

CASE REPORT

The difference between karyotype analysis and chromosome microarray for mosaicism of aneuploid chromosomes in prenatal diagnosis

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Abstract

Objective: To compare karyotype and chromosomal microarray (CMA) analysis of aneuploid chromosome mosaicism in amniocentesis samples.

Materials and methods: A total of 2091 amniocentesis samples from pregnant women were collected from March 1, 2019, to January 31, 2020. Karyotype analysis was performed using G-banding and CMA analysis used the Affymetrix CytoScan 750K SNP microarray.

Result: Thirteen cases with aneuploid chromosome mosaicism were detected and compared between the karyotype and CMA methods. Seven of these cases were trisomic mosaicism, and the levels of mosaicism calculated from CMA were higher than those detected from karyotype analysis; noting three cases of trisomy mosaicism were not detected by karyotype analysis. Four cases exhibited monomeric mosaicism, and the levels of mosaicism detected in three of these cases were higher in karyotype compared with CMA analysis; one case had equivalent levels of monomeric mosaicism from both karyotype and CMA analysis. Two other cases from karyotype analysis were a mix of monosomic and trisomic mosaicism, whereas the CMA result was restricted to monosomic mosaicism for these cases.

Conclusion: Both karyotype and CMA analysis can be used to detect aneuploid chromosome mosaicism. However, the two methods produced different results. CMA and karyotype analysis have their own advantages in detecting aneuploid mosaicism, and the combination of these methods provides a more rigorous diagnosis.

KEYWORDS

aneuploidy, CMA, karyotype analysis, mosaicism, prenatal diagnosis

1 | INTRODUCTION

Newborns with congenital diseases can increase the physical and mental burden on a family and the parents.¹ Chromosomal

abnormalities are the main cause of congenital diseases, which can be manifested as multiple malformations, including physical and mental development disorders and other serious clinical symptoms.²⁻⁴ There is currently no specific treatment for such diseases.

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Timely prenatal diagnosis and intervention are important to reduce serious congenital birth defects.

Chromosomal aneuploidy is an important cause of congenital diseases. The degree of aneuploid chromosome mosaicism has been positively correlated with the severity of the disease. A lower level of mosaicism leads to a milder phenotype,⁵ and higher mosaicism produces a more severe phenotype. Therefore, it is important to accurately detect the level of chromosome mosaicism during pregnancy to determine the severity of a potential congenital disease.

Karyotype and chromosome microarray (CMA) analysis are two prenatal diagnostic methods for chromosome analysis that have been widely used in recent years.^{6,7} Karyotype analysis is an established technique, whereas CMA is a relatively new molecular diagnostic technology.⁸ The time required for diagnosis by karyotype analysis is longer than that of CMA, because the former needs cultured amniotic fluid cells, whereas CMA can use DNA directly extracted from amniotic fluid cells without culture. CMA can detect micro-deletions and micro-duplications of chromosomes, but cannot detect balanced structural abnormalities, such as balanced translocation and inversion of chromosomes. Therefore, clinics often combine the two methods for prenatal diagnosis.⁹

Karyotype and CMA analysis can both detect aneuploid chromosomes. However, differences between the two methods may lead to different results in the diagnosis of aneuploid chromosomes. Karyotype analysis may detect mosaicism while CMA analysis indicates homozygosity, or vice versa, or both methods may detect different levels of mosaicism. The purpose of this study was to compare the differences between karyotype and CMA analysis for the detection of aneuploid chromosome mosaicism and to discuss the causes of any differences.

2 | MATERIAL AND METHODS

2.1 | Patients

Two thousand and ninety-one amniocentesis samples were collected from pregnant women from March 1, 2019, to January 31, 2020, at the Center for Prenatal Diagnosis of the First Hospital of Jilin University. Karyotype and CMA analysis was performed on 1864 samples, and 13 cases of aneuploid chromosomal mosaicism were detected and compared between the two methods. This study was approved by the Ethics Committee of the First Hospital of Jilin University, and written informed consent was obtained from all pregnant women and their families.

2.2 | Karyotype analysis

After a preoperative examination without contraindications, amniocentesis was performed under ultrasound guidance. Thirty millilitre of amniotic fluid was collected in three 15-mL sterile centrifuge

tubes, and 20 and 10 mL were used for karyotype and CMA analysis, respectively. Twenty millilitre of amniotic fluid was cultured in a specialized amniotic fluid culture bottle in a carbon dioxide incubator. After approximately 8 days of culture, adherent cells were examined; then, colchicine was added for 3-4 hours, and cells were harvested according to standard laboratory procedures. G-banding staining and karyotype analysis were performed using our previously published method.¹⁰ Fifty karyotypes were counted to detect mosaicism in each sample.

