



Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Prenatally diagnosed *de novo* segmental amplification or deletion by microarray-based comparative genomic hybridization: A retrospective studyHsiu-Huei Peng^{a, b}, Chien-Hong Lee^c, Sheng-Yuan Su^{a, b}, Kuan-Ju Chen^{a, b}, Yen-Chang Lee^{a, b}, Shu-Han You^{a, b}, Wen-Fang Lee^{a, b}, Po-Jen Cheng^{a, b, *}^a Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Linkou Medical Center, Tao-Yuan, Taiwan^b Chang Gung University College of Medicine, Kwei-Shan, Tao-Yuan, Taiwan^c Department of Laboratory Medicine, Chang Gung Memorial Hospital, Linkou Medical Center, Tao-Yuan, Taiwan

ARTICLE INFO

Article history:

Accepted 14 June 2019

Keywords:

Prenatal diagnosis

Array CGH

de novo

ABSTRACT

Objective: Prenatal diagnosis of *de novo* segmental amplification or deletion by microarray-based comparative genomic hybridization (array CGH) is uncommon. The study aimed to know about the incidence, abnormal ultrasound findings, and pregnancy outcomes of prenatally diagnosed *de novo* segmental amplification or deletion by array CGH.

Materials and methods: Between January 2014 and December 2017, we analyzed pregnant women who received prenatal array CGH (SurePrint G3 Human CGH Microarray Kit, 8 × 60K) at Chang Gung Memorial Hospital, Taiwan. Clinical data on maternal age, reason for fetal karyotyping, sonographic findings, gestational age at delivery, newborn birth weight, and associated anomalies, if any, were obtained by chart review.

Results: A total of 836 specimens (814 amniotic fluid samples, 4 cord blood samples, 18 chorionic villi samples) were analyzed by array CGH during the study period. Of the 56 cases with abnormal array CGH results, 40 had segmental amplification or deletion, 12 had trisomy, three had monosomy, and one had sex chromosome aneuploidy. Of these 40 cases with segmental amplification or deletion, 30 were inherited and 10 were *de novo* occurrences. The incidence of *de novo* segmental amplification or deletion was 1.2% (10/836). Abnormal prenatal ultrasound findings occurred in 40% (4/10) of *de novo* segmental amplification or deletion cases. Among these 10 pregnancies, nine were voluntarily terminated between 22 and 26 weeks of gestation and one was delivered at term.

Conclusions: Prenatal diagnosis of *de novo* segmental amplification or deletion by array CGH raises important genetic counseling issues. In our series, the incidence of *de novo* segmental amplification or deletion in prenatal samples was 1.2%. Abnormal prenatal sonographic findings occurred in 40% of these *de novo* segmental amplification or deletion cases. Of these *de novo* segmental amplification or deletion pregnancies, 90% were voluntarily terminated.

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Introduction

Prenatally diagnosed segmental amplification or deletion by microarray-based comparative genomic hybridization (array CGH) is uncommon, and it is either inherited from the parents

or occurs as a *de novo* phenomenon. Genetic counseling following prenatal diagnosis of *de novo* segmental amplification or deletion is based on pathological or benign findings of the involved region, and the supporting data for the diagnosis at times is scarce. The present study aimed to know about the incidence of prenatally diagnosed *de novo* segmental amplification or deletion by array CGH, the associated fetal anomalies, and the pregnancy outcomes.

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Material and methods

Case enrollment

Pregnant women who received prenatal array CGH at the Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Taiwan, between January 2014 and December 2017, were enrolled in the study. The study was approved by the Institute Review Board (IRB1803280006) of Chang Gung Memorial Hospital. A total of 836 cases that underwent amniocentesis (814 cases), cordocentesis (4 cases), and chorionic villus sampling (18 cases) were analyzed by array CGH.

Specimen collection

Of the 836 cases, amniocentesis was performed in 814 cases between 16 and 18 weeks of gestation, cordocentesis was performed in four cases between 28 and 33 weeks of gestation, and chorionic villus sampling was performed in 18 cases between 11 and 12 weeks of gestation. We collected 30 ml of amniotic fluid following amniocentesis, 5 ml of cord blood following cordocentesis, and 10 gm of chorionic villi from each of the chorionic villus sampling cases. In cases with result indicating segmental amplification or deletion, maternal and paternal peripheral blood were further collected for array CGH.

Array CGH analysis

We used SurePrint G3 Human CGH Microarray Kit, 8 × 60K (Agilent Technologies, Santa Clara, California, USA) as per the protocol. DNA extraction was performed using QIAamp DNA blood mini Kit (Qiagen, Hilden, Germany). DNA was labeled using Sure Tag DNA Labeling Kit (Agilent Technology, Santa Clara, CA, USA) with Cy3-dUTP. The sex-matched reference human genomic DNA was labeled with Cy5-dUTP. Subsequently, slides scanned using SureScan Microarray scanner (Agilent Technology, Santa Clara, CA, USA) and were analyzed with Feature Extraction Software v11.5 (Agilent Technology, Santa Clara, CA, USA) under designed parameters using human Genome build hg19.

