Journal of Clinical Laboratory Analysis

WILEY

RESEARCH ARTICLE OPEN ACCESS

Application of Chromosomal Karyotype Analysis Combined With Chromosomal Microarray Analysis in the Amniotic Fluid of Advanced Maternal Age

Caihong Liu | Yan Lu | Baohui Zhang | Liucui Yu | Jing He | Yan Ji

Department of Prenatal Diagnostic Centre, Huizhou Central People's Hospital, Huizhou, Guangdong, China

Correspondence: Yan Ji (19257621379@163.com)

Received: 28 December 2024 | **Revised:** 19 February 2025 | **Accepted:** 17 March 2025

Keywords: advanced matemal age | amniotic fluid | chromosomal microarray analysis | chromosome karyotype analysis

ABSTRACT

Objective: To explore the application and value of chromosomal karyotype analysis combined with Chromosomal Microarray Analysis (CMA) in the amniotic fluid of advanced maternal age.

Methods: A total of 817 advanced maternal age (AMA) who underwent amniocentesis at the Prenatal Diagnosis Center of Huizhou Central People's Hospital between January 2018 and July 2024 were enrolled in this study. The women were grouped based on different age ranges and prenatal diagnosis factors. These groups were used to compare the detection rates and differences between chromosomal karyotype analysis and CMA.

Result: The overall chromosomal abnormality rates detected by karyotype analysis in the 35–39 years age group and the ≥40 years age group were 8.81% and 13.79%, respectively, with a statistically significant difference ($p < 0.05$). For CMA, the overall abnormality rates in the same age groups were 10.79% and 15.33%, respectively, but the difference was not statistically significant ($p > 0.05$). The non-solely advanced-age group (those with additional factors beyond just advanced age) had higher overall chromosomal abnormality rates, aneuploidy rates, structural abnormality rates, and mosaicism rates compared to the solely advanced-age group, with statistically significant differences ($p < 0.05$). Additionally, the non-solely advanced-age group had a higher overall abnormality rate detected by CMA compared to the solely advanced-age group, with a statistically significant difference ($p < 0.05$). However, there were no statistically significant differences between the two groups in terms of the detection of pathogenic, likely pathogenic, and variants of uncertain significance ($p > 0.05$). In this study, a total of 68 cases were identified where the results of karyotype analysis and CMA were inconsistent.

Conclusion: The overall abnormal rate of chromosomal karyotype analysis increases with maternal age, while the overall abnormal rate of CMA shows no significant correlation with maternal age. The abnormal rates are significantly higher in AMA with additional factors. The combination of chromosomal karyotype analysis and CMA can validate and complement each other, thereby improving the detection rates of chromosomal abnormalities in amniotic fluid samples of AMA. This provides a diagnostic basis for subsequent pregnancy choices, which effectively reduces the birth of fetuses with chromosomal abnormalities and enhances population quality.

Advanced maternal age (AMA) refers to people whose delivery age is 35 years or older [1]. With the gradual liberalization of China's two-child and three-child policies, changes in lifestyle, differences in marital and fertility concepts, the increase of educational attainment for women, and the development of assisted reproductive technologies, the number of women getting

pregnant at a later age is continuously increasing [2, 3]. The risk of chromosomal abnormalities in germ cells during cell division is directly related to maternal age [4]. Amniocentesis is a commonly used technique for diagnosing fetal chromosomal abnormalities. It is typically performed during Weeks 15–18 of pregnancy and can also be conducted in the late stages of pregnancy [5].

China has one of the highest incidences of birth defects in the world; the incidence of birth defects in China is about 5.6%, but there are significant differences in the prevalence and types of birth defects across different geographical regions [6, 7]. There are limited detailed reports on the application of karyotype analysis combined with chromosomal microarray analysis (CMA) in the amniotic fluid of AMA in second-tier cities of southern China. This paper retrospectively analyzes the chromosomal karyotype and CMA results of amniotic fluid cells from AMA and explores the significance of these two methods in prenatal diagnosis in Huizhou, a city in Guangdong Province in China. Additionally, it provides a basis for clinical genetic counseling and prenatal diagnosis.

1 | Materials and Methods

1.1 | General Information

A total of 817 cases of AMA who underwent amniocentesis at the Prenatal Diagnosis Center of Huizhou Central People's Hospital in Guangdong Province between January 2018 and July 2024 were collected. The indications for amniocentesis included AMA with or without abnormal Down Syndrome screening, NIPT (abnormal noninvasive prenatal testing), abnormal fetal ultrasound screening, adverse obstetric history, chromosomal abnormalities in either parent, etc. The delivery age of the pregnant people was 35 years or older, and the gestational age ranged from 14 to 25 weeks. Detailed medical histories were collected and recorded for all cases. All pregnant people were informed of the relevant risks and signed the informed consent form for prenatal diagnosis.

1.2 | Research Methods

1.2.1 | Karyotype Analysis of Chromosomes

Amniocentesis was performed under strict sterilization and ultrasound guidance to collect amniotic fluid. The Zeiss automated slide scanning system is used to obtain karyotype images, and karyotype analysis is conducted according to industry standards.