2.3 | CMA analysis

The CMA analysis was performed using an Affymetrix CytoScan 750K chip (included 550 000 copy number variation markers and 200 000 single nucleotide polymorphism markers). Ten millilitre of amniotic fluid sample was centrifuged for 10 minutes at 0.3 g; then, the supernatant was discarded leaving approximately 200 μ L of cell precipitate. DNA was extracted from amniotic fluid cells using an extraction kit (QIAamp DNA Blood Mini Kit). A 5 μ L sample (250 ng total DNA) was digested with *NspI* to obtain genomic DNA with sticky terminals. An adapter that specifically recognized the sticky ends was added to the *NspI*-digested DNA, and primers for this adapter were used for PCR amplification to obtain genomic DNA with fragment sizes of 150-2000 bp. PCR amplification products DNA were purified using a magnetic bead method. Genomic DNA was enzymatically cut into smaller fragments (25-125 bp). The TDT connect and hybridization solutions were added to samples, which were then incubated at 49°C for 10 minutes, and then hybridized with an Affymetrix CytoScan 750K chip for 16-18 hours. Chips were processed for washing and dyeing on a Gene Chip Fluidics Station 450Dx and then scanned to detect copy number variation and loss of heterozygosity of the human genome (with ≥ 50 probe labels and ≥ 100 kb resolution). Results were analyzed by ChAS analysis software. The level of mosaicism was obtained from the median Log2Ratio value calculated by the software.

3 | RESULTS

Karyotype and CMA analysis of the pregnant women found 13 cases of aneuploid mosaicism. The results of CMA and karyotype analysis, the level of aneuploid mosaicism are shown in Table 1. Cases 1-7 were all trisomic mosaicism. In cases 1-4, the level of trisomic mosaicism calculated from the CMA results was higher than those from karyotype analysis. Unlike CMA analysis, karyotype analysis did not detect trisomic mosaicism in cases 5-7. Cases 8-11 were all monomeric mosaicism. In cases 8-10, the levels of monomeric mosaicism calculated from karyotype analysis were higher than those from CMA analysis. The level of monomeric mosaicism for case 11 was same to the two methods. Karyotype analysis showed that cases 12 and 13 exhibited a mixture of trisomic and monosomic mosaicism, whereas CMA only detected monomeric mosaicism. The levels of

TABLE 1 Methods and results of aneuploid mosaicism analysis

Case no.	Karyotype results	The percentage of aneuploid mosaicism detected by karyotype analysis	CMA results	The percentage of aneuploid mosaicism detected by CMA (%)
1	47,XN,+21[19]/46,XN,[31]	38%	arr (21)×2-3	84
2	47,XN,+22[1]/46,XN,[49]	2%	arr (22)×2-3	35
3	46,XN	/	arr (2)×2-3 arr (3)×2-3	36 33
4	47,XN,+21[3]/46,XN[47]	6%	arr(21)×2-3	47
5	46,XN	/	arr(16)×2-3	16
6	47,XN,+21[5]/46,XN[45]	10%	arr(21)×2-3	15
7	46,XN	/	arr(15)×2-3	14
8	45,X[13]/46,XY[37]	26%	arr(1-22)×2, (X,N)×1	/
9	45,X[24]/46,XX[26]	48%	arr (X)×1-2	46
10	45,X	/	arr (X)×1-2	82
11	45,X[5]/46,XX[45]	10%	arr (X)×1-2	10
12	45,X[43]/47,XXX[7]	86%/14%	arr (X)×1-2	49
13	45,X[48]/47,XY[2]	96%/4%	arr(Y)×0-1	50

TABLE 2 Karyotype and CMA analysis of aneuploid mosaicism in the literature

References	Karyotype results	The percentage of aneuploid mosaicism detected by karyotype analysis	CMA results	The percentage of aneuploid mosaicism detected by CMA
Chen et al ¹⁶	47,XY,+21[4]/46,XY[17]	19%	Mosaic trisomy 21	23.1%
Wu et al ¹⁷	46,XX	/	arr (22) ×3	30%
Chen et al ¹⁸	47,XY,+15[2]/46,XY[17]	10%	Mosaic trisomy 15	30%
Chen et al ¹⁹	46,XX	/	mosaic trisomy 2	NA
Tang et al ²⁰	47,XX,+3[3]/46,XX[35]	8%	mosaicism trisomy 3	10%
Luo Yu-qin et al ²¹	47,XY,+9[11]/46,XY[39]	22%	mosaicism trisomy 9	40%
Tian Yuan et al ²⁴	45,X[4]/46,XX[34]	10%	normal	/
Prakash et al ²⁵	45,X	/	45,X/46XX	83%

monosomic mosaicism calculated from CMA analysis were much lower than those from karyotype analysis.

