Received: 16 August 2019 | Revised: 15 October 2019 | Accepted: 26 October 2019

DOI: 10.1002/jcla.23117

RESEARCH ARTICLE

WILEY

Chromosomal microarray analysis for pregnancies with or without ultrasound abnormalities in women of advanced maternal age

Xiaoqing Wu | Gang An | Xiaorui Xie | Linjuan Su | Meiying Cai | Xuemei Chen | Ying Li | Na Lin | Deqin He | Meiying Wang | Hailong Huang | Liangpu Xu

Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

Correspondence
Liangpu Xu, Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, No. 18 Daoshan Road, Gulou District, Fuzhou 350001, China.
Email: xiliangpu@fjmu.edu.cn

Funding information
The present study was supported by The Fujian Provincial Natural Science Foundation (Grant No. 2017J01238).

Abstract

Background: Chromosomal microarray analysis (CMA) has been suggested to be routinely conducted for fetuses with ultrasound abnormalities (UA), especially with ultrasound structural anomalies (USA). Whether to routinely offer CMA to women of advanced maternal age (AMA) without UA when undergoing invasive prenatal testing is inconclusive.

Objective: This study aimed to evaluate the efficiency of CMA in detecting clinically significant chromosomal abnormalities in fetuses, with or without UA, of women with AMA.

Methods: Data from singleton pregnancies referred for prenatal CMA due to AMA, with or without UA were obtained. The enrolled cases were divided into AMA group (group A) and AMA accompanied by UA group (group B). Single nucleotide polymorphism (SNP) array technology and conventional karyotyping were performed simultaneously.

Results: A total of 703 cases were enrolled and divided into group A (N = 437) and group B (N = 266). Clinically significant abnormalities were detected by CMA in 52 cases (7.4%, 52/703; the value in group A was significantly lower than that in group B (3.9% vs 13.2%, $P < .05$); no statistic difference was observed with respect to submicroscopic variants of clinical significance between the two groups (0.9% vs 2.6%, $P > .05$).

Conclusions: Chromosomal microarray analysis should be available to all women with AMA undergoing invasive prenatal testing, regardless of ultrasound findings.

KEYWORDS

advanced maternal age, chromosomal microarray analysis, conventional karyotyping, ultrasound abnormalities

1 | INTRODUCTION

Women with advanced maternal age (AMA) refer to pregnant women older than 35 years. The number of women with AMA has increased year by year worldwide. The proportion of women with AMA in China rose from 10.1% in 2011[1] to 20.5% in 2016.[2] For decades, AMA has been a leading indicator of invasive prenatal testing. In recent years, many pregnant women with AMA are willing to accept non-invasive prenatal testing (NIPT) that can identify the most common fetal aneuploidies (trisomy 21, trisomy 18, and trisomy 13), and sex chromosome aneuploidies (SCAs)[3]; however, there are still a large number of women with AMA opting for invasive testing.

Conventional karyotyping can identify the majority of fetal chromosome abnormalities, at a resolution of greater than 10 Mb.[4] With the implementation of chromosomal microarray analysis (CMA), many chromosomal abnormalities that were undetectable by karyotyping were found in either prenatal or postnatal testing.[5-7] Microdeletions and microduplications have been reported in approximately 6% of pregnancies with a structural fetal abnormality and approximately 1.7% of other high-risk pregnancies.[8,9] Wapner et al[9,10] suggest that all pregnant women, regardless of age, would equally benefit from invasive testing by fetal microarray analysis. Previous reports support CMA for all women undergoing prenatal invasive testing regardless of the presence or absence of fetal abnormality. It is well known that AMA women are at increased risk for aneuploidy due to non-disjunction, while non-AMA pregnancies have a higher risk for submicroscopic pathogenic aberrations than for Down syndrome.[11] Therefore, AMA women without fetal ultrasound abnormalities (UA) always express hesitation about performing CMA. Other concerns include the theoretical risk of detecting variants of unknown clinical significance (VOUS), and variants in susceptibility loci (SL),[12,13] as well as the higher cost of CMA compared to that of karyotyping. The purpose of this study was to evaluate the efficiency of CMA in the prenatal diagnosis of AMA women with or without UA, to provide further practical evidence for pre-testing consultation.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

This retrospective study reviewed 764 singleton pregnant women with AMA who underwent invasive prenatal CMA in the second or third trimester from March 2016 to October 2018. Sixty-one women were excluded from this study for familial structural chromosome aberrations, adverse reproductive histories such as having a previous child with chromosome anomalies or dysplasia, or abnormal serological screening. As a result, 703 cases were enrolled. The mean age at delivery was 37.8 ± 2.6 years, and the mean gestational age at prenatal invasive testing was 21.4 ± 3.3 weeks. The specimens were comprised of amniotic fluid obtained during 18 and 24 gestational weeks, and fetal cord blood obtained during 25 and 35 gestational weeks (Table 1). The resulting 703 cases enrolled were divided into the AMA group (Group A: n = 439) and the AMA accompanied by UA group (Group B: n = 266). The UA included soft markers (n = 146), ultrasound structural abnormalities (USA) (n = 84), and non-structural anomalies (fetal growth restriction, FGR, or polyhydramnios) (n = 36). This study was approved by the local Ethics Committee of the Fujian Maternal and Child Health Hospital. Written informed consent to participate in the study was obtained from each patient.