1.2.2 | CMA

Extract genomic DNA according to the kit instructions. The extracted DNA was then hybridized following the operational procedures of the CytoScan 750 K. The sequencing information was compared with the human reference genome using alignment software, and the nature of the detected CNVs (CNVs refer to structural variations larger than 1 kb in genomic DNA, which represent submicroscopic changes in chromosome structure that cannot be resolved by traditional G-banding karyotype analysis) was determined by searching online public databases and consulting relevant references. The CNVs were classified into five categories according to the guidelines of ACMG (the American College of Medical Genetics and Genomics): pCNVs (pathogenic CNVs), LP CNVs (likely pathogenic CNVs), VUS CNVs (variants of unknown significance CNVs), LB CNVs (likely benign CNVs), and B CNVs (benign CNVs).

1.2.3 | Statistical Methods

The raw data were organized using Excel spreadsheets, and the data were analyzed and processed using SPSS 26.0 statistical software. The data are presented as number of cases (percentage) [n (%)]. Comparisons between groups were made using the χ^2 test, and a $p < 0.05$ was considered statistically significant.

2 | Results

2.1 | Abnormalities in Chromosome Karyotypes of Amniotic Fluid Cells From Different Age Groups With AMA

Among 817 AMA, the total abnormal rate of chromosome karyotype analysis in amniotic fluid cells was 10.4%, with the rate of chromosomal aneuploidy (7.71%) being higher than that of chromosomal structural abnormalities (1.96%) and mosaicism abnormalities (0.73%). The women were divided into two age groups: 35–39 years and ≥ 40 years. The abnormal rates of chromosome karyotype analysis were 8.81% and 13.79%, respectively, and the difference was statistically significant ($p < 0.05$). The total abnormal rate of chromosome karyotype analysis, the rate of chromosomal aneuploidy, the rate of T21 (Trisomy 21 abnormalities), and the rate of T18 (Trisomy 18 abnormalities)were all higher in the ≥ 40 years group than in the 35–39 years group, and the differences were statistically significant ($p < 0.05$). However, there were no statistically significant differences in the rates of T13 (Trisomy 13 abnormalities), sex chromosome abnormalities, mar (marker chromosome), structural abnormalities, and mosaicism between the two groups. Details are shown in Table 1.

2.2 | Results of CMA on Amniotic Fluid Cells From Different Age Groups With AMA

A total of 817 AMA underwent both chromosomal testing and CMA on amniotic fluid cells. The results for T21, T18, T13, and sex chromosome abnormalities were consistent between the two methods. Among them, 556 women were aged 35–39 years, and 261 were aged ≥ 40 years. The overall abnormal rate of CMA was 12.24%, with the rate of chromosomal aneuploidy (7.47%) being higher than that of mosaicism abnormalities (0.86%) and CNVs abnormalities (3.92%). The total abnormal incidence rates of CMA for the 35–39 years, ≥ 40 years, and combined age groups were 10.79% and 15.33%, respectively, with no statistically significant difference ($p > 0.05$). The rates of chromosomal aneuploidy and autosomal abnormalities were higher in the ≥ 40 years group than

TABLE 1 | Analysis of chromosome karyotype abnormalities in amniotic fluid cells from different age groups with AMA [*n* (%)].

Age groups	Cases	Aneuploidy [*n* (%)]						Structural abnormalities [*n* (%)]				Mosaicism [*n* (%)]
		Total	T21	T18	T13	Sex chromosome abnormalities	Mar	Total	Balanced translocation	Inversion	Others	Total	
35~39	556	49 (8.81)	16 (2.88)	5 (0.90)	2 (0.36)	7 (1.26)	2 (0.36)	32 (5.76)	8 (1.44)	3 (0.54)	1 (0.18)	12 (2.16)	5 (0.90)
≥40	261	36 (13.79)	19 (7.28)	8 (3.07)	0	4 (1.53)	0	31 (11.88)	2 (0.77)	1 (0.38)	1 (0.38)	4 (1.53)	1 (0.38)
Total	817	85 (10.40)	35 (4.28)	13 (1.59)	2 (0.24)	11 (1.35)	2 (0.24)	63 (7.71)	10 (1.22)	4 (0.49)	2 (0.24)	16 (1.96)	6 (0.73)
χ^2		4.726	8.394	5.321	0.941	0.1	0.941	9.354	0.665	0.089	0.301	0.362	0.649
p		0.03*	0.004**	0.021*	0.332	0.752	0.332	0.002**	0.415	0.765	0.584	0.547	0.42

*$p < 0.05$ result is statistically significant at the 5% level.
**$p < 0.01$ result is statistically significant at the 1% level.
***$p < 0.001$ result is highly significant at the 0.1% level.

in the 35–39 years group, and the differences were statistically significant ($p < 0.05$). However, there was no statistically significant difference in the abnormal rate of CNVs between the two groups. Details are shown in Table 2.

2.3 | Chromosomal Karyotype Abnormalities in AMA or With Different Prenatal Diagnostic Indications

The detection of chromosomal abnormalities in karyotype analysis of amniotic fluid cells from 817 cases of AMA or with various prenatal diagnostic indications is shown in Table 3. A total of 6 cases (1.5%) of chromosomal abnormalities were detected in the isolated AMA group, including 4 cases (1.01%) of non-aneuploidy and 2 cases (0.50%) of structural abnormalities. Ten cases (7.87%) of chromosomal abnormalities were detected in the group with abnormal Down Syndrome screening results. Thirty-three cases (53.23%) of chromosomal abnormalities were detected in the group with abnormal NIPT results, including 28 cases (45.16%) of non-aneuploidy, 1 case (1.61%) of structural abnormality, and 4 cases (6.45%) of mosaicism. Sixteen cases (17.02%) of chromosomal abnormalities were detected in the group with abnormal fetal ultrasound findings. Eight cases (34.78%) of chromosomal abnormalities were detected in the group with chromosomal abnormalities in one spouse, while only 2 cases (2.41%) of chromosomal abnormalities were detected in the group with a history of adverse pregnancy outcomes. Ten cases (33.33%) of chromosomal abnormalities were detected in the group with more than two prenatal diagnostic indications, including 9 cases (30.00%) of non-aneuploidy and 1 case (3.33%) of structural abnormality. The overall chromosomal abnormality rate, non-aneuploidy rate, structural abnormality rate, and mosaicism rate were all higher in the non-isolated AMA group compared to the isolated AMA group, and the differences were statistically significant ($p < 0.05$).

