Journal of Genetics (2019) 98:6
https://doi.org/10.1007/s12041-018-1050-5

RESEARCH ARTICLE

Cytogenetic microarray in structurally normal and abnormal foetuses: a five years experience elucidating increasing acceptance and clinical utility

MEENAKSHI LALLAR, PRIYANKA SRIVASTAVA, ARCHANA RAI, DEEPTI SAXENA, KAUSIK MANDAL and SHUBHA R. PHADKE*

Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226 014, India
*For correspondence. E-mail: shubharaophadke@gmail.com.

Received 7 March 2018; revised 6 July 2018; accepted 24 September 2018; published online 12 February 2019

Abstract. The aim of the present study was to evaluate the diagnostic yield of prenatal cytogenetic microarray (CMA) in structurally normal and abnormal foetuses and record the acceptance rate of CMA for prenatal diagnosis over a course of five years. In 128 structurally normal and abnormal foetuses, CMA was performed along with foetal karyotype, after exclusion of aneuploidy by quantitative fluorescence polymerase chain reaction. The microarray was able to detect the pathogenic variants in 5.5% cases; the diagnostic yield in structurally abnormal foetuses was 8.8% and 4.7% in foetuses with a high aneuploidy risk. Balanced and unbalanced translocations, and low level mosaicism were detected. Reanalysis of variants of uncertain significance identified pathogenic variant. The study shows higher diagnostic yield in structurally abnormal cases, the importance of foetal karyotype and reanalysis in microarray. The acceptance rate of prenatal CMA increased five-fold over a period of five years.

Keywords. cytogenetic microarray; prenatal; foetal structural abnormalities; karyotype; variants of uncertain significance.

Introduction

Prenatal cytogenetic testing began with foetal karyotyping and gradually evolved into high resolution using less time and labour intensive molecular cytogenetic techniques like fluorescence *in situ* hybridization (FISH), quantitative fluorescence polymerase chain reaction (QFPCR) and cytogenetic microarray (CMA). Very high-resolution coverage of the whole genome by CMA has led to replacement of traditional karyotyping by CMA for genomic imbalances. The advent of single-nucleotide polymorphism (SNP) array has further expanded the use of CMA to detect uniparental disomy and mosaicism. The analysis of CMA for copy number variations (CNV) is objective and computerized; but interpretation about pathogenic nature of the CNVs requires expertize. Not all CNVs are pathogenic, in some, the interpretation about their pathogenic nature is impossible. Counselling for such variants of uncertain significance (VOUS) is a challenge in prenatal and postnatal CMA studies.

CMA has 6–7% additional yield over conventional karyotype in foetuses with ultrasonographically detected malformations and 1.7% additional yield in foetuses with normal ultrasonographic findings (Wapner *et al.* 2012). The American College of Obstetrics and Gynaecology (ACOG) has recommended CMA as a first line test in foetuses with major ultrasonographic anomalies (ACOG 2013). In this study, we report our five years demographic and clinical experience of prenatal amniotic fluid CMA in 128 cases.

Materials and methods

This descriptive study includes 128 patients who were evaluated at our centre from 2013 to 2017. The study was approved by the Hospital ethics committee. All the patients who opted for invasive prenatal testing by amniocentesis for various indications were offered CMA after pre-test counselling and informed consent. All the families opting for CMA were informed that only pathogenic CNVs and likely pathogenic CNVs causing developmental disabilities like intellectual disability and autism will be reported. Copy number variants for susceptibility to adult onset diseases, neoplasia, carrier status for recessive diseases and Y chromosome polymorphisms were

not reported. When required, as to aid the interpretation of CNV results, the option of parental CMA was provided. The patients who opted for CMA after pre-test counselling were divided into groups according to the primary indication of prenatal diagnosis—high risk in aneuploidy screening, increased maternal age, previous history of genetic disease or family history of genetic disease, major foetal anomalies or soft markers identified by ultrasonography and structural chromosomal rearrangements in parents.

