parental origin

## 1 | INTRODUCTION

Copy number variations (CNVs) are copies of DNA sequence that are typically larger than 1 kb in size, resulting in microdeletions, microduplications, and complex

rearrangements of the genome (Iafrate et al., 2004; Zong, Lu, Chapman, & Xie, 2012). On average, each person has more than 1,000 CNVs, accounting for 4.8%–9.5% of the entire genome (Zhang, Gu, Hurles, & Lupski, 2009). These CNVs may contain one or more genes, either as recessive

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or dominant alleles, which disrupt the coding region or alter the gene effect dose (Abel & Duncavage, 2013; Wheeler et al., 2013). With the rapid development of genetic technology, CNVs have been found to play a critical role in much human diseases, such as autism, schizophrenia, depression, epilepsy, etc (Miller et al., 2010). (Stosic, Levy, & Wapner, 2018) showed that approximately 6% of pregnancies with ultrasound anomalies exist clinically significant CNVs, making chromosomal microarray analysis (CMA) the current standard of cytogenomic analysis.

The low-coverage massively parallel CNV sequencing (CNV-seq) technology is based on next-generation sequencing technology for whole-genome sequencing of sample DNA (a potential genome resolution of approximately 0.1 Mb), comparing the sequencing results with the human reference genome, and biometric analysis to discover possible chromosomal abnormalities in the sample (Alkan, Coe, & Eichler, 2011; Ku et al., 2011; Xie & Tammi, 2009). The CNV-seq technology is wider and more sensitive than that of CMA, and detects structural abnormalities larger than 100 bp and aneuploid chimerism larger than 10% (Margulies et al., 2005; Valouev et al., 2008).

In clinic, the fetal CNVs reports, especially clinical interpretation of variants of uncertain significance (VUS), as well as the lack of parental-origin test, which adds significant challenges for clinical genetic counseling (Kearney, Thorland, Brown, Quintero-Rivera, & South, 2011). In this study, CNV-seq was performed on 141 core families to detect CNVs variants sources, variants types, and parental origin on fetal clinical interpretation, and finally provide theoretical basis for clinical consultation.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

From November 2017 to August 2018, a total of 141 pregnant women, who treated in the department of genetics and prenatal diagnosis of the first affiliated hospital of Zhengzhou university, were recruited. The average age of the women was 30, range from 22 to 40. All pregnant women were divided into six groups described as below, 53 cases were abnormal with positive ultrasonography soft marker (USM), 23 cases were high risk of noninvasive prenatal testing (NIPT), 14 cases were advanced maternal age (over the year of 35 at the expected date of birth) (AMA), 23 cases were poor maternal history, 12 cases were high-risk maternal serum screening, 8 cases were voluntary request, and 8 cases were mixed indications (Tables S1 and S2). All core families were tested for CNVs. All subjects were signed informed consent for prenatal genetic investigation, and were approved by the Zhengzhou University Ethics Committee.

### 2.2 | Amniocentesis

Amniocentesis was performed according to literature description (Huang, Jiang, & Liu, 2015; Izetbegovic & Mehmedbasic, 2013). DNeasy Blood and tissue kit (Qiagen) were used to extract genomic DNA of amniocytes, which were collected by centrifugation and washed by PBS. Quantitative fluorescent PCR (QF-PCR) were used as quality control to detect all DNA contamination. Short tandem repeat (STR) markers were used for chromosome 21 (D21S1435, D21S1411, D21S11), chromosome 18 (D18S1002, D18S391, D18S535, D18S386), chromosome 13 (DXS981, DXS6809, DXS22), and sex chromosomes X and Y according to the operating procedures (Wang et al., 2018).

### 2.3 | Next-generation sequencing

CNV-seq was performed as previously described (Liang et al., 2014). Finally identified CNVs (blast with hg19) were inquired against public database, such as Decipher, DGV, OMIM, UCSC, and ClinGen and were assessed pathogenicity according to the guidelines outlined by the American College of Medical Genetics (ACMG; Richards et al., 2015). CNVs were classified as five levels, benign, likely benign, VUS, likely pathogenic, and pathogenic (Tables S1 and S2).

## 3 | RESULTS

### 3.1 | 72.3% of fetal CNVs were inherited from parents, and 27.7% were new mutations

It was found that 58 cases were inherited from mother, 38 cases were inherited from father, 6 cases were inherited from both parents, and 39 cases were new mutations after the verification of parents' CNVs (Tables S1 and S2). That was, 72.3% of fetal CNVs were inherited from parents and 27.7% were new mutations. Among parents, 59.8% were inherited from mother and 40.2% were inherited from father.