2.4 | CMA Results for AMA or Merged With Different Prenatal Diagnostic Indications

The results of CMA on amniotic fluid cells from 817 cases of AMA or with various prenatal diagnostic indications are shown in Table 4. A total of 19 cases (4.77%) of CMA abnormalities were detected in the isolated AMA group, including 4 cases (1.01%) of chromosomal non-aneuploidy, 5 cases (1.26%) of pCNVs, 1 case (0.25%) of LP CNVs, 9 cases (2.26%) of VUS CNVs, and a total of 16 cases (4.02%) of copy number variants (CNVs). Eleven cases (8.66%) of CMA abnormalities were detected in the group with abnormal Down Syndrome screening results. Thirty-nine cases (62.90%) of CMA abnormalities were detected in the group with abnormal NIPT results, including 28 cases (45.16%) of chromosomal non-aneuploidy, 6 cases (9.68%) of mosaicism, 2 cases (3.23%) of pCNVs, and 3 cases (4.84%) of VUS CNVs. Seventeen cases (18.09%) of CMA abnormalities were detected in the group with abnormal fetal ultrasound findings. Three cases (13.04%) of CMA abnormalities were detected in the group with chromosomal abnormalities in one spouse, while only 2 cases (2.41%) of chromosomal abnormalities were detected in the group with a history of

TABLE 2 | CMA results of amniotic fluid cells from AMA in different age groups [*n* (%)].

Age groups	Cases	Aneuploidy [*n* (%)]				Mosaicism [*n* (%)]	CNVs [*n* (%)]			
		Total [*n* (%)]	Autosomal abnormalities	Sex chromosome abnormalities	Total		pCNVs	LP CNVs	VUS CNVs	Total
35~39	556	60 (10.79)	23 (4.14)	7 (1.26)	30 (5.40)	6 (1.08)	9 (1.62)	2 (0.36)	13 (2.34)	24 (4.32)
≥40	261	40 (15.33)	27 (10.34)	4 (1.53)	31 (11.88)	1 (0.38)	3 (1.15)	0	5 (1.92)	8 (3.07)
Total	817	100 (12.24)	50 (6.12)	11 (1.35)	61 (7.47)	7 (0.86)	12 (1.47)	2 (0.24)	18 (2.20)	32 (3.92)
χ^2		3.4	11.915	0.1	10.801	1.013	0.27	0.941	0.147	0.739
p		0.065	0.001**	0.752	0.001**	0.314	0.603	0.332	0.701	0.39

Note: $^{**}p < 0.01$ result is statistically significant at the 1% level.

adverse pregnancy outcomes. Nine cases (30.00%) of chromosomal abnormalities were detected in the group with more than two prenatal diagnostic indications. The overall detection rate of CMA abnormalities, chromosomal non-aneuploidy rate, and mosaicism rate were all higher in the non-isolated AMA group compared to the isolated AMA group, and the differences were statistically significant ($p < 0.05$). However, there were no statistically significant differences between the two groups in the detection rates of pCNVs, LP CNVs, and VUS CNVs ($p > 0.05$).

2.5 | Discordance Between Chromosome Karyotype Analysis and CMA Results in Amniotic Fluid Cells From AMA

Among the 817 AMA who underwent both chromosome karyotype analysis and CMA on their amniotic fluid cells, there were 68 cases where the results of the two tests were discordant. In 13 cases, the CMA results were normal, but the karyotype analysis showed polymorphism, with inversions on chromosome 9 being the most common. There were 2 cases where the CMA results were normal, but the karyotype analysis revealed an mar. In 13 cases, the CMA results were normal, yet the karyotype analysis indicated structural abnormalities (4 cases of inversion abnormalities and 9 cases of balanced translocations). One case had a balanced translocation according to karyotype analysis, but the CMA result revealed a micro-duplication on another chromosome. Another case had a deletion according to karyotype analysis, but the CMA result also revealed a micro-duplication on another chromosome. There was one case where the CMA result was normal, but the karyotype analysis showed mosaicism, two cases where the karyotype analysis was normal but the CMA result showed mosaicism, and five cases where the proportion of mosaicism detected by karyotype analysis and CMA was inconsistent. Lastly, there were 30 cases where the karyotype analysis was normal, but the CMA results were abnormal (14 cases of micro-deletions, 15 cases of micro-duplications, and 1 case of uniparental disomy). Details are shown in Table 5.

