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ORIGINAL ARTICLE

Prenatal Diagnosis by Chromosome Microarray Analysis, An Indian Experience

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Abstract

Background Karyotyping has been the gold standard for prenatal chromosome analysis. The resolution should be higher by chromosome microarray analysis (CMA). The challenge lies in recognizing benign and pathogenic or clinically significant copy number variations (pCNV) and variations of unknown significance (VOUS). The aim was to evaluate the diagnostic yield and clinical utility of CMA, to stratify the CMA results in various prenatal referral groups and to accumulate Indian data of pCNVs and VOUS for further interpretation to assist defined genetic counseling.

Methods Karyotyping and CMA were performed on consecutive referrals of 370 prenatal samples of amniotic fluid ($n=274$) and chorionic villi ($n=96$) from Indian pregnant women with high maternal age ($n=23$), biochemical screen positive ($n=61$), previous child abnormal ($n=59$), abnormal fetal ultrasound ($n=205$) and heterozygous parents ($n=22$).

Results and Conclusion The overall diagnostic yield of abnormal results was 5.40% by karyotyping and 9.18% by CMA. The highest percentage of pCNVs were found in the group with abnormal fetal ultrasound (5.40%) as compared to other groups, such as women with high maternal age (0.81%), biochemical screen positive (0.54%), previous abnormal offspring (0.81%) or heterozygous parents group (1.62%). Therefore, all women with abnormal fetal ultrasound must undergo CMA test for genotype–phenotype correlation. CMA detects known and rare deletion/duplication syndromes and characterizes marker chromosomes. Accumulation of CNV data will form an Indian Repository and also help to resolve the uncertainty of VOUS. Pretest and post-test genetic counseling is essential to convey benefits and limitations of CMA and help the patients to take informed decisions.

Keywords Prenatal · Microarray · CNV · VOUS · Microdeletions · Microduplications

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Introduction

Since 1960, karyotyping has been the gold standard for prenatal chromosome analysis in pregnant women with risk of chromosomal defects [1]. Karyotyping detects genomic imbalances, such as aneuploidies and unbalanced derivative chromosomes, all visible at the microscopic level (size ≥ 4 Mb). Chromosomal microarray analysis (CMA) detects microscopic and additional submicroscopic imbalances (size <4 Mb) in the form of copy number losses and gains collectively called copy number variations (CNVs). CMA reveals the exact size, the gene content and the cytoband of the deletion or duplication [2]. The resolution and the yield of chromosome abnormalities from the whole genome should be higher by using CMA than by karyotyping. The challenge lies in the interpretation of CNVs as benign (with normal phenotype), pathogenic or clinically significant (pCNVs) or as variations of unknown

significance (VOUS). CMA can also detect loss of heterozygosity (LOH) which may be caused due to consanguinity or uniparental disomy.

Objectives

1. The scarcity of publications from India on Prenatal CMA in large cohorts reflects that classical karyotyping is still a preferred gold standard. The objective was to highlight the benefits of prescribing CMA due to increased diagnostic yield, molecular characterization into the gene content, size, exact cytoband and pathogenic significance of the chromosome defect and to recommend the approach to integrate it into prenatal diagnostic practice in the Indian setting.
2. CMA can diagnose the clinically significant submicroscopic aneusomies (e.g., DiGeorge, Williams Beuren, Smith–Magenis, Prader–Willi syndrome, etc.) currently not detected by karyotyping. These known microdeletion/duplication syndromes have been reported to be rare (1:1000 to 1:25000). Our study emphasizes the efficacy of CMA to detect these syndromes at a much higher frequency, suggesting that their prevalence rates should be re-defined.
3. Data accumulated by the West (multiple peer reviewed reports and databases) are being used for interpretation of CNVs as pathogenic, benign and VOUS. Accumulated Indian data can be deposited in the global pool. As more accumulated VOUS get associated with a common phenotype, their uncertainty will resolve. The data generated will contribute to personal or Indian repository for a more accurate and complete reference for interpretation of CNVs.
4. CMA will also discover novel or rare microdeletion and duplications.

Material

We studied 370 prenatal samples of amniotic fluid ($n=274$) and chorionic villi ($n=96$) received as consecutive referrals from pregnant women with high maternal age ($n=23$), biochemical screen positive ($n=61$), previous child abnormal ($n=59$), fetus with ultrasound abnormality ($n=205$) and heterozygous parents ($n=22$). Informed consent was obtained from all women undergoing the prenatal tests. Ethical clearance was obtained from our institution's ethical committee (EC/11/12/435).

Blood samples of parents were requested to establish inheritance, whenever possible.

Method

All 370 prenatal samples were first processed for Fluorescence Insitu Hybridisation (FISH) for five common aneuploidies of chromosomes 13, 18, 21, X and Y as per standard protocol [3]. The samples which showed whole chromosome aneuploidy for any of these five chromosomes were excluded from the study.