### 3.2 | The ratio of deletion and duplication of fetal CNVs was approximately about 1:1

Among 141 fetal CNVs, 63 cases were heterozygous deletions, 70 cases were threefold duplication, 6 cases were multiple variants (deletion and duplication), and 2 cases were fourfold duplication (Tables S1 and S2). In conclusion, 44.7% of abnormal CNVs were heterozygous deletions, 49.6% were threefold duplication, 4.3% were multiple variants, and 1.4% were fourfold duplication. The ratio of deletion and duplication was approximately about 1:1.

### 3.3 | The clinical interpretation of 78.4% parental-derived fetal CNVs changed and tended to be benign

In 102 cases of parental-derived fetal CNVs, before parent-origin test, the clinical significance was divided into 62 cases with VUS, 15 cases with likely benign, 20 cases with likely pathogenic, and 5 cases of pathogenic (Table 1). According to guidelines of ACMG and our experience, those fetal CNVs derived from parents are inclined to be benign, so we reassessed the clinical significance of fetal CNVs after parent-origin test. The clinical significance was changed into 12 VUS cases, 89 likely benign cases, 1 likely pathogenic case, and 0 pathogenic case, as showed in Table 1. Table 2 showed that after parents-origin test, 62 VUS cases were changed into 55 likely benign cases, 1 likely pathogenic case and 6 cases were maintained as original judgment; 15 likely benign cases were altered into 1 VUS case and 14 cases remain the same; 20 likely pathogenic cases were changed into 17 likely benign cases and 3 VUS cases; and 5 pathogenic cases were altered into 3 likely benign and 2 VUS. In summary, 78.4% of fetal CNVs detection changes and tend to be benign after parental source verification.

### 3.4 | The pregnancy outcome of the normal phenotypic parental fetal tended to be benign

In 102 parents-origin cases, the clinical significance of fetal CNVs was dominated by likely benign (89/102), and followed by VUS (12/102). In this study, we followed up most families and the details were in Tables S1 and S2 and as showed in Table 3. In the acknowledged 73 cases, 70 fetal were born healthy, 1 were unhealthy with unable to walk, and 2 were induced labor. The genetic counseling of likely benign cases was relatively simple and the most pregnancy outcomes tend to be benign as proved in our data. Table 3 showed that 58 likely benign cases gave birth to healthy baby, 1 case with unhealthy baby, 2 cases performed inducing labor, and 28 cases without informed information. It is important to point out parents-origin case P117 is a secondary funding. The result of CNV-seq is a heterozygous deletion of

**TABLE 1** Fetal CNVs clinical significance distribution before and after parental-origin test

Clinical significance of fetal CNVs	Before parental-origin test	After parental-origin test
VUS	62	12
LB	15	89
LP	20	1
Pathogenic	5	0

Abbreviations: CNVs, copy number variations; LB, likely benign; LP, likely pathogenic; VUS, variants of uncertain significance.

1 240 000 bp in 17p12 (14120000–15360000), which was covered in Hereditary Liability to Pressure Palsies syndrome (chr17:14097915–15470903; OMIM#162500) with phenotypes of abnormality of the motor neurons and motor conduction block. However, the pregnant exists the same site CNVs but with no symptoms. We speculated that the fetal may also have no symptoms after postnatal.

However, the genetic counseling of VUS cases was a great challenge for the first-line doctors. But in 12 VUS cases, 11 fetal were born healthy. In conclusion, 95.8% parents-origin fetal abnormal CNV tends to be benign.

### 3.5 | The pregnancy outcomes of eight VUS cases of new mutations tended to be benign

As showed in Table 4, in the 39 cases of abnormal fetal CNVs with new mutations, there were 13 cases of VUS, 10 cases of likely pathogenic, 11 cases of pathogenic, 4 cases of likely benign, and 1 case of benign. According to our follow-up results, the clinical significance of patients with pathogenic or likely pathogenic was mostly abortion or induction of labor, and 30.3% fetuses were born healthy. However, in 13 cases of VUS, 8 fetuses were born healthy, 4 were induced labor, and 1 case with atrial septal defect. In summary, the pregnancy outcomes of 61.5% VUS cases of new mutations tend to be benign.

## 4 | DISCUSSION

According to the guide of ACMG (Kearney et al., 2011), the pathogenicity of CNVs was divided into five grades, which were pathogenic, benign, VUS, likely pathogenic, likely benign. The interpretation and clinical genetic counseling of VUS or likely pathogenic were a difficulties and

**TABLE 2** The details of fetal CNVs clinical significance distribution before and after parental-origin test

Clinical significance of fetal CNVs	Before parental-origin test	After parental-origin test
VUS (62)	VUS	6
	LB	55
	LP	1
LB (15)	VUS	1
	LB	14
LP (20)	VUS	3
	LB	17
Pathogenic (5)	VUS	2
	LB	3

Abbreviations: CNVs, copy number variations; LB, likely benign; LP, likely pathogenic; VUS, variants of uncertain significance.