3 | Discussion

In this study, 817 cases of amniotic fluid cells from AMA underwent both chromosome karyotype analysis and CMA. Both detection methods revealed that fetal chromosomal abnormalities in these AMA were predominantly non-integer multiples of the haploid, with T21 being the most common type of chromosomal Aneuploidy, having an abnormality rate of 4.28%. Furthermore, the incidence increased with the mother's age, which is consistent with the report by Zhu et al. [8]. T21 is primarily associated with the nondisjunction of the corresponding chromosome during meiosis in the parental mature oocyte [9]. T21 syndrome can lead to moderate intellectual disability and various congenital malformations. Children with this syndrome may face a range of structural and functional issues across different organ systems, with heart abnormalities being a common occurrence [10]. In addition, T18 was also common in this study, with an abnormality rate of 1.59%. The risk of T18, as well as all fetal aneuploidies, is closely related to maternal age, and the risk increases significantly with increasing maternal age. This is also

TABLE 3 | Chromosomal karyotype abnormalities in AMA or with different prenatal diagnostic indications [*n* (%)].

	Indicators for prenatal diagnosis	Cases	Aneuploidy [*n* (%)]	Structural abnormalities [*n* (%)]	Mosaicism [*n* (%)]	Total [*n* (%)]
Solely AMA		398	4 (1.01)	2 (0.50)	0	6 (1.51)
Non-solely AMA	Abnormal Down Syndrome screening	127	7 (5.51)	2 (1.57)	1 (0.79)	10 (7.87)
	Abnormal NIPT	62	28 (45.16)	1 (1.61)	4 (6.45)	33 (53.23)
	Abnormal fetal ultrasound findings	94	13 (13.83)	3 (3.19)	0 (0.00)	16 (17.02)
	History of adverse pregnancy outcomes	83	1 (1.20)	1 (1.20)	0	2 (2.41)
	Chromosomal abnormalities in one spouse	23	1 (4.35)	6 (26.09)	1 (4.35)	8 (34.78)
	≥2 indicators	30	9 (30.00)	1 (3.33)	0	10 (33.33)
	Total	419	59 (14.08)	14 (3.34)	6 (1.43)	79 (18.85)
χ^2			49.042	8.567	5.741	65.892
p			0***	0.003**	0.017*	0***

Note: *$p < 0.05$ result is statistically significant at the 5% level. **$p < 0.01$ result is statistically significant at the 1% level. ***$p < 0.001$ result is highly significant at the 0.1% level.

consistent with the report by Kim et al. [4]. However, there was no significant correlation between maternal age and the abnormality rates of T13, sex chromosomes, or marker chromosomes among the aneuploidies. The incidence of mosaicism was also not influenced by maternal age, which might be related to the relatively small number of corresponding cases in this study. Furthermore, the study showed that structural abnormalities did not significantly increase with age ($p = 0.362$) and had no significant correlation with maternal age, which is consistent with some literature reports [2, 4]. Balanced translocations and inversions were common among these structural abnormalities. Additionally, there was no significant difference in the abnormality rate of CNVs between the two age groups ($p = 0.065$), indicating that the occurrence of fetal CNVs is not correlated with maternal age. This is consistent with Opinion No. 581 from the American College of Obstetricians and Gynecologists [11].

Among the 817 AMA in this study, 398 were classified as AMA only, while the remaining 419 had additional indications such as abnormal Down Syndrome screening, abnormal NIPT, abnormal fetal ultrasound, etc. By comparing the results of chromosome karyotype analysis and CMA between purely AMA and non-purely AMA, it was found that the overall fetal chromosome abnormality rate, aneuploidy rate, structural abnormality rate, and mosaicism rate were significantly higher in the non-purely elderly group compared to the purely elderly group. Similarly, Shi et al. [2] also reported a statistically significant difference in the increased abnormality rate in the group of women with AMA with additional clinical indications, especially for numerical abnormalities. However, our study did not observe any differences in pCNVs, LP CNVs, and VUS CNVs between the two groups. This may be due to the insufficient number of cases in our study to detect such differences. By analyzing the correlation between different high-risk factors and fetal chromosomal abnormalities in the non-solely AMA group, we found that the abnormal rates for chromosomal karyotype analysis (45.16%) and CMA (62.90%) were both highest among AMA with abnormal NIPT results. Therefore, we recommend that AMA who have additional clinical indications, especially those with abnormal NIPT results to undergo prenatal diagnosis to determine whether their fetuses have chromosomal abnormalities.

Karyotype analysis is a commonly used technique for identifying chromosomal abnormalities and prenatal diagnosis of chromosomal disorders. It can detect chromosomal aneuploidy, polyploidy, mosaicism, and structural abnormalities such as translocations, inversions, deletions, and duplications with a resolution greater than 5–10 Mb. However, it also has limitations, such as low resolution and a long detection period. CMA can detect microdeletions or microduplications that are not detectable by traditional karyotype analysis, with a detection rate that can detect levels as low as 50 to 100 kb. However, this technology cannot detect chromosomal rearrangements without genetic material alterations, such as translocations or inversions, and some low-proportion mosaics may also be undetectable [12–15].

In this study, a retrospective analysis was conducted on the results of fetal chromosomal karyotype analysis and CMA in amniotic fluid samples from 817 AMA. Both methods detected 35 cases of T21, 13 cases of T18, 2 cases of T13, and 11 cases of sex chromosome abnormalities, indicating that the combination of the two methods can validate each other for these non-integer multiple abnormalities. Karyotype analysis detected 13 cases of polymorphism, 2 cases of mar, 10 cases of balanced translocations, and 4 cases of inversions that were not detected by CMA. A total of two cases of mar were detected in this study, one of

TABLE 4 | CMA results in AMA or with different prenatal diagnostic indications [*n* (%)].