All 370 prenatal samples were cultured [4], processed for GTG banding [5] and karyotyping using International System for Human Cytogenomics Nomenclature (ISCN, 2016) [6].

CMA was performed using standard AGILENT protocol (www.agilent.com) for 4x180K array slide containing ~120,000 CGH and 60,000 SNP probes, including the entire 60,000 ISCA genes coverage and ability to detect loss of heterozygosity (LOH) due to the presence of a deletion, consanguinity or uniparental disomy (UPD). Human genome version GRCh 37:Feb 2009(hg19) was used for data annotation. CMA data analysis by CytoGenomics (v4.0.3.12) software enabled detection of CNVs as deletions (≥200 kb) and duplications (≥400 kb). CNVs of minimum size 50 kb were reported if they had clinically significant genes. Using standard guidelines [7, 8], the CNVs were interpreted as follows:

- Pathogenic or pCNVs: disease causing (documented in public databases OMIM, ISCA, ECARUCA, NHS UK, GARD, NLM NIH, PUBMED, etc.).
- VOUS: potential functional significance remains unknown.
- Benign CNVs: no major clinical phenotype.

Results

1. An overall higher diagnostic yield of the clinically significant abnormal results was obtained by CMA (9.18%) (34/370) as compared to that by karyotyping (5.40%) (20/370), as CMA detected an additional 3.78% (14/370) samples with submicroscopic (<4 Mb) clinically significant copy number changes which were not visible in the karyotype (Table 1, Fig. 1a).
2. The present data were strategically analyzed in all referral groups with different indications of the test. The analysis showed that the women with abnormal fetal ultrasound had the highest yield (5.40%) (20/370) of pCNVs in the form of deletions or duplications, as compared to other referral groups of women with high maternal age (0.81%) (03/370), biochemical screen positive (0.54%) (02/370), previous abnormal offspring (0.81%) (03/370) or the heterozygous parents group (1.62%) (06/370) (Table 1, Fig. 1b).

3. Clinically significant or pCNVs (Table 2): thirty (8.10%) (30/370) fetuses had at least one CNV which represented a common established genomic disorder documented in OMIM. These included some known microdeletion/duplication syndromes which karyotyping could not predict or detect. FISH was a targeted approach to confirm or validate some of these syndromes. The remaining four patients had CNVs which were rare or novel.
4. Structural aberrations (Table 2): Total 20 samples had only one CNV but there were 14 samples which had two CNVs denoting probable structural chromosomal defects such as derivative chromosomes, with unbalanced rearrangements. These accounted for total 48 CNVs, of which 28 were losses and 20 were gains including four supernumerary marker chromosomes (SMCs) such as der(11), der(13) and two of der(15), respectively.
5. VOUS: There were seven (1.89%) (7/370) VOUS which were less than 1 Mb in size (Table 3a).
6. Benign CNVs: We documented eight recurrent benign CNVs, which were also listed in DGV or dgVar as benign (Table 3b).
7. LOH: Eight regions of LOH in seven (1.89%) (7/370) samples had no imprinting gene (Table 3). Therefore, no pathogenicity was established.

Table 1 Percentage of abnormal results by karyotyping and by CMA in total 370 prenatal samples with various indications of the test

Category	Indication of test (total samples) ($N=370$)	Samples (n) with abnormal karyotype	Samples (n) with pCNVs (detected by CMA)	Samples (n) with VOUS (detected by CMA)
1	Advanced maternal age ($n=23$)	2 (0.54%)	3 (0.81%)	0 (0%)
2	Biochemical screening positive ($n=61$)	0 (0%)	2 (0.54%)	2 (0.54%)
3	Previous child with abnormality ($n=59$)	1 (0.27%)	3 (0.81%)	1 (0.27%)
4	Heterozygous parents ($n=22$)	6 (1.62%)	6 (1.62%)	0 (0%)
5	USG abnormal ($n=205$)	11 (2.97%)	20 (5.40%)	4 (1.08%)
Overall diagnostic yield		20 (5.40%)	34 (9.18%)	7 (1.89%)

