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

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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 sub-microscopic 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
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 to 20.5% in 2016. 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); 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. With the implementation of chromosomal microarray analysis (CMA), many chromosomal abnormalities that were undetectable by karyotyping were found in either prenatal or postnatal testing. 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. Wapner et al 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. 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) 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

| 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 1 Demographic characters for 703 women of AMA
| 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,XXY            | ——               | 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 | 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.
| Case number | Ultrasound findings                                                                 | CMA results                       | Karyotype results | Parental testing | Related syndrome          | Outcome  |
|-------------|-------------------------------------------------------------------------------------|-----------------------------------|-------------------|------------------|---------------------------|----------|
| 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         |                 |                           | TOP      |
| 26          | Choroid plexus cyst                                                                |                                   |                   |                 |                           |          |
| 27          | Nuchal translucency thickness                                                        | arr(13)×3                         | 47,XX,+13         |                 |                           | TOP      |
| 28          | Nuchal translucency thickness, Ventricular echogenicity, single umbilical artery     | arr(X)×1                          | 45,X              |                 |                           | TOP      |
| 29          | Nuchal translucency thickness                                                        | arr(X)×3                          | 47,XXX            |                 |                           | 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**

| Case number | Ultrasound findings                                                                 | CMA results                       | Karyotype results | Parental testing | Related syndrome          | Outcome  |
|-------------|-------------------------------------------------------------------------------------|-----------------------------------|-------------------|------------------|---------------------------|----------|
| 33-34       | CHD                                                                                 | arr(18)×3                         | 47,XX,+18         |                 |                           | TOP      |
| 35          | CHD, Cranial dysplasia                                                             | arr(18)×3                         | 47,XY,+18         |                 |                           | TOP      |
| 36          | CHD                                                                                 | arr(21)×3                         | 47,XY,+21         |                 |                           | TOP      |
| 37          | CHD                                                                                 | arr(X)×1                          | 45,X              |                 |                           | TOP      |
| 38          | Spinal dysplasia                                                                   | arr(8)×3[0.7]                     | 47,XY,+8/46,XY    |                 |                           | TOP      |
| 39          | CHD                                                                                 | arr[GRCh37] 4q25q28.1(112192577_127874789)×1 15.6 Mb | 46,XX,del(4)(q25q28) |                 |                           | TOP      |
| 40          | CHD, Cranial dysplasia                                                             | arr[GRCh37]13q31.3q34(94929201_115107733)×1 20.1 Mb | 46,XX,r(13) (7p11q32)/45,XX,-13 |                 |                           | TOP      |
| 41-42       | CHD                                                                                 | arr[GRCh37] 22q11.1q11.2(16888899_18649190)×4 1.7 Mb | 47,XY,+mar        | De novo          |                           | TOP      |
| 43          | CHD, Biped varus, severe pulmonary stenosis                                        | arr[GRCh37] 5p15.33p11(113576_46242541)×3 46 Mb | 47,XX,+mar        | De novo          |                           | TOP      |

(Continues)
(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: 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 chorionicisentesis 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%,
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. They may be categorized as pathogenic/likely pathogenic findings in spite of their incomplete penetrance and variable expressivity, even inheritance from normal parents. 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.

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 4 Types of CMA findings and their frequencies in 703 fetuses

|                        | Group A (n = 437) | Group B (n = 266) | Ultrasound anomalies (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%)   | 2 (1.4%)  | 3 (3.6%)   | 2 (5.6%)  |
| 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.

### 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(14304328_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(154453034_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. The penetration of the variants was estimated to be 10.4%, and the prevalence in patients with mental retardation, ID, and/or multiple congenital anomalies was about two to fourfold of that in control population. 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.

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. 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. 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. The VOUS rate detected by CMA shows considerable variability. 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.

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**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.

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**DATA AVAILABILITY STATEMENT**

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

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