Amniocentesis was performed in all patients after 16 weeks of gestation. After discarding the first 2 mL of amniotic fluid (to avoid maternal cell contamination) around 20 mL amniotic fluid was sent to the cytogenetic laboratory. Amniotic fluid culture was established for all samples and rapid aneuploidy testing by QFPCR for common trisomies was performed. Cases with aneuploidy identified with QFPCR were not processed for CMA. In the samples with normal QFPCR results, CMA was then performed. Optimal foetal DNA concentration for CMA was taken as more than 50 ng/μL. In the case of blood-stained amniotic fluid or low foetal DNA concentration, foetal DNA was extracted from the cultured amniotic fluid cells.

CMA

Initially CMA was performed by Cytoscan HD array on 14 amniotic fluid samples. This high density includes 750,000 genotypeable SNPs and 1.9 million nonpolymorphic probes. Further in the next 112 patients, Affymetrix CytoScan 750 K Array was used. This array contains greater than 750,000 markers including 200,000 genotypeable SNPs and 550,000 nonpolymorphic probes which provide high resolution copy number, accurate breakpoint estimation, and loss of heterozygosity detection.

CNV

The laboratory cut off for reporting copy number gains and losses was defined as 400 and 200 kb, respectively, unless the CNV involved a known clinically significant disorder or morbid gene. Analysis of CNVs was done using a UCSC genome browser (GRCH 37/hg19 assembly), ISCA, OMIM, DECPIHER and in house database. The report format was based on International System for Human Cytogenic Nomenclature (ISCN) nomenclature (2013). The CNVs were interpreted and reported in accordance with American College of Medical Genetics (ACMG) guidelines as—benign, likely benign, VOUS, likely pathogenic and pathogenic (South *et al.* 2013). CNVs previously reported in the literature and databases as benign, with high population frequency were classified as benign. CNVs were classified as likely benign, in the following circumstances: regions with no genes, heterozygous duplications with no known morbid OMIM genes, CNVs overlapping with segmental duplications or polymorphic regions and CNVs reported as likely benign in the literature and databases. The CNVs which are associated with known microdeletion/microduplication syndrome and large *de novo* variants with genes associated with phenotypes like autism, epilepsy, intellectual disability or other significant neurological dysfunctions were classified as pathogenic and likely pathogenic, taking into consideration the size, exact breakpoints, penetrance, variable expressivity and inheritance pattern. A VOUS was defined as an alteration of unclear clinical relevance that has not been previously identified and reported in the medical literature, has not been found in publicly available databases, and/or does not contain any known disease-causing genes.

Results

Table 1 lists the patient groups according to the test indication. The most common indication for opting for CMA

Table 1. Indications of prenatal testing and outcomes.

	Indications	Number of patients ($n = 128$)	CNV		
			Pathogenic	Likely pathogenic	VOUS
1	Trisomy 21 risk	39 (30.5%)	2		1
2	Previous child with genetic disease/malformation	23 (18%)	1	1	0
3	Increased maternal age	4 (3.1%)	0	0	0
4	Family history of genetic disease	3 (2.3%)	0	0	0
5	Translocation in parents	2 (1.6%)	0	0	0
6	Abnormal ultrasonographic finding	57 (44.5%)		0	
	Soft markers	20 (15.6%)			1
	Major malformations	37 (28.9%)	3		