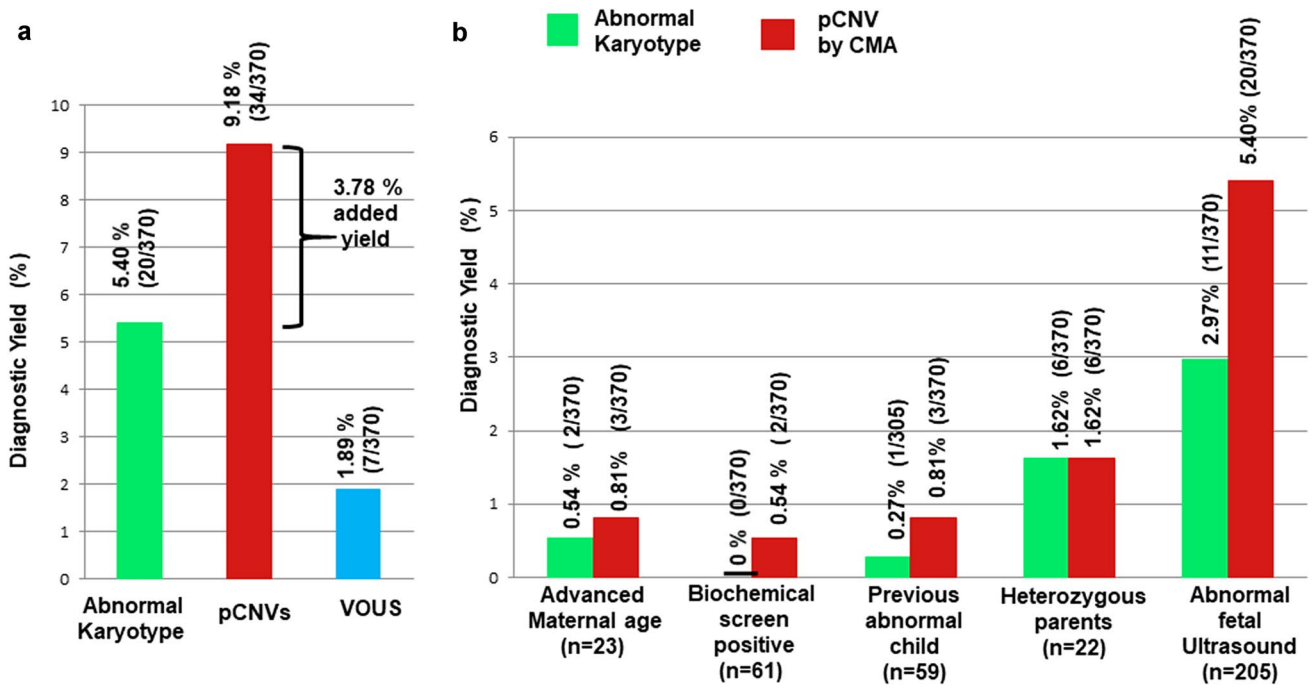

Fig. 1 **a** Overall diagnostic yield (%) by karyotyping and CMA, **b** highest diagnostic yield of abnormal results in referrals with abnormal fetal ultrasound versus other prenatal referrals

Table 2 Clinically significant CNVs (pCNVs)

PI	Age	Indication of test	Inheritance	Karyotype	Deletions and duplications detected by CMA	Multiple/critical genes	Clinical significance/syndromes	Previous reports
Advanced maternal age								
1	39 Y	AMA	ND	46,N	384 kb; 1q21.1 (145,415,190–145,799,602)x1	Multiple/*RBM8A*	Thrombocytopenia-absent radius syndrome	OMIM 274000
2	35 Y	AMA	De novo	mos 47, + mar [37]/46,N[13]	7.8 Mb; 11p12p11.12 (42,922,228–50,768,675)x3	Multiple/*EXT2, ALX4*	11p12p11.12 duplication	DECIPHER 255428,291037 (Hulya et al. 2009)
3	37 Y	AMA	De novo	47, + mar	10.1 Mb; 15q11.2q13.3 (22,765,628–32,899,558)x3	Multiple/*SNRPN, UBE3A*	15q11-13 duplication syndrome	OMIM 608636
Biochemical screen positive								
4	34 Y	Quadruple test positive	ND	46,N	1.6 Mb; 6p12.3p12.1 (51756953–53436038) x1	*Multiple GCLC*	Gamma glutamyl-cysteine synthetase deficiency	OMIM 230450
5	33 Y	Triple test positive	ND	46,N	1.6 Mb; Xp22.31 (6,488,721–8,097,511) x1	*Multiple/STS, VCX*	Ichthyosis, X-linked	OMIM 308100
Previous abnormal child								
6	33 Y	Previous child with GDD	ND	46,N	817 kb; 8p22p21.3 (18,585,309–19,402,086)x3	Multiple	ACC	OMIM 217990
7	33 Y	Previous child with Autism	De novo	46,N	267 kb; 14q24.3q31.1 (79187922–79455420) x1	*NRXN3*	ASD	OMIM 600567
8	29 Y	Previous pregnancy with MCA	Mat	46,der(13)t(2;13) (q35;q14)mat	10.0 Mb; 2q35q36.3 (216,351,479–226,369,944)x3 26.9 Mb; 13q14.11q21.33 (42,693,451–69,644,520) x1	Multiple Multiple/*RB1*	2q35 duplication syndrome 13q14 deletion syndrome	OMIM 185900 OMIM 613884
Heterozygous parent								
9	29 Y	Father carrier 46,XY,ins(2) (p21.3;q34q32.1)	Pat	46,del(2)(q32q34)	26 Mb; 2q32.1q34 (183,011,802–209,359,004)x1	Multiple/*SATB2*	Chromosome 2q32q33 deletion syndrome	OMIM 612313
10	33Y	Father carrier 46,XY,t(3;7) (p25.3;p22)	Pat	46,der(3)t(3;7) (p25.3;p22)pat	9.4 Mb; 3p26.3p25.3 (93,949–9,532,443)x1 4 Mb; 7p22.3p22.2 (92,532–4,176,031)x3	Multiple/*CHL1,CNTN6* Multiple	3pter deletion syndrome 7p22.3p22.2 duplication syndrome	OMIM 613792 (Devin et al. 2015)

