

Chromosome Analysis and Postnatal Follow-Up of Fetuses With Abnormal Ultrasound Findings

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Research Article

Keywords: fetal abnormalities, ultrasound, follow-up, chromosome analysis, copy number variations, chromosome microarray analysis, copy number variations sequencing

Posted Date: January 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-139022/v1>

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Abstract

Objective—To analyze the correlation between copy number variations (CNVs) and prenatal ultrasound abnormalities, characterize the CNVs in diverse prenatal phenotypes, and provide detailed follow-up results for prenatal diagnosis.

Method—The results obtained in 1152 fetuses referred for chromosome testing due to different ultrasound anomalies were analyzed. CNV detection was performed by chromosomal microarray analysis (CMA) and copy number variation sequencing (CNV-seq). The postnatal outcomes of the fetuses were followed up.

Result—Chromosomal abnormalities were detected in 149 of 1152 pregnancies (12.9%), including 84 (7.3%) macroscopic and 65 (5.6%) submicroscopic anomalies. The highest proportion of chromosomal defects was found in fetuses with hydrops (38.5%), followed by fetuses with multiple abnormalities (32.1%). The most common CNV number variation was 22q11 deletion (0.7%), followed by 17q12 deletion (0.4%) and 15q11.2 deletion (0.4%). Bilateral hyperechogenic kidneys were identified in all fetuses with the 17q12 deletion, and all cases of 15q11.2 deletion were positive only for soft markers. On postnatal follow-up of 362 live births, 2 cases of esophageal atresia and 2 cases of craniosynostosis were found that were not accurately diagnosed by prenatal ultrasound.

Conclusion—we identified chromosomal abnormalities in approximately one eighth of the pregnancies with abnormal ultrasound findings. More emphasis should be placed on the improvement of prenatal detection of esophageal atresia and craniosynostosis.

Introduction

Fetal structural abnormalities are found in approximately 3% of pregnancies¹. Ultrasonography can identify fetal abnormalities ranging from a single minor defect to severe multisystem anomalies. They may be associated with chromosomal aneuploidy or copy number variants (CNVs). Chromosomal microarray (CMA)^{2,3}, and CNV sequencing (CNV-Seq)^{4,5} have been increasingly adopted in prenatal diagnosis to detect submicroscopic pathogenic copy number variations (PCNVs). The addition of CMA testing to karyotyping increases the frequency of detection of chromosomal abnormalities by 3–5%^{2,3}.

Certain CNVs correlate with specific prenatal phenotypes^{6–9}. The frequency of chromosomal anomalies varies with the specific structural anomaly and the number of ultrasound-detected defects. However, more data are needed to clarify the correlation between CNVs and prenatal abnormalities. Moreover, the follow-up studies of the cases with a congenital abnormality are frequently restricted to a single disease^{10–12}; therefore, these results may not be generalizable. In our study, samples of 1152 fetuses with various ultrasound findings were analyzed by CMA and CNV-Seq to determine the correlation between CNVs and prenatal ultrasound abnormalities, including soft markers and structural anomalies. Additionally, we conducted a detailed follow-up of fetuses with abnormal ultrasound findings to gain information for prenatal diagnosis.

Materials And Methods

Patients and Ultrasonography

Our study included 1152 pregnant women who received fetal chromosome examination by CMA or CNV-seq tests due to abnormal ultrasonographic findings, including positive soft markers and structural anomalies. The women were admitted to the Xiangya Hospital of Central South University and Hunan Jiahui Genetic Hospital between January 2013 and June 2019. All pregnant couples provided written informed consent. The mean gestational age of mothers was 28.2 years (range, 22–45 years), and the mean age of fetuses at the invasive procedure was 20.3 weeks (range, 11–37 weeks). The study was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University and Hunan Jiahui Genetic Hospital. All methods were performed in accordance with Medical Ethics Committee of Xiangya Hospital of Central South University and Hunan Jiahui Genetic Hospital.