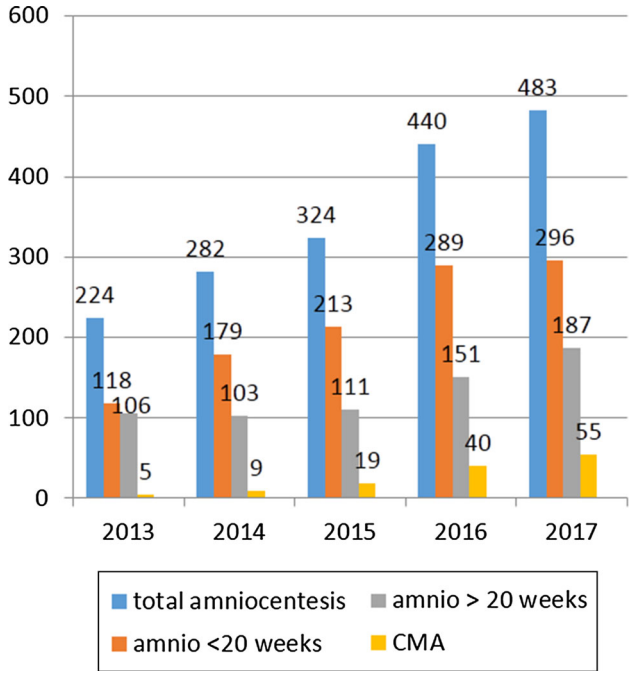

Figure 1. Increasing number of amniocentesis and CMA over a five years period (termination of pregnancy is not legally allowed in India after 20 weeks of gestation).

was abnormal ultrasonographic findings (44.5%), followed by trisomy 21 risk (30.5%). Figure 1 shows the number of amniocentesis and CMA from 2013 to 2017. In five years of study, 128 women of total 1753 undergoing amniocentesis, i.e. 7.3% opted for CMA. There was a gradual increase in the number of amniocentesis as well as CMA (CMA opted by 2.2% women undergoing amniocentesis in 2013 versus 11.4% women in 2017) (figure 1).

Table 2 shows the characteristics of CNV detected on CMA. No CNV (deletions and duplications) was detected in 17 amniotic fluid samples (13.3%), in 111 samples CNVs were identified (86.7%). In most cases, single or two variants were identified. The average numbers of variants detected per sample in the study were 1.6. The total number of benign CNVs reported were 47 (36.7%), of which 38 patients had 14q32.33 region gain ranging in size from 400 to 928 kb. This has been previously identified as a recurrent benign polymorphism in our in-house database (Boggula *et al.* 2015). But a very high frequency is speculated to be a design artefact associated with CMA technique (Redon *et al.* 2009). Likely benign CNVs were detected in 55 (43%) of the patients. The overall yield of CMA in terms of detecting clinically significant CNV was 5.5% (7/128) (pathogenic and likely pathogenic variants were 4.7 and 0.8%, respectively). The yield of CMA for detecting pathogenic variants in patients with high risk for aneuploidy was 4.7% (2/43) and in the presence of structural abnormalities ultrasound findings was 8.1% (3/37).

Six pathogenic CNVs were detected (table 3). Two pathogenic CNVs were detected in patients with high risk of trisomy 21 on aneuploidy screening; in both heterozygous gain of size 750.8 and 711 kb, respectively, were detected on chromosome 16p11.2 region. In a patient with previous child with Down's syndrome (47,XX,+21) amniocentesis was done as there is a small risk of recurrence ~1%. In this patient a pathogenic CNV was identified, a 584.5 kb heterozygous gain on 2p16.3 involving the NRXN1 gene. Parental CMA was done to check if this variant was inherited or *de novo*. As, the parental CMA was normal, *de novo* inheritance was established. In another foetus with multiple anomalies on ultrasound (Intra uterine growth retardation (IUGR), oligohydramnios, agenesis of corpus callosum, ventricular septal defects (VSD), mitral atresia and multicystic kidneys); 3.8-Mb heterozygous loss and 3.2-Mb heterozygous gain on chromosome regions 4p16.3 and 11p15, respectively were identified. In this case, the karyotypes of parents were normal. Another pathogenic variant, 272.6-kb heterozygous loss on chromosome region 7q36.2, containing gene *DPP6*, was noted in a foetus with symmetrical IUGR. This variant was detected when CMA was done in 2013 and reported as VOUS at that time. But now during the reanalysis of the CMA data, it was reclassified as pathogenic. The likely pathogenic CNV was a 171.4-kb heterozygous loss on 2p16.3 involving the *NRXN1* gene. Two VOUS were

Table 2. Number of CNVs per case, interpretation and diagnostic yield of CMA.