2.2 | CMA platforms and data interpretation

Genomic DNA was extracted from uncultured or cultured amniotic fluid and fetal cord blood with QIAGEN kit (Qiagen) according to its manufacturer's instructions. Chromosomal microarray analysis was performed with an Affymetrix CytoScan 750K array

TABLE 1 Demographic characters for 703 women of AMA

	All (n = 703)	Group A (n = 437)	Group B (n = 266)	*P* value
Age at delivery (y): mean ± SD	37.8 ± 2.6	37.5 ± 2.7	37.2 ± 2.4	>.05
Gestation age at invasive testing (wk): mean ± SD	21.4 ± 3.3	20.0 ± 1.6	23.8 ± 3.9	<.05
Specimens				
Amniotic fluid n (%)	623 (88.6%)	428 (97.9%)	195 (73.3%)	<.05
Cord blood n (%)	80 (11.4%)	9 (2.1%)	71 (26.7%)	
Outcomes				
Ongoing/Live born n (%)	650 (92.5%)	422 (96.6%)	228 (85.7%)	<.05
TOP n (%)	53 (7.5%)	15 (3.4%)	38 (14.3%)	

Note: Group A: AMA without UA group; Group B: AMA accompanied with UA group.

Abbreviations: AMA, advanced maternal age; n, number of cases; TOP, termination of pregnancy; UA, ultrasound abnormality.

TABLE 2 List of clinical significant CMA findings for Group A

Case number	CMA results	Copy number changes	Karyotype results	Parental testing	Related syndrome	Outcome
Microscopic						
1-3	arr(21)×3	Dup	47,XX+21	——	Down's syndrome	TOP
4-5	arr(21)×3	Dup	47,XY,+21	——	Down's syndrome	TOP
6	arr(18)×3	Dup	47,XX,+18	——	Edwards syndrome	TOP
7	arr(X)×2, (Y)×1	Dup	47,XXY	——	Klinefelter's syndrome	TOP
8	arr(X)×1, (Y)×2	Dup	47,XYY	——	Jacobs syndrome	TOP
9	arr(X)×1	Loss	45,X	——	Turner syndrome	TOP
10	arr(9)×3[0.5]	Mosaic dup	46,XY,+9,rob(13;21)(q10;q10) /45,XY,rob(13;21)(q10;q10)	De novo	Mosaic trisomy 9	TOP
11	arr[GRCh37] 12p11.21q12(31269113_42349971)×3	Dup (11 Mb)	47,XY,+mar	——	Non-syndromic	TOP
12	arr[GRCh37] 12p13.33p11.1(173786_34835641)×3	Dup (34 Mb)	47,XX,+mar	——	Non-syndromic	TOP
13	arr[GRCh37] 12p13.33p11.1(173786_34759042)×2-3 20p13p11.1(186793_26129447)×2-3	Mosaic Dup (34.5 Mb, 25.9 Mb)	47,XY,+mar/46,XY	De novo	Non-syndromic	TOP
Submicroscopic						
14	arr[GRCh37] 2q32.3q33.1(195940640_199623902)×1	Del (3.6 Mb)	46,XY	De novo	Non-syndromic	TOP
15	arr[GRCh37] 15q11.2(22770421_23625785)×1	Del (855 kb)	46,XX	De novo	15q11.2 deletion syndrome	Live born
16	arr[GRCh37] 16p13.11(15481747_16278133)×3	Dup (796 kb)	46,XX	De novo	16p13.11 recurrent microduplication syndrome	Live born
17	arr[GRCh37] 1p32.1p31.1(60575608_71024736)×3	Dup (10.4 Mb)	46,XX	De novo	Non-syndromic	TOP

Abbreviations: Del, deletion; Dup, duplication; TOP, termination of pregnancy.

