Practical Applications of Chromosomal Microarray in Prenatal Diagnosis

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J Fetal Med 2023;10:23–28.

Abstract

G-banded karyotyping is the most common approach for the detection of genomic alterations. However, this is unable to detect genomic changes of less than 5 Mb. The ability of fluorescence in situ hybridization (FISH) to detect cryptic chromosomal rearrangements exceeds the resolution of routine karyotype. However, conventional FISH is for targeted regions only, whereas the chromosomal microarray is a whole-genome copy number evaluation technique with a resolution of 10 to 20 kb. In this article, we discuss the application of chromosomal microarray 750 K to 384 consecutive prenatal diagnosis cases. Overall diagnostic yield is 15.36%, and chromosomal microarray accounts for a 3.6% additional detection rate. We suggest applying this technique in routine prenatal diagnosis as a first-tier test in prenatal diagnosis along with a backup culture in all cases.

Keywords
- advanced maternal age
- chromosomal microarray
- fetal anomaly
- noninvasive prenatal screening
- prenatal diagnosis

Introduction

Chromosomal abnormalities occur in approximately one in 150 live births, and several methods can be employed to identify these changes.[1] G-banded karyotype analysis has remained a gold standard for detecting fetal chromosomal abnormalities.[2] However, several chromosomal defects associated with moderate-to-severe clinical conditions cannot be resolved using the G-banding technique, which has a resolution of less than 5 to 10 Mb. Chromosomal microarray (CMA) is a molecular cytogenetic technique and has become the test of choice in current times.[3] Indications of CMA have been well characterized in postnatal settings, such as developmental delay/intellectual disability cases, and offers a much higher diagnostic yield (15–20%) than karyotyping and is thus recommended as the first test for these conditions. Improved detection of submicroscopic genetic aberrations holds the best application in the prenatal context. Unfortunately, most of these genetic aberrations result in a phenotype with no available treatment options.

However, the introduction of microarray testing in the prenatal context has been slow for various reasons. The technical limitations include obtaining sufficient and good-quality DNA for microarray results. Additionally, the interpretation of results is challenging due to the restriction of the availability of population-based data for deletions and duplication that are then categorized as variants of uncertain significance (VOUS). Incomplete penetrance and variable

article published online
May 12, 2023

DOI https://doi.org/10.1055/s-0043-57021.
ISSN 2348-1153.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

expressivity further add to the interpretation challenges. The identification of late-onset disorders may also reveal a potentially affected parent. Despite these limitations, several recent extensive prospective studies have demonstrated the feasibility, and utility of microarray in the prenatal setting, showing an increased diagnostic yield over karyotype for all indications for testing and, in particular, for referrals with sonographic abnormalities. A study funded by the National Institute of Child Health and Human Development (NICHD; demonstrated that CMA is more beneficial than karyotyping for fetuses with abnormal ultrasound findings with clinically significant copy number variants (CNVs) identified in 6% (45/755) of this group of fetuses.[4] As per the American College of Obstetricians and Gynaecologists (ACOG) and Society for Maternal-Fetal Medicine (SMFM) guidelines, CMA should be recommended in all cases with ultrasound-detected fetal anomalies.[5,6] Microarray can also be offered in patients with soft markers and/or positive biochemical screening without structural ultrasound anomalies as it has an increased detection rate (1.7%) compared with karyotype.[4]

We present our experience of applying microarray to all prenatal cases where invasive testing was indicated and its clinical utility as a method of choice in prenatal diagnosis in 384 pregnant women who underwent chorionic villus sampling (CVS) or amniocentesis during the study period.

Materials and Methods

Informed consent was taken from all participants enrolled in the study from January 2018 to December 2020 at two tertiary care referral centers involved in the comprehensive fetal diagnosis. All pregnant women referred for genetic counselling and indicated invasive testing were explained about their being at "high risk" for fetal chromosomal abnormalities and the methods available for sampling and analyses. The indications for invasive testing were high risk for chromosomal aneuploidy on a first- or second-trimester screening, high risk on noninvasive prenatal screening (NIPS), increased nuchal translucency (NT), soft markers on ultrasound, fetal structural abnormality detected on ultrasound, couples with balanced translocation carrier, advanced maternal age, and previous child with aneuploidy or CNV.