Array CGH data analysis

Data analysis was performed using the Agilent Cytogenomics software available on the company's website (<https://www.genomics.agilent.com/en/CGH-Microarray-Data-Analysis/CytoGenomics-Software/?cid=AG-PT-111&tabId=AG-PR-1017>, Agilent Cytogenomics v2.7.8.0).

Conventional chromosome analysis

Conventional chromosome analysis using *in situ*, cover-slip culture method and Giemsa-trypsin banding technique for metaphase spread was also performed for fetal karyotyping.

Data collection

Among the cases with abnormal results of prenatal array CGH results, for cases diagnosed with segmental amplification or deletion, paternal and maternal blood were further analyzed by array CGH to determine if segmental amplification or deletion was inherited or *de novo*. Data on maternal age, cause for fetal karyotyping, detailed anatomic sonographic findings, gestational age at delivery, newborn birth weight, and associated anomalies, if any, were obtained by chart review.

Ultrasound

Ultrasound examination was performed by two obstetricians between 20 and 24 weeks of gestation for all patients. Specific attention was directed to the fetal face, head, spine, heart, urinary tract, and gastrointestinal tract, and to the amniotic fluid index.

Results

Of the 836 cases, 56 cases (6.70%) had abnormal results (Fig. 1), which included 40 cases (4.82%) with segmental amplification or deletion, 12 cases (1.40%) with trisomy, three cases (0.36%) with monosomy, and one case (0.12%) with sex chromosome aneuploidy; the rest 780 patients (93.30%) had normal results. Case numbers and results of prenatal array CGH by the three sampling methods are summarized in Table 1. Of the 40 cases with segmental amplification/deletion, 30 were inherited and 10 were *de novo* occurrences. Thus, the incidence of *de novo* segmental amplification/deletion is 1.2% (10/836). Of the 40 cases with segmental amplification/deletion, 7 cases were detected by conventional karyotype analysis (Table 2) and 31 cases were not.

Table 3 summarizes the clinical characteristics of the cases with *de novo* segmental amplification or deletion. Of the 10 pregnancies with *de novo* segmental amplification or deletion, abnormal fetal sonographic findings were observed in 40% (4/10) of the cases. Of these 10 pregnancies, nine pregnancies were voluntarily terminated between 22 and 26 weeks of gestation, and one was delivered at term with normal development.

A detailed anatomical survey with ultrasonography was performed by two obstetricians between 20 and 24 weeks of gestation. There are four cases with abnormal fetal sonographic findings, including polycystic kidney disease with intrauterine growth retardation in a case of *de novo* chromosome 17q12 (34856055_36248918) microdeletion; ventricular septum defect with umbilical cord consisting of one artery and one vein in a case of *de novo* chromosome 7q11.23 (72,766,313_74,133,332) microdeletion; congenital heart disease, short femoral length, with neck edema in a case of *de novo* chromosome 11p15.5p14.3 (196966_25033896) amplification/chromosome 11q24.3q25 (129510272_134868407) deletion; and short femoral length in a case of *de novo* chromosome 12p13.33p11.1 (230421_34756209) amplification.

Discussion

Prenatal chromosomal microarray analysis has been shown to have a higher detection rate for chromosomal abnormalities than conventional karyotyping alone [1]. It offers additional diagnostic benefits by revealing submicroscopic imbalances (microdeletions and microduplications) that are too small to be observed using standard G-banded chromosome karyotyping [2]. These submicroscopic imbalances involving specific genomic regions are associated with clinical anomalies. In our study, of the 56 cases with abnormal results, 23 were also detected by conventional karyotype analysis. Thus, the array CGH incremented in diagnostic yield by 3.9% (33/836), which is higher than the 0.9% previously reported [3].

The frequency of prenatally diagnosed segmental amplification or deletion using array CGH was previously reported to be 1.5% [4]. In our series, 4.8% (40/836) fetuses had segmental amplification or deletion, and 1.2% (10/836) fetuses had segmental amplification or deletion that were *de novo* occurrences.