TABLE 3 Details of the fetuses with clinical significant CMA findings in Group B

Case number	Ultrasound findings	CMA results	Karyotype results	Parental testing	Related syndrome	Outcome
Soft markers						
18-20	Nuchal translucency thickness	arr(21)×3	47,XX,+21	—	Down's syndrome	TOP
21	Ventricular echogenicity					
22	Nuchal translucency thickness, cerebral ventriculomegaly	arr(21)×3	47,XY,+21	—	Down's syndrome	TOP
23	Nuchal translucency thickness, Ventricular echogenicity, Pyelic separation					
24	Ventricular echogenicity, echogenic bowel, echogenic kidneys					
25	Nuchal translucency thickness	arr(18)×3	47,XY,+18	—	Edwards syndrome	TOP
26	Choroid plexus cyst					
27	Nuchal translucency thickness	arr(13)×3	47,XX,+13	—	Patau syndrome	TOP
28	Nuchal translucency thickness, Ventricular echogenicity, single umbilical artery	arr(X)×1	45,X	—	Turner syndrome	TOP
29	Nuchal translucency thickness	arr(X)×3	47,XXX	—	Trisomy X	TOP
30	Ventricular echogenicity, Mild tricuspid regurgitation	arr[GRCh37] 15q11.2(22770421_23277436)×1 507 kb	46,XX	Paternal	15q11.2 deletion syndrome	Live born
31	Nuchal translucency thickness	arr[GRCh37] 16p13.11(14910158_16508123)×1 1.6 Mb	46,XY	De novo	16p13.11 recurrent microdeletion syndrome	Live born
32	Nuchal translucency thickness	arr[GRCh37] 1q21.1q21.2(145829473_148520164) ×1 2.7 Mb	46,XX	De novo	1q21.1 microdeletion syndrome	TOP
Structural abnormalities						
33-34	CHD	arr(18)×3	47,XX,+18	—	Edwards syndrome	TOP
35	CHD, Cranial dysplasia	arr(18)×3	47,XY,+18	—	Edwards syndrome	TOP
36	CHD	arr(21)×3	47,XY,+21	—	Down's syndrome	TOP
37	CHD	arr(X)×1	45,X	—	Turner syndrome	TOP
38	Spinal dysplasia	arr(8)×3[0.7]	47,XY,+8/46,XY	—	Mosaic trisomy 8 syndrome	TOP
39	CHD	arr[GRCh37] 4q25q28.1(112192577_127874789)×1 15.6 Mb	46,XX,del(4)(q25q28)	—	Non-syndromic	TOP
40	CHD, Cranial dysplasia	arr[GRCh37]13q31.3q34(94929201_115107733)×1 20.1 Mb	46,XX,r(13)(?p11q32)/45,XX,-13	—	Non-syndromic	TOP
41-42	CHD	arr[GRCh37] 22q11.1q11.21(16888899_18649190)×4 1.7 Mb	47,XY,+mar	De novo	Non-syndromic	TOP
43	CHD, Biped varus, severe pulmonary stenosis	arr[GRCh37] 5p15.33p11(113576_46242541)×3 46 Mb	47,XX,+mar	De novo	Non-syndromic	TOP

(Continues)

TABLE 3 (Continued)

Case number	Ultrasound findings	CMA results	Karyotype results	Parental testing	Related syndrome	Outcome
44	CHD, Gallbladder enlargement	arr[GRCh37] 16p13.3(85880_536631)×1, 17q24 .2q25.3(64966574_81041823)×3 451 kb, 16 Mb	46,XX,add(16)(p13.3)	De novo	Non-syndromic	TOP
45-46	CHD	arr[GRCh37] 22q11.21(18648855_21800471)×1 3.1 Mb	46,XY	De novo	DiGeorge syndrome	TOP
Non-structural abnormalities						
47	FGR	arr(21)×3	47,XX,+21	—	Down's syndrome	TOP
48	FGR	arr(X)×1, (Y)×2	47,XYY	—	Jacobs syndrome	TOP
49	FGR	arr[GRCh37] 9p24.3q13(208454_68216577)×4 68 Mb	47,XY,+psuidic(9)(q12)	—	Tetrasomy 9p syndrome	TOP
50	FGR	arr (13)×3, (20)×3[0.4]	48,XY,+13,+20/46,XY	—	Non-syndromic	TOP
51	FGR	arr(22)×3[0.3]	46,XX	—	Mosaic trisomy-22 syndrome	TOP
52	FGR	arr[GRCh37] 7q11.23(72713282_74154209)×1 1.4 Mb	46,XY	De novo	Williams syndrome	TOP

Abbreviations: CHD, congenital heart defect; FGR, fetal growth restriction; TOP, termination of pregnancy.

(Affymetrix Inc), which includes 200 000 probes for single nucleotide polymorphisms and 550 000 probes for copy number variations (CNVs) distributed across the entire human genome. To analyze the results, Chromosome Analysis Suite software (Affymetrix) and human genome version GRCh37 (hg19) were used. A resolution was generally applied: gains or losses 400 kb and loss of heterozygosity (LOH) 10 Mb. All detected CNVs were compared with in-house and national public CNV databases as following: Database of Genomic Variants (DGV), Database of Chromosome Imbalance and Phenotype in Humans Using Ensemble Resources (DECIPHER), International Standards for Cytogenomic Arrays Consortium, and Online Mendelian Inheritance in Man (OMIM). The CNVs were classified five levels according to the American College of Medical Genetics (ACMG) definitions[14]: pathogenic, benign, likely pathogenic, likely benign, and variants of unknown significance (VOUS). All of these results were reported to patients.