No. of CNV detected per case						
No. of CNV per case	Number of cases ($n = 128$)		Interpretation	No. of cases	Overall diagnostic yield (pathogenic and likely pathogenic) (7/128) (5.5%)	
0	17 (13.3%)					
1	55 (43%)	Cases with CNV ($n = 111$)	Benign	47 (36.7%)	With aneuploidy risk 4.7%	With major structural malformation 8.1%
2	33 (25.8%)		Likely benign	55 (43%)		
3	15 (11.7%)		VOUS	2 (1.6%)		
4	5 (3.9%)		Likely pathogenic	1 (0.8%)		
>4	3 (2.3%)		Pathogenic	6 (4.7%)		

Table 3. Pathogenic, likely pathogenic and VOUS CNVs detected in prenatal microarray in 128 cases.

	Indication	Chromosome band	Size	Breakpoint	Gene	CNV	Interpretation and outcome
1	Trisomy 21 risk	16p11.2†	750.8-kb Heterozygous gain	16:29580020–30330881	OMIM genes: 20	Pathogenic	16p11.2 microduplication is associated with developmental delay, intellectual disability, and/or autism spectrum disorder. In both the cases, pregnancy was terminated.
2	Trisomy 21 risk	16p11.2	711.0-kb Heterozygous gain	16:29591326–30302348	OMIM genes: 20	Pathogenic	
3	Previous child with trisomy 21	2p16.3	584.5-kb *De-novo* heterozygous loss	2:50824330–51408871	OMIM gene: 1 – *NRXN1*	Pathogenic	*NRXN1* deletions with overlapping breakpoints previously reported with neurodevelopmental abnormalities. The pregnancy was terminated. Foetal autopsy-flat facial features and midface hypoplasia.
4	Corpus callosum agenesis, VSD mitral atresia, severe IUGR and oligohydramnios.	4p16.3, Heterozygous loss 11p15.5 3181.8-kb Heterozygous gain	3844-kb	4:68345–3912660 11:230680–3412551	OMIM genes: OMIM genes: 70 40	Pathogenic	Intrauterine death at 24 weeks. The facial dysmorphism, structural abnormalities, severe IUGR were consistent with the detected 4p deletion—Wolf Hirschhorn syndrome. Parental karyotype normal.
5	Symmetric IUGR‡	7q36.2	272.6-kb Heterozygous loss	7:154258998–154531649	OMIM gene: *DPP6*	Pathogenic	*De novo* point mutations and deletions of *DPP6* are associated with microcephaly and intellectual disability. Postnatal phenotype consistent with above findings.
6	Perimembranous VSD	Xp22.3, Heterozygous gain 20q13.2,	578-kb 12.6-Mb	X:524439–1102585 20:50071393–62745089	OMIM gene: *SHOX* 87 genes	Pathogenic	Preterm birth with—micrognathia, microcephaly, preauricular tag, cleft palate with perimembranous VSD and left sided dysplastic kidney.

Table 3. (*contd*)