We discussed the benefits, limitations, and turnaround time for results in routine analysis (karyotype and quantitative-fluorescent polymerase chain reaction [QF-PCR]) and CMA with all couples. In addition, the possibility of detecting a higher number of genomic alterations on microarray than conventional karyotype was discussed. We also discussed the possibility of VOUS and the limitations of microarray in not being able to detect monogenic disorders. All women who opted for QF-PCR/FISH (fluorescence in situ hybridization) and microarray 750K were enrolled in the study.

Fetal samples were obtained by amniocentesis or CVS depending on gestational age. Post-test genetic counselling was done for all cases with normal or abnormal results. Parental CMA analysis was done in cases of VOUS. The primary outcome of this study was the detection of clinically relevant CNVs and aneuploidy.

Single Nucleotide Polymorphism Array

CMA was performed using Affymetrix microarray technology. This microarray consists of 750,000 markers for copy number analysis consisting of 550,000 unique nonpolymorphic probes and approximately 200,000 single nucleotide polymorphism (SNPs) that fully genotype with greater than 99% accuracy. Affymetrix designs this microarray and associated software (Chromosome Analysis Suite) to identify DNA copy number gains and losses related to large chromosomal imbalances. The cutoff filter setting for the CMA test analysis was 400KB for clinically relevant gain/loss and greater than 4 Mb size for loss of heterozygosity. The laboratory follows the American College of Medical Genetics (ACMG) guidelines for reporting microarray findings.[7] All results were correlated with clinical history before reporting. All VOUS were informed if they were found relevant to clinical history. An unrelated pathogenic or likely pathogenic finding was reported if there was sufficient evidence for its involvement in a disorder. Maternal cell contamination was detected by the pattern of SNP markers on the microarray. In selected instances, it was also confirmed by performing variable number tandem repeats-based analysis.

Limitations were discussed again in post-test counselling, especially single gene disorders due to point mutations not being detected on a microarray. All women were followed up, and outcomes were collected either from hospital records or telephonically from referring obstetricians and/or parents. Outcomes were obtained for all pregnancies.

Results

Three-hundred and eighty-four patients were included in the study (►**Table 1**). The mean maternal age for women in the study was 31.9 years. The mean gestational age at which invasive prenatal testing was done was 18.28 weeks.

CVS was done for 66 pregnancies, and amniocentesis was done for 319 cases. One case had to undergo both CVS and amniocentesis to exclude confined placental mosaicism.

Indications for invasive procedures are mentioned in ►**Table 2**. The positive biochemical screening was the most common indication for invasive prenatal testing in 158 cases (41.1%). Invasive testing for soft markers was done in 147 (38.28%) patients. These included both the first and second trimesters. One-hundred and eleven (28.9%) women in this study were of advanced maternal age. There was an overlap in these indications for most women. Forty-three (11.19%) invasive procedures were performed for fetal

Table 1 Baseline characteristics

Characteristics	Number
Maternal age in years (mean ± SD)	31.95 ± 4.92
Gestational age in weeks (mean ± SD)	18.28 ± 4.68
Chorionic villus sampling	66
Amniocentesis	319

Abbreviation: SD, standard deviation.

Table 2 Indications and results of prenatal diagnosis, $n = 384$

Indication	Total cases	Abnormal (*n* %)	Aneuploidy (%)	Abnormal CMA (%)
Positive screen	158	17 (10.75)	13 (76.47%)	4 (23.5%)
USG-soft markers	147	31 (21.08)	24 (77.41%)	7 (22.5%)
USG-Fetal anomaly	43	14 (32.5)	7 (50%)	7 (50%)
AMA	111	23 (20.7)	19 (82.6%)	4 (17.39%)
Positive NIPS	12	8 (54.55)	8(66.67%)	–
Balanced translocation carrier	6	1 (16.66)	–	1
Previous child with ID	13	1 (7.69)	–	1
Indications for prenatal invasive testing in women with AMA (35 years and above), $n = 111$				
Indication	**Abnormal**	**Normal**		
Only AMA	0	7		
AMA + NT > 95th centile	7	0		
AMA + high risk on serum biochemistry	5	57		
AMA + NIPS high risk	4	1		
AMA + soft markers	4	16		
AMA + balanced translocation	0	3		
AMA + structural abnormality	3	2		
AMA + previous child with T21	0	2		
Total (*n*, %)	23 (20.7)	88 (79.3)		

Abbreviations: AMA, advanced maternal age; CMA, chromosomal microarray; NIPS, noninvasive prenatal screening; NT, nuchal translucency; USG, ultrasonography.

anomalies detected on the ultrasound. A positive NIPS was the reason for amniocentesis in 12 cases.