In general, microscopically visible variants (with net imbalance ≥ 10 Mb) and submicroscopic (<10 Mb) pathogenic/likely pathogenic CNVs as clinical significant findings. However, we are aware that submicroscopic chromosome abnormalities sometimes involved low-level mosaicism with size >10 Mb. Parental CMA was recommended to determine the inheritance of CNVs. In general, CNVs inherited from normal phenotype parents were regarded as likely benign, whereas de novo fetal mutations were regarded as likely pathogenic. If the CNVs have been reported to have incomplete penetrance and/or variable expressivity, we consider as likely pathogenic variants, even though it is inherited from a parent with normal phenotype.

2.3 | Conventional karyotyping

Conventional karyotyping consisted of cell culture and G-banded karyotyping was performed according to the standard protocols in our laboratory. Cultured amniotic fluid or fetal cord blood then arrested in metaphase and finally Wright's stain was used for G-banding at a resolution of 320-500 bands.

2.4 | Statistical analysis

SPSS software v19.0 (SPSS Inc, Chicago, IL, US) was used for statistical analysis of the data. Statistical comparisons were performed using the independent samples *t* test and chi-square test. $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Patient clinical characteristics

In total, 703 cases were enrolled in the study. Demographic data are shown in Table 1: no significant differences were found between

groups A and B with respect to age at delivery; conversely, significant differences between group A and B were observed in gestational age at invasive testing, in type of specimens, and in pregnancy outcomes.

3.2 | CMA findings for the two groups

All 703 prenatal samples were processed in parallel using both CMA and conventional karyotyping. Abnormal CMA findings were detected in 60 (8.5%) fetuses. Among them, 52 (7.3%, 52/703) cases of clinically significant variants, 3 (0.4%, 3/703) cases of VOUS, 3 (0.4%, 3/703) cases of likely benign CNVs, and 2 (2.8%, 2/703) cases of LOH were identified.

In group A, 17 out of 437 (3.9%) cases yielded clinically significant results (Table 2). Of those, 13 (2.9% of the cohort) were microscopic and 4 (0.9%) were submicroscopic CNVs detected by CMA only. Thirteen microscopic aberrations involved 9 (2.1%) cases of aneuploidies, 1 case of mosaic trisomy 9, whose cytogenetic karyotyping returned an additional CMA-undetectable Roberson translocation (Case 10). As for another 3 cases (case 11-13), cytogenetics found non-mosaic or mosaic small supernumerary marker chromosomes (sSMC), while CMA revealed duplication in region 12p11.21q12, region 12p13.33p11.1, as well as mosaic duplication both in region 12p13.33p11.1 and 20p13p11.1, respectively. Four submicroscopic CNVs (Case 14-17) sized from 796 kb to 10.4 Mb. Two were related to known syndrome with variable penetrance: 15q11.2 deletion syndrome (Case 15) and 16p13.11 recurrent microduplication syndrome (Case 16). Case 14 showed a de novo 3.6 Mb deletion in the 2q32.3q33.1 region, containing 12 OMIM genes. This deletion may cause stunted development, mental retardation, and characteristic facial features. Based on this result combined with the fetal cerebral ventriculomegaly found by ultrasonography at 30 weeks of gestation, the patient chose to terminate the pregnancy. Case 17 revealed a 10.4 Mb duplication in the region of 1p32.1p31.1, which maybe also related to developmental delay, mental retardation, poor language, and cognitive impairment.

In group B, the frequency of clinically significant findings was 13.2% (35/266) (Table 3), which was significantly higher than that in group A (13.2% vs 3.9%, $P < .05$). Seven cases were only detected by CMA, contributing to a detection rate of 2.6% (7/266) for clinically significant submicroscopic findings, and it showed no statistic difference from group A (2.6% vs 0.9%, $P > .05$). These 7 cases involved 6 cases of microdeletion sized from 507 kb to 3.1 Mb and 1 case of low-level of mosaic trisomy 22 (Case 51). Case 51 underwent amniocenteses twice during a 3-week period; the CMA results from uncultured amniotic fluid were always mosaic trisomy 22, while the karyotypes from cultured amniotic fluid were normal. The fetus manifested with progressive fetal growth restriction (FGR), which was consistent with the phenotype of mosaic trisomy 22. The remaining 28 (10.5%) cases were karyotype detectable, including 15 cases of autosomal aneuploidy, 4 cases of sex chromosome aneuploidy, 2 cases of mosaic trisomy, 3 cases that harbored at least one duplication/deletion sized more than 10 Mb, as well as 3 cases of sSMC. In the 3 sSMCs, 2 were reported partial tetrasomy of chromosome 22q11.1 (Case 41-42), and 1 returned a result of duplication in region 5p15.33p11 by CMA (Case 43). Trisomy 21 was the most common aneuploidy, especially in the subgroup of soft markers, trisomy 21 was the most frequent abnormalities (Table 4). In addition to clinically significant findings, group B yielded 3 (1.1%) cases of VOUS (Table 5), while no VOUS was detected in group A, contributing to an overall detection rate of 0.4%.