Ultrasound scanning of fetuses was performed between 11 and 35 weeks of gestation. The positive soft markers included screening for thickened nuchal translucency or nuchal fold (greater than 95th percentile), absence or dysplasia of nasal bone, short long bones (more than 2 SDs below the mean), hyperechogenic bowel, tricuspid regurgitation, mild ventricular expansion (10–15mm), intracardiac echogenic focus, choroid plexus cyst, single umbilical artery, enlarged cisterna magna (10–15mm), hydronephrosis (>4mm before 28 weeks, >7mm after 28 weeks), persistent left superior vena cava, and aberrant right subclavian artery. Fetal structural anomalies were categorized into 10 phenotypic classes based on their location, including the cardiovascular system, central nervous system, genitourinary system, musculoskeletal, gastrointestinal, respiratory, facial, abdominal wall system, hydrops fetalis, and multiple malformations involving two or more systems [Supplement 1].

Sample Collection and Preparation

Fetal DNA was obtained from amniotic fluid, cord blood, or fetal skin. The genomic DNA was prepared using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The quality and concentration of genomic DNA in the samples were assessed by agarose gel electrophoresis and a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

SNP Array Analysis and CNV-seq

Chromosome CNV analysis was used before 2017 and was performed using the HumanCytoSNP-12 BeadChip array (Illumina, San Diego, CA, USA), which comprises about 300,000 SNP probes with an average marker spacing of approximately one probe every 10 kb. CNV-seq was used from 2017 and utilized the HiSeq 2000 platform (Illumina). All sequencing procedures were performed as previously described^{4,13,14}.

Copy number variations (CNVs) were called at an effective minimal resolution of 100 kb involving at least 10 contiguous probes.

The CNVs were classified based on their size. CNVs ≥ 10 Mb were defined as chromosomal macroscopic anomalies, which could be identified by traditional karyotyping. By contrast, CNVs < 10 Mb were defined as chromosomal microscopic anomalies (microdeletions/microduplications).

Detected CNVs were evaluated based on a review of scientific literature and the following public databases: Database of Genomic Variants (<http://projects.tcag.ca/variation/>), DECIPHER (<http://decipher.sanger.ac.uk/>), UCSC (<http://genome.ucsc.edu/>), and OMIM (<http://www.ncbi.nlm.nih.gov/omim>). Variants of uncertain significance (VOUS), and pathogenic CNVs. CNVs coinciding with known polymorphic CNVs reported in multiple peer-reviewed publications or databases as benign variants were considered benign. Conversely, 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, or 3) the CNV was > 5 Mb long and no well-established cytogenetic heteromorphisms were present in this region. CNVs that did not fit any of the above criteria were considered as VOUS.

Parental study

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

Postnatal Follow-up and Statistical Analysis

Follow-up was conducted by phone calls. The median time of follow-up was 10 months (range 3-24). The follow-up content based on medical reports included gestational age at birth, age, height, weight, physical exam results, large motor skills development, and other positive results. The correspondence between the postnatal exam and prenatal ultrasound was defined as an agreement (same diagnosis), additional (additional findings undetected by ultrasound), or unconfirmed (false positive and false negative ultrasound results). Categorical data are presented as numbers and proportions. Comparison between groups was performed using the Chi-square test. A *P*-value of < 0.05 was considered statistically significant. All statistical analyses were done using the SPSS 16.0 software (SPSS, Chicago, IL, USA).

Results

Chromosome Analysis

Fetal chromosome examination using CMA or CNV-seq tests was performed in 1152 fetuses with abnormal ultrasonographic findings, including 405 positive soft markers and 747 structural anomalies. The analysis employed CMA in 367 cases and CNV-seq in 785 cases. Fetal DNA was obtained from amniotic fluid in 880 (76.4%) fetuses, skin samples after pregnancy termination in 212 (18.4%) fetuses, and cord blood in 60 (5.2%) fetuses.