Abnormal CMA results were obtained in 15.36% of cases (59/384; ►**Fig. 1**). Nearly two-thirds of these (45/59, 76.27%) involved aneuploidy that could have been detected by conventional karyotyping. However, the remaining 14 patients (23.7%) had deletions or duplications beyond the resolution limit of karyotyping. Out of the 76.27% abnormal cases of aneuploidy, the majority were trisomy 21 (30/45, 66.7%), followed by Turner syndrome and trisomy 18 (4 cases each). One patient had confined placental mosaicism of trisomy 10 on CVS, but fetal CMA on amniotic fluid was normal.

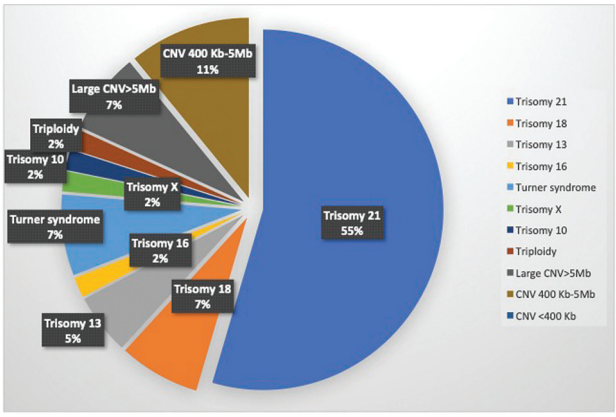

Fig. 1 Types of aneuploidy/copy number variant (CNV).

Pathogenic CNVs were seen in 12/59 (20.33%). VOUS was present in 2/59 (3.38%). Additional details, including clinical findings and CMA results, are detailed in ►**Table 3**. There was an unexpected finding of deletion of the Duchene muscular dystrophy (DMD) gene in two cases where amniocentesis was done for agenesis of the corpus callosum in one case and isolated cleft lip in the second case. We also had two instances of microduplication involving chromosome 15 and one microdeletion on chromosome 15.

Discussion

CMA can detect microdeletions and microduplications with a higher resolution than conventional karyotyping.[8] The resolution of karyotype is 7 to 10 Mb, whereas the resolution of CMA can be as low as 20 Kb. The resolution of karyotyping is also limited by the banding techniques that generally influence the quality of the chromosome preparation; thus, it is not always possible to achieve the desired resolution in routine prenatal banding analysis. It is even more significant when performing karyotyping from CVS samples.

Prior studies have reported a 1:300–1:600 (0.33–0.16%) chance of finding a CNV on using CMA. Microarray also helps identify precise breakpoints involving genes that may cause a serious disability that cannot be otherwise detected by traditional karyotyping.[9] Moreover, the test does objective evaluation rather than subjective analysis. SNP arrays can also identify uniparental disomy, loss of heterozygosity, triploidy, and maternal cell contamination. Other advantages of CMA

Table 3 Clinical, ultrasound, and CMA details of cases other than common and rare trisomy