It is worth noting that CMA confirmed the nature and origin of 8 (1.1%, 8/703) cases of sSMC. Of these, four were from group A: three were clinically significant variants (Case 11-13) and one (not listed) was normal by CMA analysis; another four cases from group B revealed three pathogenic aberrations (Case 41-43), and one (not listed) with no change in genetic material.

4 | DISCUSSION

There have been various reports on the efficacy of CMA in high-risk (mainly USA) and low-risk populations.[15,16] However, in the population of AMA women, comparison between pregnancy with and without ultrasound findings was rarely reported. The purpose of this study was to investigate the necessity to perform CMA in women with AMA undergoing invasive prenatal testing.

We found significant differences in gestational age at diagnosis, types of specimens, and pregnancy outcomes between the two experimental groups in this study. Amniotic fluid was the predominant specimen in group A, whereas the proportion of cord blood in group B was significantly higher. This was closely related to the gestational age at the time of diagnosis. Majority of pregnant women in group A readily preferred amniocenteses because compared with choriocentesis and cordocentesis, amniocentesis is more acceptable. Accordingly, they underwent amniocentesis at the proper time. Ultrasonic structure screening is generally conducted during the 20th and 24th gestational week, and amniocentesis is generally performed before 24 weeks of gestation. Therefore, several pregnant women may be detected with fetal ultrasound problems at a later gestational age after amniotic fluid testing. This was the circumstance for case 15. The patient made the decision to terminate the pregnancy, taking into account the CMA results and subsequent ultrasound findings. This further indicates the importance of offering CMA when performing amniocentesis, that is, to avoid later cordocentesis and save time for the patient as well.

As expected, trisomy was the most frequent abnormal findings in this study. Chromosomal microarray analysis showed a detection rate of 0.9% for submicroscopic findings with clinical significance in the group of AMA without UA, lower compared with 1.0%-2.1% reported in other literatures.[17-20] It has been reported that incremental diagnostic yield of CMA for fetuses with UA ranged from 5.2% to 10%, fetuses with ultrasound structural abnormalities ranged from 3.1% to 7.9%.[16] In our study, the value was 2.6% and 3.6%,

TABLE 4 Types of CMA findings and their frequencies in 703 fetuses

			Ultrasound anomalies (n = 266)		
	Group A (n = 437)	Group B (n = 266)	Soft markers (n = 146)	Structural anomalies (n = 84)	Non-structural anomalies (n = 36)
Clinical significant variants	17 (3.9%)	35 (13.2%)	14 (9.6%)	15 (17.9%)	6 (16.7%)
Microscopic	13 (3.0%)	28 (10.5%)	12 (8.2%)	12 (14.3%)	4 (11.1%)
T21	5 (1.1%)	9 (3.4%)	7 (4.8%)	1 (1.2%)	1 (2.8%)
T18 and T13	1 (0.2%)	6 (2.3%)	3 (2.1%)	3 (3.6%)	0 (0.0%)
SCA	3 (0.7%)	4 (1.5%)	2 (1.4%)	1 (1.2%)	1 (2.8%)
Mosaicism	2 (0.5%)	2 (0.8%)	0 (0.0%)	1 (1.2%)	1 (2.8%)
Deletion/duplication	2 (0.5%)	7 (2.6%)	0 (0.0%)	6 (7.1%)	1 (2.8%)
Submicroscopic	4 (0.9%)	7 (2.6%)	2 (1.4%)	3 (3.6%)	2 (5.6%)
Microdeletion	2 (0.5%)	6 (2.3%)	2 (1.4%)	3 (3.6%)	1 (2.8%)
Microduplication	2 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mosaicism	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (2.8%)
VOUS	0 (0.0%)	3 (1.1%)	1 (0.7%)	1 (1.2%)	1 (2.8%)
Likely benign CNVs	0 (0.0%)	3 (1.1%)	2 (1.4%)	1 (1.2%)	0 (0.0%)
Non-CNVs	2 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Abbreviation: T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; SCA, sex chromosomal aneuploidy; VOUS, variants of unknown significance.