Table 2 (continued)

PI	Age	Indication of test	Inheritance	Karyotype	Deletions and duplications detected by CMA	Multiple/critical genes	Clinical significance/syndromes	Previous reports
11	29Y	Father carrier 46,XY,t(4:5) (p16;p15)	Pat	46, der(4),t(4:5) (p16;p15)pat	8.6 Mb; 4p16.3p16.1 (85,040–8,732,731)x1 296 kb; 5p15.33 (50,093–346,336)x3	Multiple/*LETM1,WHSC1, WHSC2* Multiple/*SDHA*	Wolf–Hirschhorn syndrome Mitochondrial respiratory chain complex ii deficiency	OMIM 194190 OMIM 600857
12	32Y	Mother carrier of inv(10) (p12q26)	mat	46,rec(10)dup(10p) inv(10)(p12q26)	29.0 Mb; 10p15.3p12.1 (232,737–29,288,132) x3 9.2 Mb;10q26.13q26.3 (126,161,478–135,434,178)x1	Multiple *ZMYND11* Multiple	Mental retardation 10q26 deletion syndrome	OMIM 616083 OMIM 609625
13	29 Y	Mother carrier 46,XX,t(10;13) (q26;q12.3)	Mat	47, + mar	10.1 Mb; 10q26.13q26.3 (125,266,022–135,434,178)x3 7.9 Mb; 13q11q12.13 (19,463,637–27,376,648)x3	Multiple Multiple	GDD GDD	nssv582217, 582218 nssv578643
14	29 Y	Father carrier 46 XY,(11;12) (q23;p13)	Pat	46,der(12)t(11;12) (q23;p13)pat	16.7 Mb; 11q23.3q25 (118,158,163–134,934,196)x3 2.9 Mb; 12p13.33 (162,848–3,112,486)x	Multiple Multiple *WNK1*	GDD GDD Hereditary Sensory and autonomic neuropathy	nssv578607 nssv707362 nssv1494954 OMIM 201300
Ultrasound abnormality								
15	30Y	NT increased (> 3.5 mm)	De novo	46,inv(1)(p22q12),t(1;7) (q12;p15.3), del(4) (q32.3q34.2)	10.1 Mb; 4q32.3q34.2 (166,220,800–176,350,810)x1	Multiple *TLL1, HAND2, HPGD*	Intellectual disability GDD	DECIPHER 257358 DECIPHER 286735
16	32 Y	NT increased (> 3.5 mm)	De novo	46,N	1.4 Mb; 7q11.23 (72,726,572–74,133,332)x3 (validated by FISH)	Multiple/*ELN*	Williams Beuren region duplication syndrome	OMIM 609757
17	31 Y	NT increased (> 3.5 mm)	De Novo	47, + mar	12.2 Mb; 15q11.1q13.3 (20,190,548–32,408,319)x3	Multiple/*SNRPN*	15q11-13 duplication syndrome	OMIM 608636
18	28 Y	NT increased (> 3.5 mm)	ND	46,N	2.7 Mb; 22q11.21 (18,729,944–21,440,514)x1	*Multiple/TBX1*	DiGeorge syndrome	OMIM 188400

Table 2 (continued)