4 | DISCUSSION

Karyotype and CMA analysis are commonly used for prenatal diagnosis in hospitals.^{11,12} There are a number of differences between the two methods. Karyotype analysis requires manual selection of cultured cells, whereas CMA can use uncultured amniocytes. Karyotype analysis detects entire chromosomes, whereas CMA analyzes gene fragments using a large number of known probes to provide genomic information. These differences lead to differences in the detections of aneuploid mosaicism. For instance, cultivation of amniocytes and manual selection of cells may cause fluctuations

in the level of aneuploid chromosome mosaicism detected by karyotype analysis. For example, poor genetic stability in cells with trisomy may eliminate such cells in long-term cultures from amniotic fluid, reducing the level of trisomy mosaicism after culture.¹³ In contrast, the stability and higher survival rate of cells with monomeric aneuploidy may lead to increased levels of the monomeric mosaicism after culture.¹⁴

In comparison, CMA analyzes the information of gene segments rather than intact chromosomes and will be unable to accurately determine the level of mosaicism in cases with a mixture of monomeric and trisomic mosaicism.¹⁵ To more clearly discuss the differences between karyotype and CMA methods and demonstrate the reasons for the differences, we have listed the results of aneuploid mosaicism from karyotype and CMA analysis recently reported in the literature (Table 2).

In the current study, we found differences between the level of mosaicism found by karyotype compared with CMA analysis. For seven cases exhibiting trisomic mosaicism, the levels of trisomic mosaicism from CMA analysis were higher than the levels from karyotype analysis. These results are consistent with those reported in the literature (Table 2).^{16–21} Chen et al.^{22,23} reported that the calculated level of trisomy mosaicism in amniocytes was higher from fluorescent in situ hybridization (FISH) analysis (using uncultured amniocytes) compared with karyotype analysis. As mentioned above, these results may reflect the poor genetic stability and elimination of cells with trisomy during long-term cell culture (required for karyotype analysis), leading to the relatively reduced levels of trisomy mosaicism from karyotype analysis.

In the present study, three of the four cases of monomeric mosaicism had higher levels of mosaicism from the karyotype analysis compared with the CMA analysis. These results are similar to those reported in the literature (Table 2).^{24,25} Based on the results from our study and those in the literature, we propose that the cells with monomeric aneuploid chromosome mosaicism had a higher proliferation and/or survival rate, resulting in an increased level of cells with monomeric mosaicism after culture. We found that one case had similar levels of monomeric mosaicism calculated from both the CMA and karyotype analysis. The similarity may be due to the manual selection of cells for karyotype analysis that coincidentally exhibited a level of mosaicism equivalent to that from CMA analysis.



The current study identified two cases with a mixture of monomeric and trisomic mosaicism. Karyotype analysis indicated monomeric mosaicism predominated over trisomic mosaicism in both cases. In contrast, CMA analysis only detected monomeric mosaicism in both cases. For both cases, the level of monomeric mosaicism was 86% and 96%, and 49% and 50% from karyotype and CMA analysis, respectively. Therefore, the levels of monomeric mosaicism from the CMA analysis were much lower than those from karyotype analysis. This finding likely reflects the higher number of monomeric cells versus trisomic cells and the CMA method that compares the overall difference in DNA copy number. For instance, the gain of X chromosome material was counterbalanced by a corresponding X chromosome loss, so that the fragmented CMA analysis ultimately detects monomeric mosaicism. This also highlights the limitations of CMA methods for the detection of a mixture of trisomic and monosomic cells. For approximately the same number of trisomic and monosomic cells, the results of CMA may incorrectly show a normal ploidy. Keren Markus-Bustani reported a case of a fetus mosaic for 45,X/47,XXX. The karyotype result was 47,XXX[12]/45,X[4], but CMA analysis failed to detect the aneuploidy.¹⁵

Our study found three cases that exhibited a normal karyotype analysis, but showed trisomic mosaicism by CMA analysis. This may reflect the elimination of trisomic cells during culture, resulting in karyotype analysis missing trisomic detection. This type of case illustrates the importance of using a combination of karyotype and CMA analysis.

5 | CONCLUSION

In conclusion, both karyotype and CMA analysis can be used to detect aneuploid chromosome mosaicism; however, key differences between the two methods lead to different results. For trisomic and monomeric mosaicism, the level of mosaicism from karyotype analysis was lower and higher, respectively, than that from CMA, possibly due to the different requirements of cell culture. The CMA method is faster, as it does not require cultured amniotic fluid cells. However, for cases exhibiting both trisomic and monosomic cells, CMA may produce incorrect findings. In summary, combined detection of mosaicism by both karyotype and CMA analysis is extremely important to provide a more comprehensive and accurate screen for prenatal diagnosis and appropriate genetic counseling.

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