	Indication	Chromosome band	Size	Breakpoint	Gene	CNV	Interpretation and outcome
		22q13.33, Heterozygous gain	588-kb	22:49434634–50022320			
		17q21.32, Heterozygous loss	256-kb	17:46707528–46963847	Six genes		
		Xq28 Heterozygous loss	943-kb	X:154289273–155233098	Nine genes		
7	Previous child with ? Inborn error of metabolism (IEM)	2p16.3 Heterozygous loss	171.4-kb	2:50914045–51085471	OMIM gene: *NRXN1*	Likely pathogenic	*NRXN1* deletions with overlapping breakpoints reported with neurodevelopmental abnormalities. However, classified as likely pathogenic in view absence of parental studies and overlapping breakpoints classified as VOUS in databases. Lost to follow-up.
8	Trisomy 21 risk	Xp22.31 Gain	569.8 kb	X:7831495–8401383	OMIM gene: 2—*PNPLA4* (obesity) *VCX2* (not well characterized)	VOUS	Male foetus. Larger duplications associated with developmental delay, intellectual disability and autism spectrum disorder. In this case probable critical genes are not involved. Phenotype in such cases will also be affected by skewed X inactivation, variable expressivity and decreased penetrance. Mother was not tested for the same duplication. Follow-up: development and growth is normal. Child is one year old with normal development.
9	Single umbilical artery	7q11.23, Heterozygous loss	794-kb	7:75952095–76746141	OMIM genes: 6, *YWHAG*	VOUS	Haploinsufficiency in *YWHAG* contributes to infantile spasms in William syndrome but is not solely responsible for it. Early infantile epileptic encephalopathy is caused by *de novo* point mutations in *YWHAG* gene. Follow-up: child is 3 months old attained normal social smile and neck holding.

[†] Allelic to recurrent 16p11.2 microdeletion (nucleotides 29,606,852–30,199,855),
[‡] Detected on HD array, all the other CNV were detected on cytoscan 750k.

detected (1.6%). A 569.8-kb heterozygous gain on chromosome region Xp22.31 in a male foetus encompassing two genes (*PNPLA4* and *VCX2*) and a 794-kb heterozygous loss on chromosome region 7q11.23 containing the gene *YWHAG* was detected in a foetus with single umbilical artery in ultrasound (table 3).

Translocations

In a couple with Robertsonian translocation in the male [45,XY,der(14;15) (q10;q10)], the foetal karyotype also detected similar translocation and CMA showed no CNV. In another couple, where the male had balanced translocation, 46,XY, t(18;22)(p11;q11.2), foetal karyotype showed similar balanced translocation and normal CMA. In the third case, with choroid plexus cyst and renal pyelectasis on ultrasound, the foetus had mosaic karyotype, with deletion of chromosome 19 short arm—46,del(19)(p13.1→ter)[16]/46[14]. Here, CMA reported no pathogenic CNV. Subtelomeric multiplex ligation-dependent probe amplification (MLPA) was also performed and found to be normal. The pregnancy was terminated and on foetal autopsy facial dysmorphism (hypertelorism, coarse face) and agenesis of corpus callosum were observed (figure 2a). In this family there was history of previous intrauterine foetal demise at 30 weeks and the foetus had agenesis of corpus callosum, oedema of hands and feet, lobulation defects in lungs, interhemispheric cyst, severe oligohydramnios, single umbilical artery and IUGR. In the fourth case, ultrasonography revealed perimembranous VSD at 24 weeks and the foetal karyotype was suggestive of a balanced chromosomal rearrangement involving chromosome X and 22[46,t(X;22)(q11.2;q28)]. CMA was performed, and identified multiple CNVs—12.6-Mb heterozygous duplication on 20q13.2 and 578 kb duplication on Xp22.33 and 943 kb deletion on Xq28 which were found to be pathogenic (table 3). The couple continued the pregnancy and a preterm baby girl (30 weeks) was born with multiple congenital anomalies—micrognathia, microcephaly, preauricular tag, cleft palate, congenital heart defect (perimembranous VSD) and left sided dysplastic kidney (figure 2b). The parental karyotypes were normal.

Figure 2. (a) Hypertelorism and coarse facies in 23 week foetus with mosaic 19p deletion, normal CMA. (b) Dysmorphism in 30 weeks preterm with apparently balanced chromosome X and 22 translocation and CNVs identified on CMA.