	Indications for prenatal diagnosis	Cases	Total [*n* (%)]	Aneuploidy [*n* (%)]	Mosaicism [*n* (%)]	CNVs [*n* (%)]			
						pCNVs	LP CNVs	VUS CNVs	Total
Solely AMA		398	19 (4.77)	4 (1.01)	0	5 (1.26)	1 (0.25)	9 (2.26)	16 (4.02)
Non-solely AMA	Abnormal Down Syndrome screening	127	11 (8.66)	7 (5.51)	0	2 (1.57)	0	2 (1.57)	4 (3.15)
	Abnormal NIPT	62	39 (62.90)	28 (45.16)	6 (9.68)	2 (3.23)	0	3 (4.84)	5 (8.06)
	Abnormal fetal ultrasound findings	94	17 (18.09)	13 (13.83)	0	1 (1.06)	1 (1.06)	2 (2.13)	4 (4.26)
	History of adverse pregnancy outcomes	83	2 (2.41)	0	0	1 (1.20)	0	1 (1.20)	2 (2.41)
	Chromosomal abnormalities in one spouse	23	3 (13.04)	0	1 (4.35)	1 (4.35)	0	1 (4.35)	2 (8.70)
	≥ 2 indicators	30	9 (30.00)	9 (30.00)	0	0	0	0	0
	Total	419	81 (19.33)	57 (13.60)	7 (1.67)	7 (1.67)	1 (0.24)	9 (2.15)	17 (4.06)
χ^2			40.271	46.895	6.707	0.242	0.001	0.012	0.022
p			0***	0***	0.01*	0.623	0.971	0.912	0.882

Note: *$p < 0.05$ result is statistically significant at the 5% level. ***$p < 0.001$ result is highly significant at the 0.1% level.

TABLE 5 | Discordance between chromosome karyotype analysis and CMA results in amniotic fluid cells from AMA.

	Karyotype analysis		CMA				Cases
No.	Results	Type of abnormality	Results	Size of abnormal fragment	Nature	Type of abnormality	
1	46,XN,1qh+	Polymorphism	arr (1–22) x2, (XN)x1	—	—	—	1
2	46,XN,inv.(9)(p12q13)	Polymorphism	arr (1–22) x2, (XN)x1	—	—	—	7
3	46,XN,21pss	Polymorphism	arr (1–22) x2, (XN)x1	—	—	—	1
4	46,XN,16qh+	Polymorphism	arr (1–22) x2, (XN)x1	—	—	—	1
5	46,X,inv.(Y)(p11.2q11.23)	Polymorphism	arr (1–22) x2, (XN)x1	—	—	—	3
6	47,XN,+mar	Numerical abnormality	arr (1–22) x2, (XN)x1	—	—	—	1
7	47,XN,+mar mat	Numerical abnormality	arr (1–22) x2, (XN)x1	—	—	—	1
8	46,XN,t(1;3)(q32;p13)	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	2
9	46,XN,t(1;14)(q42.1;q22)	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	1
10	46,XN,t(3;7)(p24;p15.3)	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	1
11	46,XN,t(4;7)(q25;p12)mat	Balanced translocation	arr[GRCh37] 1q21.1q21.2(145764679_147933973)x3	2.1 Mb	pCNVs	Microduplication	1
12	46,XN,t(7;14)(p21;q21)	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	1
13	46,XN,t(9;22)(p22;q11.2)mat	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	1
14	46,XN,t(11;22)(q23.3;q12)	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	1
15	45,XN,der(13;14)(q10;q10)	Balanced translocation	arr (1–22) x2, (XN)x1	—	—	—	2
16	46,XN,inv.(1)(p13q21)	Inversion	arr (1–22) x2, (XN)x1	—	—	—	1
17	46,XN,inv.(6)(p21.q23)	Inversion	arr (1–22) x2, (XN)x1	—	—	—	1
18	46,XN,inv.(11)(p15q21)	Inversion	arr (1–22) x2, (XN)x1	—	—	—	1
19	46,XN,inv.(16)(q21q23.1)pat	Inversion	arr (1–22) x2, (XN)x1	—	—	—	1
20	46,XN,del(9)(p23)	Deletion	arr[GRCh37] 8p23.3p23.2(158049_4664396)x3 9p24.3p23(208455_12596589)x1	4.5 Mb、12.4 Mb	pCNVs	Microduplication, Deletion	1
21	mos 45,X [3],/46,XN[97]	Mosaicism	arr (1–22) x2, (XN)x1	—	—	—	1

(Continues)

TABLE 5 | (Continued)