Chromosomal abnormalities were detected in 149 (12.9%) samples, involving 84 (7.3%) macroscopic anomalies and 65 (5.6%) submicroscopic anomalies (Table 1). The frequency of chromosome macroscopic anomalies was significantly higher in hydrops fetuses (35.7%) than in the remaining cohort. While multiple abnormalities (13.9%) are most common in copy number variations, followed by the central nervous system (7.9%) and musculoskeletal system (7.3%).

Table 1
Distribution of copy number variants by ultrasonographic anomaly

	Total	Cardiovascular	CNS	GU	GI	Fetal hydrops	Musculo-skeletal	Abdominal wall	Facial	Respiratory
n	1152	192(16.7%)	89(7.7%)	106(9.2%)	37(3.2%)	70(6.1%)	41(3.6)	24(2.1%)	35(3.0%)	16(1.4%)
Total chromosome abnormalities	149(12.9%)	22(11.4%)	9(10.1%)	9(8.5%)	0(0%)	27(38.5%)	3(7.3%)	0(0%)	2(5.7%)	0(0%)
Macroscopic anomalies	84(7.3%)	11(5.7%)	2(2.2%)	3(2.8%)	0(0%)	25(35.7%)	0(0%)	0(0%)	0(0%)	0(0%)
Submicroscopic anomalies	65(5.6%)	11(5.7%)	7(7.9%)	6(5.7%)	0(0%)	2(2.8%)	3(7.3%)	0(0%)	2(5.7%)	0(0%)
PCNV	33(2.9%)	6(3.1%)	2(2.3%)	4(3.8%)	0(0%)	1(1.4%)	2(4.9%)	0(0%)	0(0%)	0(0%)
LpCNV	32(2.7%)	5(2.6%)	5(5.6%)	2(1.9%)	0(0%)	1(1.4%)	1(2.4%)	0(0%)	2(8.6%)	0(0%)
VOUS	206(17.9%)	36(18.7%)	24(26.9%)	15(14.1%)	16(43.2%)	7(10.0%)	10(24.4%)	3(12.5%)	3(8.6%)	4(25%)

CNS, central nervous system; GU, genitourinary; GI, gastrointestinal; PCNV, pathogenic copy number variant; LpCNV, likely pathogenic copy number variant.; V significance

Among the 84 chromosomal macroscopic anomalies, the three most common were trisomy 21, 18, and 45X, which comprised 77% of all karyotype-detectable aberrations. The prenatal ultrasound showed multiple malformations in 65% of fetuses with trisomy 18, and hydrops fetalis in 64.7% of cases with 45X.

Among the 65 pathogenic and likely pathogenic CNVs, 22q11 deletion (9 cases, 0.7%) was the most common, followed by 17q12 deletion (5 cases, 0.4%) and 15q11.2 deletion (5 cases, 0.4%). Of note, bilateral hyperechogenic kidneys were found in all 17q12 deletion fetuses, and in all 15q11.2 deletion cases only soft markers were positive (Table 2). However, both ultrasound manifestations (hyperechogenic kidneys and soft markers) corresponded to benign phenotypes. The five cases of 15q11.2 deletion syndrome included 4 cases of the termination of pregnancy for 15q11.2 deletion and 1 case that was found to have craniosynostosis after birth with significant global developmental delay.