PI	Age	Indication of test	Inheritance	Karyotype	Deletions and duplications detected by CMA	Multiple/critical genes	Clinical significance/syndromes	Previous reports
19	30Y	NT increased (> 3.5 mm)	ND	46,der(10) add(10)(q26)	1.8 Mb; 10q26.3 (133,536,778–135,434,178)x1 25.5 Mb; 11p15.5p14 (196,966–25,705,103) x3	Multiple Multiple	10q26 deletion syndrome 11p15.5 duplication	OMIM 609625 OMIM130650
20	26 Y	IUGR	Pat	46, der(4),t(4:10) (p16;q25)pat	25.0 Mb; 4p16.3p15.2 (72,447–25,785,945)x1 26.0 Mb; 10q25.1q26.3 (109,312,155–135,404,523)x3	Multiple/*LETM1*, *WHSC1*, *WHSC2* Multiple	Wolf–Hirschhorn syndrome Distal 10q duplication	OMIM 194190 (Xiao et al. 2012)
21	34Y	IUGR, fetal ascites	De novo	46,der(5)del(5) (p15.1)dup(5)(p12q35)	17.1 Mb; 5p15.33p15.1 (22,149–17,213,536)x1 119.9 Mb; 5q12.1q35.3 (60,785,924–180,696,806)x3	Multiple Multiple	5p15.2 deletion/cri du chat syndrome GDD	OMIM 123450 nssv579012 1602271
22	29 Y	IUGR	ND	46,N	88 kb; Xq28 (147,766,011–147,853,6190x1	AFF2	FRAXE mental retardation syndrome	OMIM 309548
23	28 Y	Ventricular septal defect, short femur	ND	46,N	1.6 Mb; 7q11.23 (72,726,572–74,339,044)x1 (validated by FISH)	*Multiple* *ELN*	Williams Beuren syndrome (WBS)	OMIM 194050
24	29 Y	Intracardiac focus & mild IUGR	De novo	46,N	3.0 Mb; 22q11.21 (18,661,724–21,704,972)x1 (validated by FISH)	*Multiple/TBX1*	DiGeorge syndrome	OMIM 188400
25	30 Y	Tetralogy of fallot	De novo	46,N	2.7 Mb; 22q11.21 (18,729,944–21,505,417) x1 (validated by FISH)	*Multiple/TBX1*	DiGeorge syndrome	OMIM 188400
26	31 Y	Holoprosencephaly, hydrops, aortic root stenosis	Pat	46,der(13)t(6;13) (p24.2;q31.3)pat	11.0 Mb; 6p25.3p24.2 (389,423–11,462,960) x3 23.2 Mb; 13q31.3q34 (91,837,820–115,092,640x1	Multiple Multiple	MCA 13q32 deletion syndrome	DECIPHER 1448, 284585, 327131 OMIM 156600
27	33 Y	AVSD, single umbilical artery, skull strawberry shape	ND	46,N	455 kb; 11q22.3 (106,959,465–107,414,218)x1 927 kb; Xp22.33 (305,449–1,232,910)x1	*ALKBH8, CWF19L2 PPP2R3B, SHOX*	Intellectual developmental disorder Short stature idiopathic	OMIM 613306 OMIM 300582

Table 2 (continued)

PI	Age	Indication of test	Inheritance	Karyotype	Deletions and duplications detected by CMA	Multiple/critical genes	Clinical significance/syndromes	Previous reports
28	28 Y	AVSD, blake pouch cyst	ND	46,der(9)t(9;11)(p23;q23)	12.4 Mb; 9p24.3p23 (172,364–12,643,569)x1 14.4 Mb;11q23 (120,519,571–134,934,196)x3	Multiple Multiple	9p deletion syndrome GDD	OMIM 158170 DECIPHER nssv578608
29	32 Y	B/L club foot, fetal bowel loop echogenic	De novo	46,del(10)(q23)	7.3 Mb; 10q22.3q23.2 (81,584,966–88,940,429)x1	Multiple/*BMPRIA*	10q22.3q23.2 deletion syndrome	OMIM 612242
30	34 Y	Single ventricle, right CTEV	De Novo	46,der(17)dup(17)(q23q25)	13.0 Mb; 17q23.1q25 (58,151,616–71,236,468)x3	Multiple	17q23.1-q23.2 duplication syndrome	OMIM 613618
31	34 Y	Blake pouch cyst, CTEV	NF	46,N	448 kb; 19p13.3 (869,455–1,317,770)x1	Multiple	19p13.3 microdeletion syndrome	OMIM 613638
32	34 Y	Depressed nasal bridge, CTEV, ACC	ND	46,N	187 Kb; 20q13.33 (62,762,136–62,949,149)x1	*MYT1, PCMTD2*	20q13.33 microdeletion syndrome	ORPHA 261311 [27]
33	30 Y	Mild Ventriculomegaly, ACC	Pat	46,der(18) add (18)(p11.3)inv(18)(p11.3q21) pat	2.1 Mb; 18p11.32 (148,963–2,286,200)x1 28.3 Mb; 18q21.2q23 (49,680,998–78,012,829)x3	Multiple Multiple	18p deletion syndrome Pitt–Hopkins Syndrome	OMIM 146390 OMIM 610954
34	35 Y	B/L Pelviectasis, ventriculomegaly	Mat	46,der(13)t(1;13)(q42;q34)mat	17.9 Mb; 1q42.2q44 (231,237,196–249,212,668)x3 4.8 Mb; 13q34 (111,645,199–115,092,648)x1	Multiple/*AKT3* Multiple/*F7*, *F10*	1q42q44 duplication Intellectual disability Factor VII deficiency	DECIPHER 253950, 282589 OMIM 227500