Case no.	Age	Gestational age	Sample	USG	Deletion	Duplication	Incidental findings	Pathogenic/ benign
1	35	24 weeks	AF	Agenesis of corpus callosum	74 kb deletion arr Xp21.1 (31,734,706–31,808,845) x1	–	Parental testing not done	Pathogenic
2	19	19 weeks	AF	Absent nasal bone and persistent right umbilical vein	160 kb deletion Xp22.33 (509457–669263)x0, hemizygous	–	Incidental findings of clinical relevance	Likely pathogenic
3	28	29 weeks and 6 days	AF	Mild ventriculomegaly, pelvis dilatation	15.1 Mb deletion arr [GRCh38] 5p15.33p15.1 (113461- 15309875)x1	21 Mb duplication arr [GRCh38] 5p15.1p13.2 (15309981 -36335344)x3	Parental karyotype not done	Pathogenic
4	28	14 weeks	CVS	High risk for trisomy 18 on dual marker	585 Kb deletion at 16p11.2 arr[GRCh38] 16p11.2 (29580005- 30165187)x1	1.1 Mb gain at 3p21.3 arr [GRCh38] 3p21.31 (48192206- 49311207)x3	Parental karyotype not done	Pathogenic
5	31	16 weeks and 5 days	AF	Husband balanced translocation carrier T (11;20)	2.4 Mb deletion at 18q23 arr[GRCh38] 18q23 (77795992- 80255845)x1	25.85 Mb gain 1q aarr [GRCh38] 1q41q44 (223221583- 248507023) x3	Parental karyotype done previously	Pathogenic
6	35	19 weeks	AF	Cleft lip	129 kb deletion, arr Xp21.1 (32,661,868–32,791,566) x0	–	Family history of DMD	Pathogenic
7	30	32 weeks	AF	Agenesis of corpus callosum	0.5 Mb deletion arr 15q11.2(22,770,421–23,282,798)x1	–		Pathogenic
8	26	12 weeks	CVS	Increased NT, bilateral CTEV	5.8 Mb deletion, 7q36.1q36.3 (150331810_156114158) x1,	9.5 Mb duplication on 15q, 15q26.1q26.3 (91444669_100976780) x3	Parental karyotype not done	Pathogenic
9	22	20	AF	Bilateral CTEV	–	344 Kb duplication arr [GRCh37] 15q11.2 (25021067_ 25365142)x3 arr[GRCh37]	Parental segregation refused by patient	VOUS
10	29	30	AF	Partial agenesis of corpus callosum	343kb deletion of chromosome 1q43-q44, arr[hg19] 1q43q44(243,969,490–244,989,179)x1	–		Pathogenic
11	31	12	CVS	NT above 95th centile	–	1.2 Mb duplication arr [GRCh37] 8q13.3 (7225752 6		Pathogenic

Table 3 (*Continued*)

Case no.	Age	Gestational age	Sample	USG	Deletion	Duplication	Incidental findings	Pathogenic/benign
12	35	17	AF	High risk on quadruple test	–	666 kb gain arr[GRCh37] 9q34.3 (139455690_140141288) x3	Parental segregation refused by patient	VOUS
13	27	22	AF	Unilateral MCDK, SUA, single hemivertebra	–	2.5 Mb duplication on 15q13.2–13.3.		Pathogenic
14	29	13	CVS	NT above 99th centile, DV reversal, previous TOP for heterotaxy syndrome	8.86 Mb deletion, arr [GRCh37] 4q28.3q31.21 (137828474- 146714962) x1	–		Pathogenic

Abbreviations: AF, amniotic fluid; CTEV, congenital talipes equinovarus, DV-ductus venosus; MCDK, multicystic dysplastic kidney; SUA, single umbilical artery; VOUS, variation of uncertain significance; USG, ultrasound.

include its ability to analyze various tissue types and no mandatory requirement for tissue culture. This results in a faster turnaround time that is crucial in countries where there are legal limits on the timing of termination of pregnancy.

In this study, 15.36% of fetuses (59/384) had chromosomal abnormalities, with CMA providing an additional yield of 3.9%. In an extensive systematic review, 2.4% of all cases (including those with and without structural abnormalities) had a clinically significant finding on CMA that was not identified by karyotype.[8] Fetal anomalies accounted for 3.6% of abnormal results (14/384) in this study, and half of them had abnormal CMA. Several large studies have reported on the yield of CMA in fetuses with ultrasound anomalies. Wapner et al reported clinically significant CNVs in 6% of fetuses with a normal karyotype and an ultrasound anomaly.[4] Srebniak et al used SNP arrays in 1,033 fetuses with ultrasound anomalies and reported 5.5% pathogenic CNVs in fetuses with normal karyotype.[3] In an extensive study of 5000 cases, of which 2462 had ultrasound anomalies, the additional yield of microarray over karyotype was 6.6% in the anomalous cohort.[10] A meta-analysis by Hillman et al found 7 to 10% more abnormalities than karyotype in pregnancies with structural abnormalities.[11] Thus CMA is currently the test of choice when a structural abnormality is detected on ultrasound.