Table 2
 Ultrasonographic Anomaly of the three most common copy number variants

n	CNV position [hg19]	Size (Mb)	Ultrasound	methods	Parental study	interpretation
1	arr22q11.21(18,874,965 – 21,464,479)x1	2.6	Thickened nuchal fold	CMA	de novo	22q11 deletion associated with DiGeorge syndrome
2	arr22q11.21(18,844,632 – 21,800,444)x1	2.9	Unilateral ureteral dilatation,Hydronephrosis,VSD;aortic stenosis single umbilical artery,	CMA	de novo	
3	arr22q11.21(18,847,953 – 21,855,411)x1	3.0	IAA,VSD;unilateral renal agenesis ;Unilateral ureteral dilatation, ureterocele	CMA	de novo	
4	arr22q11.21(18,895,703 – 21,462,353)x1	2.6	Clubfeet,Oligohydramnios	CMA	de novo	
5	arr22q11.21(18,856,942 – 21,736,471)x1	2.88	Truncus arteriosus,VSD;Right aortic arch	CMA	de novo	
6	seq[hg19] 22q11.21(18.92 Mb- 21.48 Mb)x1	2.56	Single umbilical artery	CNV-seq	de novo	
7	seq[hg19]22q11.21(18.92 Mb- 21.92 Mb)x1	3.0	Truncus arteriosus,VSD;Right aortic arch	CNV-seq	de novo	
8	seq[hg19] 22q11.21(18.9 Mb- 21.46 Mb)x1	2.56	Complex congenital heart disease	CNV-seq	de novo	
9	seq[hg19] 22q11.21(18.9 Mb- 21.46 Mb)x1	2.56	Complex congenital heart disease	CNV-seq	de novo	
10	arr17q12(34815551– 36351076)x1	1.5	Hyperechogenic kidneys Enlarged kidney	CMA	maternal inherited	17q12 deletion syndrome
11	seq[hg19] 17q12(34.84 Mb- 36.26 Mb)x1	1.42	Hyperechogenic kidneys Enlarged kidney	CNV-seq	de novo	
12	seq[hg19] 17q12(34.84 Mb- 36.26 Mb)x1	1.42	Polycystic kidney Oligohydramnios	CNV-seq	de novo	
13	seq[hg19] 17q12(34.82 Mb- 36.26 Mb)x1	1.44	Hyperechogenic kidneys Clubfeet	CNV-seq	de novo	
14	seq[hg19] 17q12(34.8 Mb- 36.5 Mb)x1	1.7	Hyperechogenic kidneys Unilateral renal cyst	CNV-seq	de novo	
15	arr15q11.2(21974835– 23226254)x1	1.3	Thickened nuchal translucency	CMA	de novo	15q11.2deletion syndrome
16	seq[hg19] 15q11.2(23.6 Mb- 29.14 Mb)x1	5.54	Thickened nuchal translucency	CNV-seq	de novo	
17	seq[hg19] 15q11.2(22.76 Mb- 23.1 Mb)x1	0.34	Ventriculomegaly	CNV-seq	de novo	
18	seq[hg19] 15q11.2(22.76 Mb- 23.08 Mb)x1	0.32	Short long bones	CNV_seq	de novo	
19	seq[hg19] 15q11.2(22.76 Mb- 23.1 Mb)x1	0.34	Choroid plexus cyst	CNV-seq	de novo	
IAA interrupted aortic arch ; VSD, ventricular septal defect						

The rate of chromosomal abnormalities in the soft markers group was 8.1% (4.4% corresponded to macroscopic anomalies and 3.7% to submicroscopic anomalies), which was significantly lower than in the structural abnormalities group (15.5%, $P < 0.001$). Fetal structural anomalies were categorized into 10 phenotypic classes based on their location. The number of cases in these classes ranged from 16 (respiratory) to 192 (cardiovascular). The rate of chromosomal abnormality varied by the anatomical systems involved: the greatest fraction of chromosomal abnormalities were found in fetuses with

hydrops (38.5%), multiple malformations (32.1%), cardiovascular (11.4%), and central nervous system (10.1%) anomalies, followed by the genitourinary (8.5%), skeletal (7.3%), and facial (5.7%) anomalies; the other systems were negative.

Follow-up Results

Pregnancy outcomes were available for 533 (46.3%) of the 1152 fetuses. Of these, 165 (30.9%) were pregnancies that parents opted for termination and 6 (1.1%) died in the uterus. Additionally, there were 12 (2.3%) postnatal deaths, and 350 (65.7%) live births. Postnatal findings confirmed prenatal ultrasound in 96.9% fetuses (351/362), provided additional information in 4 (1.1%) fetuses, and did not confirm prenatal ultrasound in 7 (1.9%) fetuses. The additional findings undetected by ultrasound include 2 cases of esophageal atresia (1 with cleft palate) and 2 cases of prenatal craniosynostosis. The missed findings were 4 ventricular septal defects, and only soft markers were detected by prenatal ultrasound. The instances of misdiagnosis included 1 case of clubfoot (found to be normal after birth), 1 abdominal lymph cyst (diagnosed postnatally as intestinal atresia), and 1 duplex kidney (found to be normal after birth) (Table 3).