	Karyotype analysis		CMA				Cases
No.	Results	Type of abnormality	Results	Size of abnormal fragment	Nature	Type of abnormality	
22	mos 45,X [16],/46,XN[84]	Mosaicism	arr (1–22) X2, (x) X1-2 (The proportion of deletions is 40%)	—	pCNVs	Mosaicism	1
23	mos 47,XN,+9[21]/46,XN[29]	Mosaicism	arr (9)X2-3 (The proportion of mosaicism is 50%)	—	pCNVs	Mosaicism	1
24	mos 46,XN,del(18) (q21.2) [9]/46,XN[91]	Mosaicism	arr[GRCh37] 18q21.2q23(50872623_78013728)x1-2 (The proportion of mosaicism is25%)	27.1 Mb	pCNVs	Mosaicism, Deletion	1
25	mos 47,XN,21[16]/46XN[84]	Mosaicism	arr (21)X2-3 (The proportion of mosaicism is55%)		pCNVs	Mosaicism	
26	mos 47,XN,+21[8]/46,XN[92]	Mosaicism	arr (21)X2-3 (The proportion of mosaicism is25%)	—	pCNVs	Mosaicism	1
27	46,XN	Normal	arr (15) X2-3 (The proportion of mosaicism is30%)	—	pCNVs	Mosaicism	1
28	46,XN	Normal	arr (7)x3[0.1] (The proportion of mosaicism is10%)	—	pCNVs	Mosaicism	1
29	46,XN	Normal	arr[hg19] 17p12(14, 099, 564–15, 482,833)x1	1.4 Mb	pCNVs	Microdeletion	1
30	46,XN	Normal	arr[GRCh37]Xq28(154109414_154555853)x2	446 kb	pCNVs	Microduplication	1
31	46,XN	Normal	arr[GRCh37] 17p12(14060336_15484335)x1	1.4 Mb	pCNVs	Microdeletion	1
32	46,XN	Normal	arr[GRCh37] 10q11. 22q11.23(46293591_51904377)x1	5.6 Mb	pCNVs	Microdeletion	1
33	46,XN	Normal	arr[GRCh37] Xp22.31(6455152_8135568)x1	1.68 Mb	pCNVs	Microdeletion	1
34	46,XN	Normal	arr[GRCh37] 15q13.3q23(33205490_70171958) x2 hmz	36.9 Mb	pCNVs	Uniparental Disomy, UPD[upd(15)]	1
35	46,XN	Normal	arr[GRCh37] 7q11. 23(72621346_74154209)x3	1.5 Mb	pCNVs	Microduplication	1
36	46,XN	Normal	arr[GRCh37] 1q21.1q21.2(145764679_147933973)x3	2.1 Mb	pCNVs	Microduplication	1
37	46,XN	Normal	arr[GRCh37] 16p11.2(28748617_29051191)x1	303Kb	pCNVs	Microdeletion	1
38	46,XN	Normal	arr[GRCh37]Xp22.31(6455152_8135568)x0	1.68 Mb	pCNVs	Microdeletion	1

(Continues)

TABLE 5 | (Continued)

	Karyotype analysis		CMA				Cases
No.	Results	Type of abnormality	Results	Size of abnormal fragment	Nature	Type of abnormality	
39	46,XN	Normal	arr[GRCh37] Xp21.1(31844713_31898005)x1	53 kb	pCNVs	Microdeletion	1
40	46,XN	Normal	arr[GRCh37] 7q11.23(74621926_76709600)x1 pat	2.1 Mb	LP CNVs	Microdeletion	1
41	46,XN	Normal	arr[GRCh37] 20p13(133900_515866)x1	382 kb	LP CNVs	Microdeletion	1
42	46,XN	Normal	arr[hg19] 5q22.1(110,251,665–111, 379,011)x3 8q12.1(57, 302, 769–58,393,873) x3	1.1 Mb、1.1 Mb	VUS CNVs	Microduplication	1
43	46,XN	Normal	arr[GRCh37] 17q21.31(41418274_42011232)x1	593 kb	VUS CNVs	Microdeletion	1
44	46,XN	Normal	arr[GRCh37]1613.11(15,058,821 16,278,133)x3	1.2 Mb	VUS CNVs	Microduplication	1
45	46,XN	Normal	arr[GRCh37] 9p24.2p24.1(3302033_5109972)x3	1.8 Mb	VUS CNVs	Microduplication	1
46	46,XN	Normal	arr[GRCh37] 4p16. 1(8327683_10228789)x3	1.9 Mb	VUS CNVs	Microduplication	1
47	46,XN	Normal	arr[GRCh37] 21q22.3(43734392_44910472)x3	1.2 Mb	VUS CNVs	Microduplication	1
48	46,XN	Normal	arr[GRCh37] 15q21.3(53065535_54235618)x3	1.1 Mb	VUS CNVs	Microduplication	1
49	46,XN	Normal	arr[GRCh37]Xq21.1(76758467_77014511)x2 Xq21.1(77130900_77507589)x2	256 kb、377 kb	VUS CNVs	Microduplication	1
50	46,XN	Normal	arr[GRCh37] 21q21.2(24563993_25751780)x1	1.18 Mb	VUS CNVs	Microdeletion	1
51	46,XN	Normal	arr[GRCh37]8p23.2p23.1(3409287_8937788)x3	5.5 Mb	VUS CNVs	Microduplication	1
52	46,XN	Normal	arr[GRCh37] 9p24.1 (5529756_6211500)x1	682 kb	VUS CNVs	Microdeletion	1
53	46,XN	Normal	arr[GRCh37] 9q34. 12(133532120_133693202)x1	161 kb	VUS CNVs	Microdeletion	1
54	46,XN	Normal	arr[GRCh37] 2q32.1(187869242_189154429)x4	1.3 Mb	VUS CNVs	Microduplication	1
55	46,XN	Normal	arr[GRCh37] 3p21.31(47192964_47405438)x3	212Kb	VUS CNVs	Microduplication	1
56	46,XN	Normal	arr[GRCh37] 16p13.11(15154357_16289059)x3	1.1 Mb	VUS CNVs	Microduplication	1
57	46,XN	Normal	arr[GRCh37] 4q12q13.1(58193592_62750005)x3	4.6 Mb	VUS CNVs	Microduplication	1
58	46,XN	Normal	arr[GRCh37] 4q34.2q34.3(177387824_177869978)x1	482 kb	VUS CNVs	Microdeletion	1