GDD global developmental delay, *MCA* multiple congenital anomalies, *NT* nuchal thickness, *IUGR* intrauterine growth retardation, *AVSD* atrioventricular septal defects, *CTEV* congenital talipes equinovarus, *ND* not done, *Mat* maternal, *Pat* paternal

Discussion

CMA is a powerful tool in pre- and postnatal diagnostics [9–12]. It is used as a first tier diagnostic test to detect genomic imbalances in children with idiopathic intellectual disabilities, autism, developmental delay and multiple congenital anomalies [13]. The western institutes have validated the test for prenatal studies of microarray in high-risk pregnancies. The analysis of datasets reported in the medical literature showed a higher incidence of pCNVs even in prenatal samples [14–20]. This has triggered the interest to apply this technology for prenatal diagnosis in India, but this required validation by comparison of the result outcome in the Indian context, as some recurrent CNVs are a source of variation in the healthy population or can be considered as benign polymorphisms occurring in communities due to evolution.

Accuracy and Efficiency of CMA with High Diagnostic Yield of pCNVs

The results from the present study showed a higher resolution for genomic imbalances (minimum 50 kb), giving a diagnostic yield of 9.18% by CMA as compared to 5.40% by karyotyping (Table 1, Fig. 1a). Another recent Asian study of CMA in the prenatal cohort [16] showed the diagnostic yield of 12.2% pCNVs. All clinically significant chromosome abnormalities detected by karyotyping were also detected by CMA. The additional submicroscopic aberrations (3.78%) which were missed by karyotyping were detected by CMA. Therefore, CMA proves to be an accurate and more efficient test to use for prenatal diagnosis of genomic imbalances.

Table 3 (a) Variations of uncertain significance; (b) recurrent benign CNVs; (c) LOH

SN	VOUS (present study)	VOUS (previous reports)
(**a**)		
1	462 kb;3p26.3p26.2 (2,685,713–3,147,222)x3	DECIPHER: 267506, 289396, 251186
2.	725 kb;4q13.3 (72,771,832–73,496,614)x3	dbVar nsv530125, ISCA nssv581162
3	301 kb;6q12 (65,625,209–65,926,900)x1	ISCA nssv579359
4	695 kb;7q33 (133,483,238–134,178,419)x3	DECIPHER 251768, 305865
5	391 kb;8q12.1 (56,946,660–57,337,802) x1	ISCA nssv1603607
6	296 kb;16q24.2 (87,504,276–7,800,485)x1	ISCA nssv582897
7	850 kb;20p12.1 (13,438,816–14,288,315)x3	dbVar: nsv531478, nsv916202, nsv531479
SN	BENIGN CNVs (present study)	BENIGN CNVs (previous reports)
(**b**)		
1	658 kb;1q21.2 (149,041,013–149,699,420)x1	ISCA nssv575615
2.	680 kb;2p11.2 (87,325,327–88,005,429)x3	ISCA nssv576269
3	316 kb;4q13.2 (69,276,372–69,592,846)x3	ISCA nssv583925
4	180 kb;9p11.2 (43,659,483–43,840,040)x1	ISCA nssv707218
5	478 kb;15q13.3 (32,031,012–32,509,926)x3	ISCA nssv576648
6	575 kb;16p11.2 (32,890,035–33,465,531)x1	ISCA nssv707486
7	498 kb;19p13.2q13.3 (43,268,069–43,580,748)x1	ISCA nssv578414
8.	498 kb;19p13.2q13.3 (43,268,069–43,580,748)x3	ISCA nssv581840
SN	Eight loss of heterozygosity regions found in seven patients	
(**c**)		
1	11.8 Mb;2q24.2q31.1 (160,406,890–172,283,604)x2 hmz.	
2.	12.4 Mb;3q11.2q13.12 (93,989,454–106,412,711)x2 hmz	
3	10.2 Mb;6q24.3q25.3 (148,651,499–158,890,892)x2 hmz	
4	12.0 Mb;7p14.1p12.1 (40,440,573–53,245,512)x2 hmz	
5	13.5 Mb;12q15q21.31 (71,054,305–84,652,424)x2 hmz	
6	11.5 Mb;17q22q24.2 (53,196,132–64,766,443)x2 hmz	
7	14.2 Mb;5q21.1 (98,925,681–113,181,521)x2 hmz, 17.7 Mb; 8q22.33q24.12 (103,572,247–121,355,690)x2 hmz	

Highest Diagnostic Yield of pCNVs in the Women with Abnormal Fetal Ultrasound

Strategic analysis of CMA results in various test indication groups (Table 1, Fig. 1b) showed that the group of women with abnormal fetal ultrasound had the highest yield (5.40%) of pCNVs as compared to the other four referral groups of high maternal age (0.81%), triple test positive (0.54%), women with previous abnormal offspring (0.81%) or heterozygous parents group (1.62%). This was comparable with other prenatal studies [12, 17]. They reported pCNVs in 6.0 to 6.5% of fetuses with structural abnormality and 1.0 to 1.5% in other indication groups with no structural abnormalities in the fetus.