Table 3
Postnatal findings inconsistent with prenatal ultrasonography

Case number	Prenatal ultrasound findings	Postnatal diagnosis	CMA or CNV-seq	Outcome
1	Microphthalmia	Microphthalmia, esophageal atresia, esophagotracheal fistula, chondrodysplasia of larynx, ASD	Normal	Live birth
2	VSD, black cyst	VSD, black cyst, esophageal atresia, esophagotracheal fistula	Normal	Infantile death
3	VSD, mild ventricular expansion, spinal anomaly	VSD, spinal anomaly, craniosynostosis, cleft palate	VOUS seq[hg19]17q21.1q21.2(38.16 Mb-38.66 Mb)×3	Live birth
4	Mild ventricular expansion	Craniosynostosis	Pathology seq[hg19] 15q11.2(22.76 Mb-23.1 Mb)×1	Live birth
5	Single umbilical artery	Ventricular septal defects	Normal	Live birth
6	Short long bones	Ventricular septal defects	Normal	Live birth
7	Aberrant right subclavian artery	Ventricular septal defects	VOUS seq[hg19]8p23.2(2.58 Mb-2.92 Mb)×3	Live birth
8	Tricuspid regurgitation	Ventricular septal defects	VOUS seq[hg19]1q23.3(162.08 Mb-162.36 Mb)×3	Live birth
9	Clubfoot	Normal	Normal	Live birth
10	Abdominal lymph cyst	Intestinal atresia	VOUS seq[hg19]Xp22.33(3.44 Mb-3.74 Mb)×2	Live birth
11	Duplex kidney	Normal	VOUS seq[hg19] 8q23.3(115.78 Mb-116.28 Mb)×3	Live birth

Discussion

In our study, chromosomal abnormalities were detected in 12.9% of pregnancies, 7.3% were macroscopic anomalies and 5.6% were submicroscopic anomalies. The rate of chromosomal abnormalities associated with structural defects (15.5%) is consistent with previous studies, which identified significant chromosomal abnormalities in 16% of fetuses in which ultrasonography indicated at least one anomaly¹⁵. The frequency of chromosomal abnormalities in fetuses with soft markers (8.1%) was higher than reported in other studies (approximately 4%). This difference may be due to the following reasons: 1) at our referral center, many pregnant women with positive soft markers are of advanced age and have a complicated pregnancy, and 2) our study included fetuses with persistent left superior vena cava and aberrant right subclavian artery. Although the rate of chromosomal abnormalities in the soft markers group was lower than in the structural abnormalities group, identifying an additional 4.4% pathogenic or likely pathogenic CNV can avert the possibility of birth of children with a chromosome microdeletion or microduplication syndrome. Certainly, the risk of pregnancy loss or other post-amniocentesis complications should be considered, even if currently the risk related to amniocentesis is approximately 1 in 800¹⁶.

The rate of chromosomal abnormalities varied among the anatomical systems involved. We detected chromosomal abnormalities more frequently in association with fetal hydrops, multiple malformations, cardiovascular and central nervous anomalies, and, to a lesser extent, abnormalities of the genitourinary and musculoskeletal systems. In a recent study using WES to identify genetic variants in fetuses with structural anomalies, the greatest proportion of variants was found in cases of hydrops¹⁷. Their relative frequency can give insight into the proportion of anomalies, such as hydrops fetalis, that potentially have a detectable underlying genetic cause.