Discussion

The overall yield for clinically significant pathogenic CNVs in our study was 5.5% (7/128); detection rate being 4.7% (2/43) in group with high aneuploidy risk, normal ultrasonographic evaluation and 8.1% (3/37) in group with significant structural abnormality in ultrasound (table 2). Various studies have reported detection rates for pathogenic CNVs for high risk of aneuploidy in the range 1.7–5.5%; concurrently the detection rates for pathogenic CNVs reported for significant structural abnormalities on ultrasound is reported higher and varied in the range 4–10% in different studies (Breman *et al.* 2012; Shaffer *et al.* 2012; Hillman *et al.* 2013; Wapner *et al.* 2012) (table 4). The detection rate of VOUS in our study was 1.6%, similar to previous studies where VOUS were detected in the range 1.7–4.2% (Breman *et al.* 2012; Shaffer *et al.* 2012; Hillman *et al.* 2013; Wapner *et al.* 2012) (table 4). The two VOUS detected were—a heterozygous gain on chromosome region Xp22.31 in a male foetus (encompassing two genes, *PNPLA4* and *VCX2*) and a heterozygous loss on chromosome region 7q11.23 containing the morbid gene *YWHAG* (Esplin *et al.* 2014; Guella *et al.* 2017). As described in table 3, these VOUS pose a great challenge in interpretation of prenatal CMA. Periodic reanalysis, with availability of newer literature and long-term follow-up of such cases is essential.

Table 4. Comparison of prenatal CMA detection rates in different studies.

	Study, year	CMA platform	Sample size	Overall pathogenic CNV (%)	Pathogenic CNV in ultrasound major structural abnormality (%)	Pathogenic CNV in high risk for aneuploidy (%)	VOUS (%)
1	Wapner *et al.* (2012)	44K oligo/SNP Genechip 6.0	4406	3.7	6	1.7	3.4
2	Breman *et al.* (2012)	BAC/44K oligo/105K oligo	1115	4.2	9.3	5.4	1.7
3	Shaffer *et al.* (2012)	Multiple	5003	5.3	6.5	5.5	4.2
4	Hillman *et al.* (2013) study	Multiple	243		4.1	–	2.1
	Meta-analysis				10		1.4
5	Present study	CytoHD array/750K	128	5.5	8.1	4.7	1.6

As shown in figure 1, over the five years study period, we observed a gradual increase in the number of patients opting for prenatal invasive testing at earlier gestation. In spite of the high costs associated with testing, the acceptance of CMA increased five times from being just 2.2% in 2013 to 11.4% in 2017 (figure 1). Abnormal ultrasonographic findings and a high risk of aneuploidy remained the chief indications for CMA testing (table 1).

The overall prevalence of clinically relevant microdeletions and duplications in structurally normal foetuses is 1–1.7% and as compared to aneuploidy does not increase with advanced maternal age (Wapner *et al.* 2012). In our study, the two cases of 16p11.2 microduplication were observed in pregnant women with a high risk of aneuploidy (table 3). With wider availability of CMA, submicroscopic 16p11.2 chromosomal rearrangements are increasingly being recognized as one of the most common genomic disorders in developmental delay and autism (Weiss *et al.* 2008). The two recurrent disorders in 16p11.2 region include—the common microdeletions/duplications of ∼600 kb in 16p11.2 (29.5–30.1 Mb position); and the second lesser common microdeletions of ∼220 kb in 16p11.2 (28.7–28.9 Mb position). Both of our patients had microduplications in chromosome region 16p11.2, 16:29580020–30330881 and 16:29591326–30302348, respectively. The recurrent microduplications/microdeletions in region 16p11.2 (29.5–30.1 Mb position), is because known low copy repeats (LCRs) ∼1.3 Mb flank the surrounding of the proximal 16p11.2 locus (chr16:29,100,000–30,400,000) (Shinawi *et al.* 2010). It is important to carefully identify and delineate such deletions from the nearby benign euchromatic variants in the 16p11.2 chromosomal region (31–32 Mb position).