respectively, this can be explained by the great proportion of aneuploidies caused by AMA women's basic risk for aneuploidy. The clinical significant findings in group B is more frequent than that in group A, but submicroscopic findings in the two groups showed no statistic difference. This may further imply that AMA women could be recommended for CMA testing just as UA group. Notably, clinically significant submicroscopic findings in group A were mostly associated with neurological or mental dysplasia. A susceptibility locus (SL) for neurodevelopmental disorders is considered the most common finding in pregnancies without UA.[20] They may be categorized as pathogenic/likely pathogenic findings in spite of their incomplete penetrance and variable expressivity, even inheritance from normal parents.[21-23] SL is generally associated with autism, learning and speech problems, mild intellectual disability (ID), or developmental delay (DD). It is well established that a second hit plays a vital role in causing an abnormal phenotype.[24] In our study, there were four cases harboring SL: 15q11.2 deletion (case 15) and 16p13.11 duplication (case 16) in group A; 15q11.2 deletion (case 30), 16p13.11 deletion (case 31) in group B. The 15q11.2 microdeletion was considered to be responsible for 15q11.2 BP1-BP2 microdeletion syndrome.

TABLE 5 Characteristics of variants without clinical significance detected by CMA

Case number	Indications	Chromosome region	Copy number changes	Inheritance	Significance
53	AMA, prefrontal skin thickens	11p11.12q11(50608808_55363330)	Gain (4.7 Mb)	De novo	VOUS
54	AMA, VSD, lateral ventriculomegaly,	11p15.1p14.3(20745930_21780075)	Gain (1.0 Mb)	De novo	VOUS
55	AMA, polyhydramnios	2q22.2(143043284_143866399)	Gain (823 kb)	De novo	VOUS
56	AMA	6q14.3q21(87299268_110741585)	LOH (23.4 Mb)	De novo	Non-CNVs
57	AMA	3p13q13.31(71435373_116447779)	LOH (45.0 Mb)	De novo	Non-CNVs
58	AMA, lateral ventriculomegaly	2q13(110498141_110980295)	Gain (500 kb)	De novo	Likely benign
59	AMA, lateral ventriculomegaly, echogenic bowl	5q33.2q33.3(154435034_156727811)	Gain (2.29 Mb)	Paternal	Likely benign
60	AMA, Right ventricular wall thickened, hydropericardium	3p22.3(33805560_35318562)	Gain (1.5 Mb)	Maternal	Likely benign

Abbreviations: AMA, advanced maternal age; VSD, ventricular septal defect.

It was previously recognized as a VOUS but now is considered as likely pathogenic/pathogenic variants in many reports.[25,26] The penetration of the variants was estimated to be 10.4%,[27] and the prevalence in patients with mental retardation, ID, and/or multiple congenital anomalies was about two to fourfold of that in control population.[27-29] It is well known that being rich in low copy repeats (LCRs) increases the occurrence of non-allelic homologous recombination resulting in duplications or deletions. This is particular for chromosome 16. Nearly 9.89% of chromosome 16 consists of LCRs. The 16p13.11 locus is a genomic hotspot encompasses a core set of eight protein-coding genes, including NDE1, the strongest candidate gene for the neurodevelopmental phenotypes. 16p13.11 deletions are considered as pathogenic for its association with multiple phenotypic manifestations, including neurodevelopmental phenotypes such as autism, epilepsy, physical dysmorphisms, and other congenital anomalies.[30-33] 16p13.11 microduplication syndrome was the most commonly detected microduplication syndrome in a postnatal cohort with unexplained DD, ID, and autistic spectrum disorder (ASD)[34]; the penetration was estimated to be 10.6%. As shown in our study, fetuses with SL can be found in ultrasound normal or soft marker group, although they were live born with normal phenotype, long-time follow-up is crucial to assess the actual risks for neurodevelopmental disorders.[35]

Chromosomal microarray analysis is of great value not only for detecting submicroscopic anomalies but also cytogenetic abnormalities with unknown origins, such as small supernumerary marker chromosomes (sSMC), whose clinical phenotype and prognosis depend on the chromosomal origin. sSMC were reported to be associated with AMA.[36] Identification of the nature and source of sSMC is the most important for pregnancy guidance. In the current study, 8 cases with de novo sSMC were diagnosed in both the groups (0.14%), 4 in each; this figure is higher than that reported previously.[37,38] Finally, one case in each group was confirmed by CMA as having no change in the genetic material, and the pregnancy was continued.