Prenatally diagnosed *de novo* amplification or deletion may present as fetal anomalies in prenatal ultrasound [5–7]. These anomalies may include intrauterine growth restriction, congenital heart disease, brain abnormalities, renal abnormalities, limbs

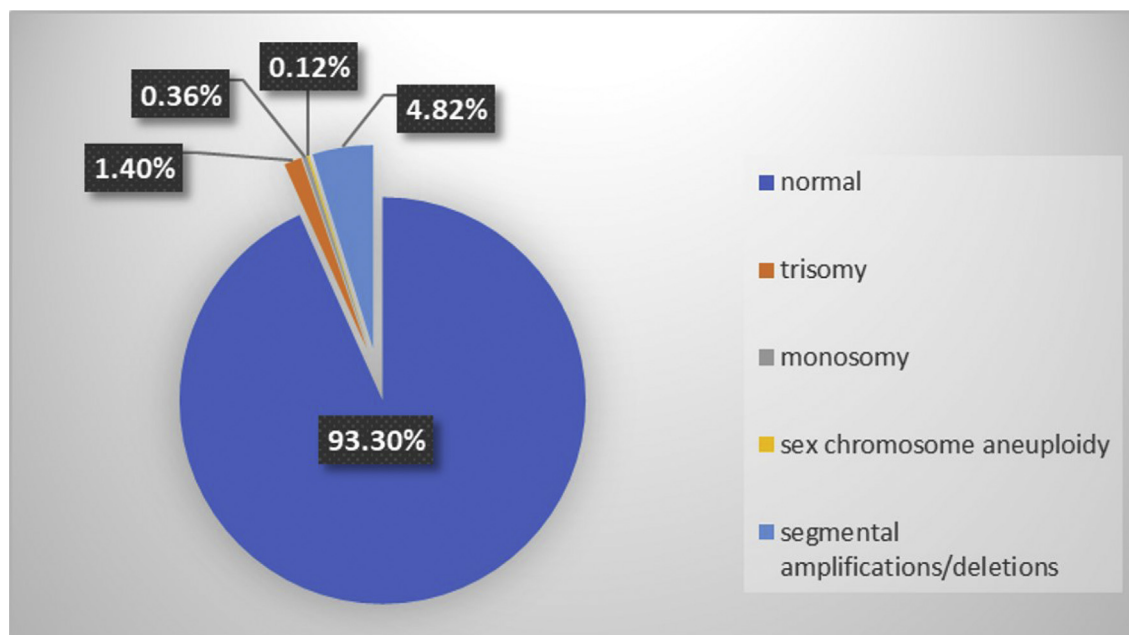


Fig. 1. Percentage of normal and abnormal results of prenatal array CGH.

Table 1

Case numbers and results of prenatal array CGH testing using amniocentesis, cordocentesis, and chorionic villus samples.

Sample source	Total (n)	Normal result (n)	Abnormal result (n)
Amniotic fluid	814	765	2 (trisomy 13) 1 (trisomy 16) 3 (trisomy 18) 3 (trisomy 21) 1 (45,X) 1 (47,XXY) 21 (segmental amplification) 14 (segmental deletion) 3 (segmental amplification/deletion)
Cord blood	4	2	1 (segmental amplification) 1 (segmental amplification/deletion)
Chorionic villi	18	13	1 (trisomy 13) 1 (trisomy 16) 1 (trisomy 18) 2 (45, X)

Table 2

Characteristics of the 7 cases with fetal segmental amplification/deletion detected by conventional karyotype analysis.

Case	Maternal age (y/o)	Results of fetal karyotype	Results of fetal array CGH	Maternal karyotype	Paternal karyotype
1	31	46,XX,der(inv.9)t(9:?) (q13:?)	9p24.3p13.1 (204,193_38,741,437) × 3	46,XX	46,XY,inv(9) (p12q13)
2	38	46,XX,der(20)t(20:?) (p11.23:?)	20p12.1p11.1 (14,567,155_25,678,253) × 3	46,XX, der(20)t(20:?) (p11.23:?)	46,XY
3	33	46,XY,add(11) (q24.2)dn	11p15.5p14.3(196966_25033896) × 3; 11q24.3q25(129510272_134868407) × 1	46,XX	46,XY
4	39	46, XY,r(21) (p12q22)	21q22.3(42,850,359_48,084,156) × 1	46,XX	46,XY
5	39	47,XX,+mar	15q11.2q13.1(22,765,628_28,691,460) × 4	46,XX	46,XY
6	38	Mos 47,XX,+mar(21)/46,XX(9)	15q11.2q13.3(22,765,628_32,411,911) × 4	46,XX	46,XY
7	33	47,XX,+l(12) (p10)	12p13.33p11.1(230421_34756209) × 4	46,XX	46,XY

abnormalities, and increased nuchal translucency [8–14]. In this study involving 10 cases of *de novo* amplification or deletion, four cases (40%) had abnormal sonographic findings.