Certain CNVs are associated with specific phenotypes. For example, cardiovascular abnormalities represent one of the pillars of clinical diagnosis of 22q11.2 deletion (DiGeorge syndrome). In our study, 50% of cases with 22q11 deletion syndrome exhibited cardiovascular defects. Bilateral hyperechogenic kidneys were found in all fetuses with 17q12 deletion, which is in line with previous studies¹⁸. The 15q11.2 BP1-BP2 deletion has been associated with variable phenotypes and low penetrance. Our results tend to suggest that the deletion confers milder phenotypes, as only soft markers were positive prenatally. A previous investigation documented that the 15q11.2 deletion is associated with congenital cardiovascular malformations¹⁹, but no such case was present in our study. There are some controversies regarding the classification of 15q11.2 deletion in prenatal management. Marshall et al.²⁰ recommended classifying the deletion as “pathogenic of mild effect size, while Maya et al.²¹ recommended that 15q11.2 deletion should not be considered pathogenic and should not be reported to the parents in the prenatal setting. In our laboratory, this deletion is classified as pathogenic variation with incomplete penetrance. Unfortunately, among the 5 cases of 15q11.2 deletion syndrome, in 4 instances the termination of pregnancy was chosen based on the report of pathogenic copy number variant, and 1 case was found after birth to have craniosynostosis with a significant global developmental delay. 15q11.2 BP1-BP2 structural variation is associated with changes in brain morphology and cognition²², possibly indicating that this CNV is more commonly associated with neurocognitive impairment than with structural anomalies. Larger studies of prenatal and postnatal phenotypic groups will be required to provide useful information for interpreting and consulting cases with 15q11.2 deletions.

Follow-up results demonstrated a high agreement between prenatal ultrasound and living birth. Not surprisingly, the most frequently missed prenatal diagnosis was the ventricular septal defect. Also, esophageal atresia and craniosynostosis are difficult to diagnose by ultrasound prenatally. Analysis of the prenatal ultrasound images of these patients revealed that 2 cases of craniosynostosis had abnormal head morphology (1 occipital flattening, 1 navicular head). However, since both fetuses had multiple malformations, the doctors focused on the obvious structural abnormalities and ignored the changes in cranial morphology. Therefore, alterations in cranial morphology provide an important clue that requires consideration to exclude the possibility of craniosynostosis.

The antenatal detection rate of esophageal atresia has been extremely poor even in expert hands, with a sensitivity ranging from 40 to 60%^{23,24}. Remarkably, in our study, 2 cases in which esophageal atresia was missed in prenatal ultrasound had co-existent esophagotracheal fistula, making a correct prenatal diagnosis more challenging. More recently, Dall'Asta et al.²⁵ demonstrated that the detection of esophageal atresia is possible when three-dimensional ultrasound and Crystal Vue rendering techniques are used. Thus, employing the most current ultrasound methods may increase the accuracy of prenatal detection of esophageal atresia.

Limitations of our study include a high rate of lost follow-up (telephone number errors or refusal to answer). Additionally, many pregnant women do not consent to the autopsy of the fetus due to cultural norms, and the prenatal structural abnormalities could not be confirmed. The absence of data regarding the inheritance (i.e., parental vs. de novo) of each alteration and the presence of many variants of unknown significance increased the difficulty of genetic counseling.

In summary, we identified chromosomal abnormalities in approximately one eighth of the pregnancies with abnormal ultrasound findings. Follow-up is recommended in the management of fetal structural anomalies since it confirms, modifies, or adds information to prenatal diagnosis.

Declarations

Acknowledgments

We thank all the patient's family members for their participation and support in this study.

Funding

This work was supported by grants from the Natural Science Foundation of Hunan Province, China (2020JJ5917).

Competing interests

There are no potential conflicts of interest to report for this paper.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University and Hunan Jiahui Genetic Hospital. All methods were performed in accordance with Medical Ethics Committee of Xiangya Hospital of Central South University and Hunan Jiahui Genetic Hospital.

Consent for publication

None

Authors' contributions

LP, MS, LD and KC design the experiment and perform the experiments. LP, MS, CC and ZZ analyzed the data. LP, ZZ, MS, LD and KC wrote the manuscript. ZZ supervised the whole study.

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