In fact, an increasing number of women has gradually accepted this relatively high-cost test and self-financed testing. Other barriers for consultants to offer CMA lie in interpreting the VOUS results and patient-related concerns such as anxiety.[39] The VOUS rate detected by CMA shows considerable variability.[40,41] In present study, VOUS was all detected from UA group and had a relatively lower detection rate (0.4%) than other literatures, this may be explained by the limited case number. From a patient's point of view, they want to exclude as many abnormalities as possible to ensure that the fetus is healthy. Whether to choose CMA mainly depends on pre-test counseling. As shown in this study and previous literature, while improving the detection rate of submicroscopic abnormalities in pregnancies of AMA, CMA also yields uncertain results which are unavoidable in prenatal testing for any other indications. Therefore, research should be focused on how the results should be managed, and how to provide reasonable consultation, rather than trying to avoid the uncertain results. A reasonable consultation is inseparable from the accumulation of experience, and prenatal findings combined with long-term postnatal follow-up are essential means to gaining experience. It is only by this method that we can provide more evidence for consultation, thus release the burdens on genetic consultants and patients.

In conclusion, CMA has a higher detection rate of chromosomal abnormality than conventional karyotyping, either in AMA women with or without UA. As for the population of AMA women, CMA should be available to all pregnancies undergoing invasive prenatal testing, regardless of ultrasound results.

ACKNOWLEDGMENTS

The authors want to thank Min Zhang and Yan Wang for their technical support on SNP analysis.

ETHICAL APPROVAL

The present study was approved by the Protection of Human Ethics Committee of Fujian Provincial Maternity and Children's Hospital, affiliated Hospital of Fujian Medical University. Written informed consent for participation was received for all patients.

ORCID

Xiaoqing Wu https://orcid.org/0000-0003-4797-952X
Hailong Huang https://orcid.org/0000-0001-5775-5082

DATA AVAILABILITY STATEMENT

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

REFERENCES

1. Liu X, Zou L, Chen Y, Ruan Y, Liu Y, Zhang W. Effects of maternal age on pregnancy: a retrospective cohort study. *Natl Med J Chin*. 2014;94(25):1984-1988.
2. Chen Y, Zheng XL, Wu SW, Zhang WY. Clinic characteristics of women with advanced maternal age and perinatal outcomes. *Zhonghua Fu Chan Ke Za Zhi*. 2017;52;508-513.
3. Zhang B, Lu BY, Yu B, et al. Noninvasive prenatal screening for fetal common sex chromosome aneuploidies from maternal blood. *J Int Med Res*. 2017;45(2):621-630.
4. Smeets DF. Historical prospective of human cytogenetics: from microscope to microarray. *Clin Biochem*. 2004;37(6):439-446.
5. Zhu Y, Lu S, Bian X, et al. A multicenter study of fetal chromosomal abnormalities in Chinese women of advanced maternal age. *Taiw J Obstetr Gynecol*. 2016;55(3):379-384.
6. Reddy UM, Page GP, Saade GR, et al. Karyotype versus microarray testing for genetic abnormalities after stillbirth. *New Engl J Med*. 2012;367(23):2185-2193.
7. Shaffer LG, Dabell MP, Fisher AJ, et al. Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies. *Prenat Diagn*. 2012;32(10):976-985.
8. Ferreira JC, Grati FR, Bajaj K, et al. Frequency of fetal karyotype abnormalities in women undergoing invasive testing in the absence of ultrasound and other high-risk indications. *Prenat Diagn*. 2016;36(12):1146.
9. Fiorentino F, Napoletano S, Caiazzo F, et al. Chromosomal microarray analysis as a first-line test in pregnancies with a priori low risk for the detection of submicroscopic chromosomal abnormalities. *Eur J Hum Genet*. 2013;21(7):725-730.
10. Hay SB, Sahoo T, Travis MK, et al. ACOG and SMFM guidelines for prenatal diagnosis: is karyotyping really sufficient? *Prenat Diagn*. 2018;38(3):184-189.