Note: CMA nomenclature is based on the International System for Human Cytogenetic Nomenclature (ISCN).

which was inherited from the mother and the other was de novo, and both were shown to be normal by CMA. Chromosomal polymorphism includes variations in heterochromatic segments, satellites, and satellite stalks [16]. Chromosomal polymorphism is usually unrelated to clinical diseases. However, in recent years, chromosomal polymorphism has been confirmed to be potentially associated with reproductive failure and recurrent spontaneous abortions [17–19]. Mar are usually equal to or smaller than chromosome 20 in the same metaphase karyotype. However, traditional chromosomal banding techniques cannot identify the origin and characteristics of small supernumerary marker chromosome (sSMCs) [20]. It is composed of euchromatic (gene-rich genetic material) and/or heterochromatic (gene-poor region) material with a primary constriction (centromere) [21]. Seventy-two percent of sSMCs originate from acrocentric chromosomes. In 30% of cases, the presence of sSMCs can lead to euchromatic imbalance [22].

In this study, the rate of chromosomal abnormalities detected by chromosomal microarray analysis (12.24%) was slightly higher than that detected by karyotype analysis (10.4%) in amniocentesis performed on advanced maternal age pregnant women in our study. However, karyotype analysis detected 10 cases of balanced translocations, including two cases of Robertsonian translocation carriers and four cases of inversions. However, except for one case of balanced translocation with a microduplication on another chromosome, the CMA results for the other translocations and inversions were all normal. This suggests that karyotype analysis should be combined with CMA to determine whether there is an increase or decrease in pathogenic genetic material in the fetal chromosomes of translocation or inversion carriers, thereby reducing misdiagnosis and missed diagnosis. True balanced translocation and inversion carriers usually do not lose or gain genetic material and typically have a normal phenotype. However, balanced translocations or inversions can lead to unfavorable pregnancy outcomes, such as recurrent pregnancy loss or congenital defects in the offspring due to an increased risk of producing unbalanced gametes during meiosis [23, 24].

This study detected seven cases of mosaicism, but two cases were not detected by karyotype analysis and one case was not detected by CMA. For the remaining four cases, the mosaic proportions detected by karyotype analysis and CMA were inconsistent. Due to the different principles of karyotype analysis and CMA, there may be some differences in mosaic proportions. Therefore, the combined use of the two methods can reduce misdiagnosis caused by a single detection method. In this study, 30 cases of smaller submicroscopic deletions and duplications, known as chromosomal CNVs, were detected by CMA but not by karyotype analysis. CNVs can cause a wide range of human diseases, including neurodevelopmental disorders and congenital anomalies such as heart defects [12].

In this study, one case was also identified with a normal chromosomal karyotype but was diagnosed as pathogenic uniparental disomy based on the combined CMA results of the fetus and its parents. Therefore, CMA technology compensates for the limitations of karyotype analysis to a certain extent. In summary, in amniotic fluid testing of AMA, both karyotype analysis and CMA have their unique advantages. The combined use of these two methods can validate each other, complement each other's deficiencies, and effectively improve the detection rate of chromosomal abnormalities. Fernan Chaves et al. [25] also addressed this discussion. Ultimately, this, in turn, will reduce the incidences of AMA giving birth to children with chromosomal disorders and lower the chances of birth defects, which enhance the quality of the human population.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. M. Afroz and M. Rajib, "Comparison of Fetomaternal Outcome of Pregnancies Between Women With Advanced Maternal Age and Younger Women," *Scholars International Journal of Obstetrics and Gynecology* 7, no. 5 (2024): 217–222, https://doi.org/10.36348/sijog.2024.v07i05.002.

2. Y. Shi, J. Ma, Y. Xue, et al., "The Assessment of Combined Karyotype Analysis and Chromosomal Microarray in Pregnant Women of Advanced Maternal Age: A Multicenter Study," *Annals of Translational Medicine* 7, no. 14 (2019): 318, https://doi.org/10.21037/atm.2019.06.63.

3. D. Ratiu, F. Sauter, E. Gilman, et al., "Impact of Advanced Maternal Age on Maternal and Neonatal Outcomes," *In Vivo* 37, no. 4 (2023): 1694–1702, https://doi.org/10.21873/invivo.13256.

4. Y. J. Kim, J. E. Lee, S. H. Kim, S. S. Shim, and D. H. Cha, "Maternal Age-Specific Rates of Fetal Chromosomal Abnormalities in Korean Pregnant Women of Advanced Maternal Age," *Obstetrics & Gynecology Science* 56, no. 3 (2013): 160–166, https://doi.org/10.5468/ogs.2013.56.3.160.

5. P. M. Miron, "Preparation, Culture,and Analysis of Amniotic Fluid Samples," *Current Protocols in Human Genetics* (2012): 8.4.1-8.4.14, https://doi.org/10.1002/0471142905.hg0804s74.

6. Ministry of Health, People's Republic of China, Report on Prevention and Treatment of Birth Defects[S] (2012).

7. H. Li, J. Hu, Q. Wu, J. Qiu, L. Zhang, and J. Zhu, "Chromosomal Abnormalities Detected by Chromosomal Microarray Analysis and Pregnancy Outcomes of 4211 Fetuses With High-Risk Prenatal Indications," *Scientific Reports* 14, no. 1 (2024): 15920, https://doi.org/10.1038/s41598-024-67123-5.