The *de novo* heterozygous *NRXN1* deletion was detected in a woman with the previous child being trisomy 21. *NRXN1* mutations (both single exon and CNVs) have been associated with a range of neurodevelopmental disorders, including autism spectrum disorders, schizophrenia, intellectual disability, speech and language delay, epilepsy and hypotonia (Curran *et al.* 2013). In a foetus with multiple malformations, foetal karyotype was reported normal and heterozygous 3.8 Mb loss and 3.2 Mb gain on chromosome regions 4p16.3 and 11p15.5 respectively, were detected (table 3).

The pathogenic CNV, a 272.6 kb heterozygous loss in *DPP6* gene in a foetus with symmetrical IUGR was initially reported as VOUS in 2013. However, now during the reanalysis, taking into consideration the latest literature (Liao *et al.* 2013) and postnatal phenotype, it was reclassified as a pathogenic variant and parental testing was advised. It is now known that loss of function variants in *DPP6*, both deletions and point mutations, are associated with autosomal dominant microcephaly and intellectual disability, with decreased penetrance and varied expressivity (Liao *et al.* 2013). This highlights the

significance of long-term follow-up and reanalysis over time. Similarly, many VOUS are expected to be reclassified as benign or pathogenic on reanalysis on long-term follow-up as more CNV data are generated in coming years.

The two cases in our study with balanced translocations in one of the parents and similar in foetus elucidate the limitation of CMA to detect balanced chromosomal rearrangements. In the third case, foetus was mosaic for deletion of chromosome 19 short arm—{46,del(19)(p13.1→ter)[16]/46[14]}. The CMA results were normal. CMA usually detects mosaicism of around 30%. But in this foetus mosaicism was missed on CMA and detected by routine karyotyping. The most probable reason could be that during culture the cells containing the deletion had a survival advantage/higher proliferation and outnumbered the normal cells, which led to them being seen in a higher number in karyotyping as compared to CMA where foetal DNA obtained from amniotic fluid was analysed. This highlights the importance of conventional karyotyping in all cases as CMA does not detect low-level mosaicism. With two foetuses with similar major malformations, karyotype was done in the second foetus and a mosaic deletion of chromosome 19 was identified suggesting that most likely either of the parents was a carrier for a balanced translocation involving chromosome 19, in a complete or mosaic form. This could be validated by karyotyping the parents and/or subtelomeric FISH studies.

In another case with VSD on prenatal, ultrasonographic evaluation foetal karyotype showed a balanced translocation between chromosomes X and 22- 46,t(X;22)(q11.2;q28) (table 3). However, CMA identified multiple CNVs—heterozygous duplication on 20q13.2, duplication on Xp22.33 and deletion on Xq28, all pathogenic. This case highlights utility of CMA in identifying small CNV in apparently balanced foetal karyotypes.

Counselling issues are of paramount significance. Pretest and post-test counselling are very important while offering prenatal CMA. The diagnosis of variations of uncertain significance and CNVs associated with susceptibility to cancers and late-onset diseases are major challenges for counselling. As mentioned in the methods, we inform the families who are opting for prenatal CMA that only pathogenic CNVs and likely pathogenic CNVs causing developmental disabilities like intellectual disability and autism will be reported. The information is also included in the pamphlet provided to the families before testing. It should be discussed that parental studies might be required, to determine *de novo* or inherited variants. Post-test counselling is relatively straightforward in pathogenic CNVs. Post-test counselling for likely pathogenic CNVs VOUS poses challenges to the counsellor and dilemma for the families. The policies regarding reporting of these variations should be in place for each laboratory and need to be conveyed to the clinician while ordering the test and to the patient/family while offering the test.