11. Srebniak MI, Joosten M, Knapen M, et al. Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis. *Ultrasound Obstetr Gynecol*. 2018;51(4):445-452.
12. Friedman JM. High-resolution array genomic hybridization in prenatal diagnosis. *Prenat Diagn*. 2009;29(1):20-28.
13. Shuster E. Microarray genetic screening: a prenatal roadblock for life? *Lancet (London, England)*. 2007;369(9560):526-529.
14. South ST, Lee C, Lamb AN, Higgins AW, Kearney HM. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med*. 2013;15(11):901-909.
15. Hillman SC, McMullan DJ, Hall G, et al. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound Obstetr Gynecol*. 2013;41(6):610-620.
16. de Wit MC, Srebniak MI, Govaerts LC, Van Opstal D, Galjaard RJH, Go ATJI. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature. *Ultrasound Obstetr Gynecol*. 2014;43(2):139-146.
17. Callaway JL, Shaffer LG, Chitty LS, Rosenfeld JA, Crolla JA. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature. *Prenat Diagn*. 2013;33(12):1119-1123.
18. Armengol L, Nevado J, Serra-Juhe C, et al. Clinical utility of chromosomal microarray analysis in invasive prenatal diagnosis. *Hum Genet*. 2012;131(3):513-523.
19. Sagi-Dain L, Cohen Vig L, Kahana S, et al. Chromosomal microarray vs. NIPS: analysis of 5541 low-risk pregnancies. *Genet Med*. 2019;21(11):2462-2467.
20. Van Opstal D, de Vries F, Govaerts L, et al. Benefits and burdens of using a SNP array in pregnancies at increased risk for the common aneuploidies. *Hum Mutat*. 2015;36(3):319-326.
21. Cooper GM, Coe BP, Girirajan S, et al. A copy number variation morbidity map of developmental delay. *Nat Genet*. 2011;43(9):838-846.
22. Kaminsky EB, Kaul V, Paschall J, et al. An evidence-based approach to establish the functional and clinical significance of copy number variants in intellectual and developmental disabilities. *Genet Med*. 2011;13(9):777-784.
23. Srebniak MI, Diderich KE, Govaerts LC, et al. Types of array findings detectable in cytogenetic diagnosis: a proposal for a generic classification. *Eur J Hum Genet*. 2014;22(7):856-858.
24. Girirajan S, Rosenfeld JA, Cooper GM, et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet*. 2010;42(3):203-209.
25. Davis KW, Serrano M, Loddo S, et al. Parent-of-origin effects in 15q11.2 BP1-BP2 microdeletion (Burnside-Butler) syndrome. *Int J Mol Sci*. 2019;20(6):1459.
26. Brabbing-Goldstein D, Reches A, Svirsky R, Bar-Shira A, Yaron Y. Dilemmas in genetic counseling for low-penetrance neuro-susceptibility loci detected on prenatal chromosomal microarray analysis. *Am J Obstet Gynecol*. 2018;218(2):247.e1-247.e12.
27. Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG. Estimates of penetrance for recurrent pathogenic copy-number variations. *Genet Med*. 2013;15(6):478-481.
28. Hannes FD, Sharp AJ, Mefford HC, et al. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet*. 2009;46(4):223-232.
29. Cox DM, Butler MG. The 15q11.2 BP1-BP2 microdeletion syndrome: a review. *Int J Mol Sci*. 2015;16(2):4068-4082.
30. de Kovel CG, Trucks H, Helbig I, et al. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. *Brain*. 2010;133(Pt 1):23-32.
31. Heinzen EL, Radtke RA, Urban TJ, et al. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet*. 2010;86(5):707-718.
32. Mefford HC, Muhle H, Ostertag P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet*. 2010;6(5):e1000962.
33. Ullmann R, Turner G, Kirchhoff M, et al. Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum Mutat*. 2007;28(7):674-682.
34. Cheng SSW, Chan KYK, Leung KKP, et al. Experience of chromosomal microarray applied in prenatal and postnatal settings in Hong Kong. *Am J Med Genet C Semin Med Genet*. 2019;181(2):196-207.
35. Govaerts L, Srebniak M, Diderich K, et al. Prenatal diagnosis of susceptibility loci for neurodevelopmental disorders - genetic counseling and pregnancy outcome in 57 cases. *Prenat Diagn*. 2017;37(1):73-80.
36. Hook EB, Cross PK. Extra structurally abnormal chromosomes (ESAC) detected at amniocentesis: frequency in approximately 75,000 prenatal cytogenetic diagnoses and associations with maternal and paternal age. *Am J Hum Genet*. 1987;40(2):83-101.
37. Ming C, Shun-Ping C, Pao-Lun Y, et al. Prenatal identification of small supernumerary marker chromosomes by FISH in an infant born with mild congenital anomalies. *Prenat Diagn*. 2010;26(4):383-387.
38. Blennow E, Bui TH, Kristoffersson U, et al. Swedish survey on extra structurally abnormal chromosomes in 39 105 consecutive prenatal diagnoses: prevalence and characterization by fluorescence in situ hybridization. *Prenat Diagn*. 2010;14(11):1019-1028.
39. Durham L, Papanna R, Stevens B, et al. The utilization of prenatal microarray: a survey of current genetic counseling practices and barriers. *Prenat Diagn*. 2019;39(5):351-360.
40. Oneda B, Baldinger R, Reissmann R, et al. High-resolution chromosomal microarrays in prenatal diagnosis significantly increase diagnostic power. *Prenat Diagn*. 2014;34(6):525-533.
41. Ganesamoorthy D, Bruno DL, McGillivray G, et al. Meeting the challenge of interpreting high-resolution single nucleotide polymorphism array data in prenatal diagnosis: does increased diagnostic power outweigh the dilemma of rare variants? *BJOG*. 2013;120(5):594-606.

How to cite this article: Wu X, An G, Xie X, et al. Chromosomal microarray analysis for pregnancies with or without ultrasound abnormalities in women of advanced maternal age. *J Clin Lab Anal*. 2020;34:e23117. https://doi.org/10.1002/jcla.23117