Fetal multicystic dysplastic kidney has been reported to be involved in several critical regions for renal cysts and diabetes syndrome, Williams–Beuren syndrome, and copy number variants of 22q11.1 duplication, 4q35.2 deletion, 22q13.33 duplication, and

1p33 duplication [15]. The 17q12 deletion syndrome is associated with renal disease, learning disability, behavioral abnormalities, epilepsy, autism, schizophrenia, structural brain abnormalities, facial dimorphism, and joint laxity [16]. Prenatal 17q12 microdeletion causes variability in phenotype resulting in renal malformations, renal echogenicity, and congenital diaphragmatic hernia [17]. In our case, *de novo* microdeletion in chromosome 17q12

Table 3Clinical characteristics of prenatally diagnosed *de novo* segmental amplification/deletion by array CGH.

Case	Maternal age (y/o)	Reason for fetal karyotyping	Sonographic findings	Results of array CGH	Gestational age at delivery	Fetal body weight(gm)	Associated anomalies
1	25	Abnormal sonographic finding	Polycystic kidney disease and intrauterine growth retardation	17q12 (34856055_36248918) × 1	26	120	Polycystic kidney
2	31	Abnormal sonographic finding	Ventricular septum defect with umbilical cord consisting of one artery one vein	7q11.23 (72766313_74133332) × 1	25	370	Williams–Beuren syndrome
3	33	Abnormal sonographic finding	Congenital diaphragmatic hernia, short femoral length, neck edema	11p15.5p14.3(196966_25033896) × 3; 11q24.3q25(129510272_134868407) × 1	22	800	Beckwith–Wiedemann syndrome
4	43	AMA	Negative findings	16p11.2 (28503803_29592842) × 1	23	N/A	nil
5	32	anxiety	Negative findings	8p23.1 (7053186_7752586) × 3	39	3015	nil
6	39	AMA	Negative findings	21q22.3(42850359_48084156) × 1	23	540	nil
7	39	AMA	N/A	15q11.2q13.1(22765628_28691460) × 4	23	420	nil
8	38	AMA	Negative findings	15q11.2q13.3(22765628_32411911) × 4	21	475	nil
9	34	AMA	Negative findings	12p13.3p13.2(255252_10198452) × 3	N/A	N/A	nil
10	33	MSS	Short femoral length	12p13.3p11.1(230421_34756209) × 4	22	532	Short limb

AMA: advanced maternal age, MSS: maternal serum screening for Down syndrome, N/A: not available.

(34856055–36248918) presented with prenatal polycystic kidney disease with intrauterine growth retardation.

Congenital heart disease is associated with chromosomal anomalies, mostly trisomy 21, trisomy 18, and 22q11 microdeletion. Fetuses with congenital heart disease are at increased risk of additional genetic anomalies including microdeletion or microduplication, such as Williams–Beuren syndrome (7q11.23), Potocki–Lupski syndrome (17p11.2 duplications), 8p deletion, 15q11.2 deletion, 16p 11.2 duplication, or monogenetic anomalies, such as Noonan syndrome [7,18]. The phenotypic features of 7q11.23 deletion varied in fetuses, children and adults, which are influenced by the genes, deletion size and breakpoint [19]. In our case of *de novo* microdeletion in chromosome 7q11.23 (72,766,313–74,133,332), the prenatal presentation included ventricular septum defect with umbilical cord consisting of one artery and one vein.

Congenital diaphragmatic hernia is a common congenital birth defect and is associated with significant morbidity and mortality. Genetic causes of congenital diaphragmatic hernia include aneuploidies, chromosome copy number variants, and single gene mutations [20,21]. Mutations or epigenetic events occurring on the genes at the chromosome 11p15.5's critical imprinting region leads to disorders such as Beckwith–Wiedemann syndrome (BWS), with prenatal presentations of overgrowth, macroglossia, visceromegaly, abdominal wall defect, and renal anomalies. In our case of *de novo* amplification in chromosome 11p15.5p14.3(196966_25033896) and deletion in chromosome 11q24.3q25(129510272_134868407), the duplicated chromosome 11p segment involved a critical region containing genes for BWS, so further QR-PCR analysis using STR markers specific for 11p15.5 on the DNA from both parents and the fetus was performed which revealed a *de novo* duplication of 11p15.5 and a paternal origin of the duplication. The prenatal presentation included congenital diaphragmatic hernia, short femoral length, and neck edema.

In conclusion, prenatal diagnosis of *de novo* segmental amplification or deletion by array CGH raises important genetic counseling issues. Our study demonstrate that the incidence of prenatal diagnosed *de novo* segmental amplification or deletion was 1.2%, abnormal prenatal ultrasound findings occurred in 40% cases, and 90% cases choose to termination of pregnancy.

Conflicts of interest

The authors declared no conflicts of interest.

Acknowledgements

The study was supported by grant CORPG3G0301 provided by Chang Gung Memorial Hospital (Tao-yuan, Taiwan).

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