American College of Obstetricians and Gynaecologists (ACOG) and Society for Maternal–Fetal Medicine (SMFM) [8] recommend CMA for prenatal diagnosis in cases with one or more fetal structural abnormalities. For patients with structurally normal fetus, either karyotype or CMA is recommended. A recent study [18] showed that 2.5% of patients will have clinically significant genomic imbalances that may be missed if the guidelines continue to suggest that karyotyping and CMA have equivalent diagnostic value for patients with no fetal ultrasound abnormality. The present study recommends that if cost is not an issue, CMA can be offered to all pregnant women undergoing invasive test.

Increased Frequency of Detection of Less Common, But Known Genomic Disorders

CMA can detect chromosome gains and losses as small as 0.05–0.1 Mb (50–100 kb) anywhere in the genome [19]. Some submicroscopic CNVs can cause recurrent or known genomic disorders such as Wolf Hirschhorn syndrome, Williams Beuren deletion syndrome and Di George syndrome. These contiguous stretches of deletions and duplications which occur between low copy repeat clusters mediated by nonallelic homologous recombination (NAHR) express variable phenotypes with incomplete penetrance, due to which their prevalence in the populations has been reported to be very low. The incidence of known microdeletion or microduplication syndromes is estimated to be 1:1000 to 1:25000 [20], is unrelated to maternal age and may manifest as moderate to severe disease. Out of 9.18% (34/370) clinically significant samples, we found 3.0% (11/370) samples with such recurrent microdeletion/duplication syndromes: 3p deletion syndrome ($n=1$) (1/370), Wolf Hirschhorn syndrome ($n=2$) (2/370), Cri du chat syndrome ($n=1$) (1/370), Williams Beuren deletion syndrome ($n=1$) (1/370), Williams Beuren region duplication syndrome ($n=1$) (1/370), 15q duplication syndrome ($n=2$) (2/370) and DiGeorge syndrome ($n=3$) (3/370). Therefore, our data also show that such less common clinically known aneusomies are actually more frequent. This calls for strategic re-evaluation of the population prevalence of these contiguous gene syndromes.

Rare or Novel pCNVs

CMA has led to the discovery of new unreported clinically significant segmental deletions and duplications. Out of 34 prenatal samples with pCNVs, 30 samples had at least one known deletion or duplication which was documented with a phenotypic association in OMIM [21] and there were four samples with rare or novel CNVs which were not documented in OMIM but were overlapping with isolated reported cases.

Case 1 (Table 1-P15)

In a CVS sample of a patient with a fetus with increased NT (4.9 mm at 11.2 weeks gestation), a distal interstitial deletion of 10.1 Mb on 4q32.3q34.2 (166,220,800–176,350,810) was a rare finding. Increased nuchal translucency can be associated with congenital heart disease (CHD) [22]. Three genes in this deletion at 4q32.3q34.2, namely *TLL1* (Tolloid-like-1), *HPGD* (15-hydroxyprostaglandin dehydrogenase), and *HAND2* (Heart and neural crest derivatives-expressed protein 2), are known to be involved in cardiac morphogenesis. There is only one report which narrowed this critical region responsible for CHD seen in 4q deletion syndrome [23]. Deletion 4q32.3q34.2 has also been reported as a pathogenic locus with intellectual disability and global developmental delay in Decipher (patient ID 257358 and 286735). The karyotype of this prenatal sample was 46,inv(1)(p22q12),t(1;7)(q12;p15.3),del(4)(q32.3q34.2) dn, which showed a cluster of multiple rearrangements: an inversion, a balanced translocation and a deletion involving four chromosomes. This complex chromosome rearrangement (CCR) had occurred de novo, as none of the parents had inv(1) or t(1;7) or del(4)(q32.3q34.2). This suggests that the chromosomes at the embryo stage may have undergone chromothripsis and chromoanagenesis, a mechanism of formation of CCR in a congenital disorder or in cancers, as described recently [24].