In this study, 7 cases of abnormal CMA and 24 cases of aneuploidy were detected in fetuses with soft markers. A recent paper by Hu et al has also reported a prevalence of 4.3% for chromosomal aberrations in fetuses with soft markers that included 40.2% numerical abnormalities, 48.6% pathogenic CNVs, and 11.2% "likely pathogenic" CNVs.[12] Thus, CMA should be offered to women with soft markers on ultrasound even in absence of structural abnormalities.

We had 38 cases of increased NT which were categorized into NT between 95th and 99th centile and NT more than 99th centile. Fourteen out of thirty-eight (36.8%) women had an abnormal result: 11/14 (78.6%) involved a common aneuploidy, out of which 10 were trisomy 21 cases. The remaining 3/14 (21.4%) had an abnormal CMA. This is similar to a prior study including 226 pregnancies with NT more than 99th centile, wherein 88% cases had aneuploidy of five common chromosomes.[13] A systematic review and meta-analysis reported that CMA provides a 5% incremental yield in detecting CNVs in fetuses with increased NT with normal karyotype.[14] With the widespread availability and acceptance of NIPS, even women with increased NT are opting for it. However, given the incremental increase in detection of pathological CNVs, it has been suggested that the NT cutoff for diagnostic testing for CMA should be 3 mm rather than 3.5 mm that is consistent with the results of this article.[15]

Our study shows some interesting though unexpected findings, such as the two cases (case 1 and case 6) with a deletion in the DMD gene. DMD is a muscular dystrophy affecting males and manifests after birth, usually at 3 to 4 years. After detecting this deletion, it is essential to find out the gender of the child as this is an X-linked recessive condition. In one case (case 6), amniocentesis and CMA were done for isolated unilateral cleft lip in the fetus, and family history suggestive of DMD could be elicited only in

retrospect. This also highlights the importance of pretest counselling that informs parents of possible unexpected pathogenic results on CMA testing.

In our study, we found three cases of CNV involving chromosome 15 which is a well-known region for recurrent CNV. We also detected four double segment exchange cases, which has further implications as this kind of unbalanced double exchange indicates balanced translocation in a couple. Hence, the parental karyotype is required for further genetic counselling.

VOUS remains the biggest challenge in counselling especially in the prenatal context. However, this uncertainty is similar to what is observed in routine prenatal counselling or any genetic testing. In the initial *The New England Journal of Medicine* article by Wapner et al, the incidence of VOUS was 2.5%.[4] Over the next 7 years, a re-review of the interpretation of the CNVs reduced this to 0.9% based on new literature.[16] This can be further decreased if parental samples are available since it could be inherited from a parent. The incidence of VOUS in our study is 3.3%. There remains a potential to miss low-level mosaicism, less than 20% with CMA, although karyotype can also miss low-level mosaicism as fewer cells are counted.

Most of the disadvantages of CMA are relative and require clinical expertise in its application in the prenatal setting, for example, CMA cannot identify balanced rearrangements. However, the clinical relevance of detecting balanced translocation is limited for the health of that individual as the main implication is an abnormal gamete at conception. Some laboratories have adopted the approach of doing both analyses. CMA is the primary diagnostic tool to expand the clinically relevant diagnostic results. The karyotype is used to avoid missing balanced changes, which will help evaluate the underlying mechanism and recurrence risk estimations. However, increasing the cost of testing with this approach remains challenging.

We acknowledge the limitations of the article as the numbers included are limited and larger studies are needed to further validate our findings.

Conclusion

Genetic technology has advanced rapidly in the past few decades. Its applications and use in caring for and counselling pregnant women are evolving and need to be updated regularly in prenatal diagnosis. The results of this study suggest that more widespread CMA testing of fetuses would result in a higher detection of clinically relevant chromosome abnormalities. We propose that this technique be used as first-tier in all cases undergoing prenatal diagnosis, the same as recommended by ACMG in postnatal cases. A backup culture should be kept in all cases to handle all major challenges expected with this approach.

Ethics Committee Statement

We did not take ethics committee permission as this was a retrospective study. We took consent from all participants before doing testing.

Funding

None.

Conflict of Interest

None declared.

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