8. Y. Zhu, S. Lu, X. Bian, et al., "A Multicenter Study of Fetal Chromosomal Abnormalities in Chinese Women of Advanced Maternal Age," *Taiwanese Journal of Obstetrics & Gynecology* 55, no. 3 (2016): 379–384, https://doi.org/10.1016/j.tjog.2016.01.002.

9. S. E. Antonarakis, M. B. Petersen, M. G. McInnis, et al., "The Meiotic Stage of Nondisjunction in Trisomy 21: Determination by Using DNA Polymorphisms," *American Journal of Human Genetics* 50, no. 3 (1992): 544–550.

10. E. Yilmaz Gulec and A. Gezdirici, "The Effect of Maternal Age on the Incidence of Major Malformations and Operations in Children With Down Syndrome," *Medeniyet Medical Journal* 37, no. 3 (2022): 226–233, https://doi.org/10.4274/MMJ.galenos.2022.09086.

11. "Committee Opinion No. 581: The Use of Chromosomal Microarray Analysis in Prenatal Diagnosis," *Obstetrics and Gynecology* 122,

no. 6 (2013): 1374–1377, https://doi.org/10.1097/01.AOG.0000438962.16108.d1.

12. L. Dugoff, M. E. Norton, and J. A. Kuller, "The Use of Chromosomal Microarray for Prenatal Diagnosis," *American Journal of Obstetrics and Gynecology* 215, no. 4 (2016): B2–B9, https://doi.org/10.1016/j.ajog.2016.07.016.

13. J. Xiang, Y. Ding, X. Song, et al., "Clinical Utility of SNP Array Analysis in Prenatal Diagnosis: A Cohort Study of 5000 Pregnancies," *Frontiers in Genetics* 11 (2020): 571219, https://doi.org/10.3389/fgene.2020.571219.

14. L. Lan, H. Wu, L. She, et al., "Analysis of Copy Number Variation by Sequencing in Fetuses With Nuchal Translucency Thickening," *Journal of Clinical Laboratory Analysis* 34, no. 8 (2020): e23347, https://doi.org/10.1002/jcla.23347.

15. S. C. Hillman, D. J. McMullan, G. Hall, et al., "Use of Prenatal Chromosomal Microarray: Prospective Cohort Study and Systematic Review and Meta-Analysis: Prenatal CMA: Cohort Study and Systematic Review," *Ultrasound in Obstetrics & Gynecology* 41, no. 6 (2013): 610–620, https://doi.org/10.1002/uog.12464.

16. L. Willatt, S. Morgan, L. G. Shaffer, M. L. Slovak, and L. J. Campbell, "ISCN 2009 an International System for Human Cytogenetic Nomenclature," *Human Genetics* 126, no. 4 (2009): 603–604, https://doi.org/10.1007/s00439-009-0726-6.

17. F. I. Sahin, Z. Yilmaz, O. O. Yuregir, T. Bulakbasi, O. Ozer, and H. B. Zeyneloglu, "Chromosome Heteromorphisms: An Impact on Infertility," *Journal of Assisted Reproduction and Genetics* 25, no. 5 (2008): 191–195, https://doi.org/10.1007/s10815-008-9216-3.

18. D. Mierla and V. Stoian, "Chromosomal Polymorphisms Involved in Reproductive Failure in the Romanian Population," *Balk J med Genet* 15, no. 2 (2012): 23–28, https://doi.org/10.2478/bjmg-2013-0003.

19. Y. Hong, Y. W. Zhou, J. Tao, S. X. Wang, and X. M. Zhao, "Do Polymorphic Variants of Chromosomes Affect the Outcome of In Vitro Fertilization and Embryo Transfer Treatment?," *Human Reproduction* 26, no. 4 (2011): 933–940, https://doi.org/10.1093/humrep/deq333.

20. H. Xue, X. Chen, M. Lin, et al., "Prenatal Diagnosis and Molecular Cytogenetic Identification of Small Supernumerary Marker Chromosomes: Analysis of Three Prenatal Cases Using Chromosome Microarray Analysis," *Aging (Albany NY)* 13, no. 2 (2021): 2135–2148, https://doi.org/10.18632/aging.202220.

21. L. Rodríguez, "Be Careful With Small Supernumerary Marker Chromosomes!," *Frontiers in Genetics* 14 (2023): 1269679, https://doi.org/10.3389/fgene.2023.1269679.

22. N. Armanet, L. Tosca, S. Brisset, T. Liehr, and G. Tachdjian, "Small Supernumerary Marker Chromosomes in Human Infertility," *Cytogenetic and Genome Research* 146, no. 2 (2015): 100–108, https://doi.org/10.1159/000438718.

23. S. Shetty, J. Nair, J. Johnson, et al., "Preimplantation Genetic Testing for Couples With Balanced Chromosomal Rearrangements," *Journal of Reproduction & Infertility* 23, no. 3 (2022): 213–223, https://doi.org/10.18502/jri.v23i3.10013.

24. K. Zhang, Y. Huang, R. Dong, et al., "Familial Intellectual Disability as a Result of a Derivative Chromosome 22 Originating From a Balanced Translocation (3;22) in a Four Generation Family," *Molecular Cytogenetics* 11, no. 1 (2018): 18, https://doi.org/10.1186/s13039-017-0349-x.

25. T. F. Chaves, N. Baretto, L. F. Oliveira, et al., "Copy Number Variations in a Cohort of 420 Individuals With Neurodevelopmental Disorders From the South of Brazil," *Scientific Reports* 9, no. 1 (2019): 17776, https://doi.org/10.1038/s41598-019-54347-z.