Our five years study shows a gradual increase in the acceptance rate of CMA for prenatal diagnosis. This study highlights the importance of CMA in detection of clinically significant pathogenic CNVs in pregnant women with foetal structural abnormalities and in women with structurally normal foetuses. It is important to maintain long-term follow-up in VOUS and reanalyse the results with availability of newer literature. Although CMA has a higher yield and identifies CNVs in apparently balanced foetal karyotypes, it is important to perform foetal karyotyping along with CMA in all the cases as CMA does not detect foetal balanced chromosomal rearrangements and low-level mosaicism.

Acknowledgements

We thank 'Indian Council of Medical Research' for funding support (ICMR grant number: 63/8/2010-BMS) and we are thankful to the patients' families for their unending support and patience.

References

American College of Obstetricians and Gynecologists Committee on Genetics 2013 Committee Opinion No. 581: the use of chromosomal microarray analysis in prenatal diagnosis. *Obstet. Gynecol.* **122**, 1374–1377.

Boggula V. R., Agarwal M., Kumar R., Awasthi S. and Phadke S. R. 2015 Recurrent benign copy number variants and issues in interpretation of variants of unknown significance identified by cytogenetic microarray in Indian patients with intellectual disability. *Indian J. Med. Res.* **142**, 699–712.

Breman A., Pursley A. N., Hixson P., Bi W. Ward P., Bacino C. A. *et al*. 2012 Prenatal chromosomal microarray analysis in a diagnostic laboratory; experience with >1000 cases and review of the literature. *Prenat. Diagn.* **32**, 351–361.

Curran S., Ahn J. W., Grayton H., Collier D. A. and Ogilvie C. M. 2013 NRXN1 deletions identified by array comparative genome hybridisation in a clinical case series—further understanding of the relevance of NRXN1 to neurodevelopmental disorders. *J. Mol. Psychiatry* **1**, 4.

Esplin E. D., Li B., Slavotinek A., Novelli A., Battaglia, Clark R. *et al*. 2014 Nine patients with Xp22.31 microduplication, cognitive deficits, seizures, and talipes anomalies. *Am. J. Med. Genet. A.* **164**, 2097–2103.

Guella I., McKenzie M. B., Evans D. M., Buerki S. E., Toyota E. B., van Allen M. I. *et al*. 2017 De Novo mutations in YWHAG cause early-onset epilepsy. *Am. J. Hum. Genet.* **101**, 300–310.

Hillman S. C., McMullan D. J., Hall G., Togneri F. S., James N., Maher E. J. *et al*. 2013 Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound Obstet. Gynecol.* **41**, 610–620.

Liao C., Fu F., Li R., Yang W. Q., Lia H. Y., Yan J. R. *et al.* 2013 Loss-of-function variation in the DPP6 gene is associated with autosomal dominant microcephaly and mental retardation. *Eur. J. Med. Genet.* **56**, 484–489.

Redon R., Rigler D. and Carter N. P. 2009 Comparative genomic hybridization: DNA preparation for microarray fabrication. *Methods Mol. Biol.* **529**, 259–266.

Shaffer L. G., Dabell M. P., Fisher A. J., Coppinger J., Bandhdz A. M., Ellison J. W. *et al*. 2012 Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies. *Prenat. Diagn.* **32**, 976–985.

Shinawi M., Liu P., Kang S. H., Shen J., Belmont J. W., Scott D. A. *et al*. 2010 Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J. Med. Genet.* **47**, 332–341.

South S. T., Lee C., Lamb A. N., Higgins A. W., Kearney H. M. *et al*. 2013 Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet. Med.* **15**, 901–909.

Wapner R. J., Martin C. L., Levy B., Ballif B. C., Christine M., Zachary I. M. *et al.* 2012 Chromosomal microarray versus karyotyping for prenatal diagnosis. *N. Engl. J. Med.* **367**, 2175–2178.

Weiss L. A., Shen Y., Korn J. M., Arking D. E., Miller D. T., Fossdal R. *et al.* 2008 Association between microdeletion and microduplication at 16p11.2 and autism. *N. Engl. J. Med.* **358**, 667–675.

Corresponding editor: H. A. Ranganath