Case 2 (Table 1-P32)

A small 187 kb subtelomeric deletion on 20q13.33 (62,762,136–62,949,149) was found in an amniotic fluid sample of a patient with fetal ultrasound showing agenesis of corpus callosum, depressed nasal bridge and congenital talipes equinovarus. 20q13.33 microdeletion syndrome was also reported in ORPHNET (orpha261311) [25] as a rare chromosomal anomaly syndrome characterized by intellectual disability, seizures and dysmorphic facial features, cognitive and language deficits, microcephaly and malformed

hands and feet. In this sample, we could identify a defined subtelomeric deletion on 20q13.33 involving genes *MYT1* and *PCMTD2*. Only a few patients have been described with a subtelomeric deletion on chromosome 20. Subtelomeric rearrangements not visible by conventional cytogenetic analysis have been reported to occur in approximately 5–7% of patients with unexplained mental retardation and physical abnormalities [26]. The smallest 20q13.33 microdeletion encompassing *MYT1* and *PCMTD2* was reported to be associated with severe mental retardation by Kroepfl et al. [27]. *MYT1* regulate neuronal transcription and are involved in the proliferation and differentiation of oligodendrocytes, cells that form the myelin sheath in the central nervous system. Therefore, *MYT1* can be associated with neurological disorders [27].

Cases 3 and 4 (Table 1-P2 and P13)

There were two novel duplications in the form of supernumerary marker chromosomes: SMC der(11) with 7.8 Mb duplication of 11p12p11.12 (42,922,228–50,768,675) and SMC der(13)t(10;13) with 10.1 Mb duplication 10q26.13q26.3 (125,266,022–135,434,178) and 7.9 Mb duplication of 13q11q12.13 (19,463,637–27,376,648) (published elsewhere) [2].

Molecular Characterization of SMCs

SMCs are detected by conventional karyotyping with GTG banding, as unexpected results in 0.072–0.075% of prenatal and 0.044% of postnatal patients [28]. They vary in size, structure, chromosome origin and gene content. Therefore, it is challenging to decide the clinical outcome of a marker chromosome. In the past, identification was attempted by FISH or spectral karyotyping. CMA has added clinical utility, as it characterized the SMCs as duplications with exact size and gene content, thus predicting their clinical outcome. CMA and karyotype detected SMC der(11) and SMC der(15) in the advanced maternal age group, one SMC der(15) in the abnormal fetal ultrasound group and one SMC der(13)t(10;13) which was a derivative chromosome inherited from a heterozygous mother who had a balanced translocation 46,XX,t(10;13) (q26;q12.3). Molecular characterization was possible for all four SMCs for better genetic counseling and future planning (published elsewhere) [2].

Limitations

Balanced translocations are not detected by CMA. However, truly balanced rearrangements do not predict an abnormal phenotype and so are carried to term by the parents.

Repository

We listed the pCNVs, VOUS and benign CNVs for the repository (Tables 2, 3a, b). We found 1.89% (7/370) of VOUS of size < 1 Mb. The results of previous studies by Shaffer et al. [12] and Wapner et al. [17] showed 2.7% and 3.4% karyotypically normal VOUS, respectively. The uncertainty of VOUS will resolve with the accumulation of larger datasets. Some CNVs (recurrent benign) are population based; therefore, formation of a repository of a database in the Indian context was attempted. Dominant gene alterations can be pathogenic with late onset, and recessive disease can result in fetuses with heterozygous deletions and a mutation in the intact allele [12]. Correlation of the phenotype, family history and ethnic background can help in such cases for counseling. It is important that both pCNVs and VOUS are conveyed to the parents with caution, and therefore, pretest and posttest counseling should be done by trained experts to reduce anxiety [29].

An increasing number of pregnancies are being screened by Non-Invasive Prenatal Screening (NIPS); however, ACMG recommends that invasive testing using either NGS or CMA will still be necessary to confirm pregnancies with the positive NIPS results where the fetus is suspected of having a chromosome abnormality [30].

Conclusion

The overall diagnostic yield of genomic imbalances was higher by CMA (9.18%) than by karyotyping (5.40%). The pCNVs were higher (5.40%) in pregnant women with abnormal fetal ultrasound than in all other referral groups with no fetal structural anomaly (average 0.94%). Therefore, CMA must be used as the first tier test in all cases with abnormal fetal ultrasound and if cost is not an issue, it can be offered to all pregnant women undergoing the invasive test, as the test results are faster and the diagnostic yield is higher by CMA than by karyotyping even in other groups.

Pretest and posttest genetic counseling by the expert team is essential.

Accumulation of more data will resolve the uncertainty of VOUS and redefine the prevalence of microdeletion and duplication syndromes in the population. Molecular characterization of the genomic imbalances by CMA allows recognition of the genes and their functions correlating with the phenotype for precise genetic counseling and discovery of novel syndromes.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interests.

Ethical Approval The study was performed in accordance with the ethical standards of the responsible committee on human experimentation and with Helsinki Declaration of 1975, as revised in 2008. The study was approved by the ethics committee of our institute (EC/11/12/435).

Informed Consent Informed consent was obtained from all the patients for being included in the study.

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