**TABLE 3** 102 parental-origin fetal CNVs followed-up results

Clinical significance of fetal CNVs	Born, healthy	Born, unhealthy	Abortion	Induce labor	N/A
VUS (12)	11	0	0	0	1
LB (89)	58	1 <sup>a</sup>	0	2	28
LP (1)	1	0	0	0	0
Total amount	70	1	0	2	29

Abbreviations: CNVs, copy number variations; LB, likely benign; LP, likely pathogenic; N/A, none information; VUS, variants of uncertain significance.

<sup>a</sup>The fetal was born but cannot walk (case P73 in Table S1).

**TABLE 4** 39 new mutation fetal CNVs followed-up results

Clinical significance of fetal CNVs	Born, healthy	Born, unhealthy	Abortion	Induce labor	N/A
Benign (1)	0	0	0	0	1
LB (4)	0	0	0	0	4
VUS (13)	8	1 <sup>a</sup>	0	4	0
LP (10)	2	0	2	5	1
Pathogenic (11)	0	0	0	11	0

Abbreviations: CNVs, copy number variations; LB, likely benign; LP, likely pathogenic; N/A, none information; VUS, variants of uncertain significance.

<sup>a</sup>The fetal was born with atrial septal defect (case P103 in Table S2).

challenges for laboratory technician and clinical genetics (Bernhardt, Kellom, Barbarese, Faucett, & Wapner, 2014; Kiedrowski, Owens, Yashar, & Schuette, 2016), mainly due to the rare effects of new mutations, gene expression degree, and penetrance. We studied the parental origin of fetal CNVs from 141 core family cases to help to interpret the VUS or likely pathogenic, then given patients a better choice about pregnancy. In the present study, 72.3% (102/141) fetal CNVs were inherited from parents. Before the parental verification, the clinical significance of fetal CNVs was mainly VUS (62/102). After parental verification, likely benign cases (89/102) were the dominant. There are very little data on parental sources. The detection of fetal parental samples to determine the source of CNVs in the fetus, as well as the comprehensive family analysis and clinical assistant examinations are helpful for the interpretation and clinical genetic counseling on VUS reports.

Wu et al. (2017) showed 27 VUS reports were proved 26 likely benign with prenatal origin and 1 pathogenic with new variants after parental verification by chromosome microarray analysis (CMA). In this study, the data were collected by CNV-seq, which was based on next-generation sequencing. And the results showed 62 VUS fetal CNVs reports were changed to 55 likely benign cases, 1 likely

pathogenic, and 6 VUS after parental verification. In a conclusion, above 90% clinical significance of the fetal VUS were changed and tended to be likely benign by two different detection methods.

All the fetuses were followed up. In the cases which were still read as VUS after parental verification, 91.6% (11 /12) fetuses were born and healthy with no clinical symptoms and 1 with no acknowledged information. The 12 cases include 7 heterozygous deletion cases and 5 duplication cases, and divided into 4 different clinical significance before parental verification. The minimal and maximum size of 12 cases were 120,000 and 14,480,000 bp.

The results indicated that the clinical outcomes of the fetus whose CNVs were genetically derived from normal phenotypic parents tended to be benign. Parental verification provided strong evidence for the interpretation and clinical genetic counseling about the VUS. It suggested that if the samples of parents and fetus were detected at the same time for the larger pregnancy week, it has a great guiding function for the clinical significance of fetal CNVs and can greatly reduce the time of verification. Combine the parental origin verification and the follow-up results, this study found the follow CNVs had a higher frequency in the population, such as the duplication of Xp22.31, 22q11.2 and deletion of 16p12.2. These results enrich the database of genotypes and phenotypes. We suggest these CNVs would be directly judged to be normal in the future work.

In summary, parental verification is critical for the clinical interpretation of prenatal fetal CNVs, especially for the fetus with VUS reports. Parental verification contributes clinical first-line physicians to make precise genetic counseling and provides strong evidence for further family diagnosis and assessment of recurrence risk. In addition, the establishment of local CNVs database, especially the VUS fetal database, using dynamic racking and follow-up, providing powerful clinical evidence for clinical reports, eliminating benign CNVs effectively, and accumulating pathogenicity and suspected pathogenic CNVs data.

## ACKNOWLEDGMENT

We thank all families investigated for their invaluable contribution to this study.

## CONFLICT OF INTEREST

None declared.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Shi P, Li R, Wang C, Kong X. Influence of validating the parental origin on the clinical interpretation of fetal copy number variations in 141 core family cases. *Mol Genet Genomic Med*. 2019;7:e944. <https://doi.org/10.1002/mgg3.944>