

Molecular characterization of supernumerary marker chromosomes found as unexpected chromosome abnormalities in nine prenatal and nine postnatal samples

Abstract

Objectives: The supernumerary marker chromosomes (SMCs) are extra structurally abnormal chromosomes that cannot be unambiguously identified or characterised by conventional karyotyping. Their clinical phenotypes are variable and are dependent on their size, gene content, inheritance and level of mosaicism. In the past, fluorescence in situ hybridization (FISH) and related techniques were used to identify the chromosome origin, but the molecular makeup was still unknown. Chromosome microarray analysis (CMA) can detect the exact genomic breakpoints and gene content of the SMC. This can be correlated with the phenotype for better genetic counselling

Methods: In the last 5 years, out of 20,000 samples referred for various reasons for karyotyping, we found 18 SMCs (0.09%) as unexpected results, nine were prenatal samples and nine were postnatal. All eighteen samples were subjected to CMA to characterize the SMCs. FISH was done for identification or validation, wherever possible.

Results: Out of 18 SMCs, 14 were successfully characterized: eight (57.14%) were acrocentric chromosomes [seven der(15) and one der(22)], five (35.71%) were non-acrocentric chromosomes [der(9), der(11), der(12), two der(18)] and one (7.14%) was a complex, novel SMC originating from the maternal translocation t(10;13). The remaining four were very small and heterochromatic with normal array reports.

Seven had SMC-related known syndromes such as 15q11q13 duplication syndrome (n=2), Cat Eye syndrome, Trisomy 9p syndrome, i(12)p Pallister Killian syndrome and the rare Trisomy 18p syndrome (n=2).

Conclusion: The results, their molecular relevance and pathological significance for genetic counselling were discussed case by case individually. The study emphasizes the usefulness of CMA in identification and characterization of the additional genetic material of the SMC which can be correlated with the phenotypes of the postnatal patient for future management and also the clinical significance conveyed to the parents when the SMC is found in the prenatal samples.

Keywords: CMA, SMC, CNV, karyotyping, mosaic, genetic counselling

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Introduction

The supernumerary marker chromosomes (SMCs) are extra structurally abnormal chromosomes that cannot be unambiguously identified or characterized by conventional karyotyping.¹ They are found as unexpected chromosomal defects in 0.072-0.075% of unselected prenatal samples and 0.044% of consecutively studied postnatal samples referred for karyotyping. Their frequencies in intellectual disability and infertility are higher, 0.288% and 0.125% respectively.² The phenotype of SMCs is highly variable, from normal to severely affected and is dependent on their size, structure, origin, gene content, level of mosaicism and whether euchromatic or heterochromatic. The presence of uniparental disomy in the sister chromosome of some SMCs may also influence the clinical phenotype.³ Therefore, it is challenging to decide the clinical outcome and render genetic counselling especially in prenatal samples. The risk for clinically abnormal phenotypes of SMCs ascertained in *de novo* prenatal cases is 7% for the SMCs from acrocentric autosomes and 28% from non-acrocentric autosomes.^{4,5} About 30- 50% of

pregnancies diagnosed with SMCs go for termination due to lack of insight about the outcome of the SMCs.²

In the past, identification of the SMCs was done by Fluorescence in situ hybridisation (FISH).^{3,5} FISH is a targeted approach and has some limitations.⁶ Sometimes multiple attempts of FISH with various centromere enumerating probes or whole chromosome paint probes may be required, sequentially, even then the gene content remains undefined. The newer approach of Chromosome Microarray Analysis (CMA) offers identification of the copy number variations (CNVs) at the whole genome level along with defined breakpoints and the gene content of the involved duplication forming the SMC. Using CMA, the molecular characterization of the SMC is possible in just one test. This approach saves time and is also cost effective.

Thus, based on the exact cytoband, size and gene content obtained by CMA, the genotype correlation with the clinical pathology arising from the SMC can be studied in postnatal referrals to attempt to close the gap of knowledge by accumulation of as many such informative cases. The problem arises when a clinical decision has to be taken

about the outcome of a pregnancy in a prenatal sample with an unexpected finding of SMC. Therefore, molecular characterization of the SMCs is of utmost importance for better understanding and rendering personalised genetic counselling in postnatal and prenatal cases.³

Material and method

In the present study we focus, case by case individually, on molecular characterization, result interpretation for clinical significance and subsequent genetic counselling for 18 SMCs found as unexpected results of chromosome analysis from 20,000 referrals of last five years.

Informed consent was obtained at the time of sample collection. The study was approved by our institutional ethical committee.

Chromosome analysis by karyotyping: For Postnatal samples, standard routine peripheral blood lymphocyte cultures were set up by adding 0.5ml of heparinized blood sample in 5ml of PB-Max karyotyping medium (GIBCO) which were incubated at 37°C for 69 hours. Thymidine was added in the cultures 16 hours before harvesting of the metaphase spreads, to synchronise the cultures to obtain long chromosomes for high resolution chromosome analysis. The metaphases were harvested and fixed on the glass slides.

For prenatal samples, using standard protocol, long term cultures were set up from 20ml amniotic fluid samples obtained at 16 weeks of gestation. The cultures were maintained at 37°C with 5.0% carbon dioxide and were monitored daily. The cultures were harvested to obtain metaphases when there were at least five clones visible by using the inverted microscope. The metaphases were fixed on the glass slides.

The metaphases obtained from both prenatal and postnatal cultures were then stained by GTG banding (G-banding by Trypsin using Giemsa stain). Twenty metaphases were analyzed and subsequently five karyotyped using computerized image analysis software (IKAROS) and International System for Human Cytogenomic Nomenclature, 2016.¹ At least, 500–550 band resolution level was maintained during analysis.

Chromosome microarray analysis: Isolated DNA of nine blood samples and nine amniotic fluid samples were subjected to CMA using standard Agilent protocol for 4x180K arrays (www.agilent.com). Whole genome microarray was performed using AGILENT 4x180K (CGH+SNP) array slide. This contains 120,000 CGH probes and 60,000 SNP probes. The extracted DNA from the patient's EDTA blood sample and a phenotypically normal sex matched reference DNA from Agilent were fragmented using restriction enzymes and labelled with different color dyes, Cy5 and Cy3 respectively. These were then co-hybridized with the CGH probes and SNPs on the array slide, which was then incubated at 67°C at 20 rpm for 24 hours. The slides were washed according to the Agilent Protocol v7.2. Agilent scanner was used to retrieve images for feature extraction. The images were then used for data analysis and interpretation with the help of Cytogenetics software (v4.0.3.12) based on UCSC build 37(hg19), using Aberration detection method2 (ADM2) algorithm with a sensitivity threshold of log2ratio and 6 CGH probes calling. Various databases such as UCSC browser, DECIPHER, ECARUCA, OMIM, ISCA, etc were used for further analysis to classify the CNV, as per ACMG recommendations.

Fluorescence in situ hybridization: Metaphase or interphase FISH was performed using standard protocol⁷ with centromeric probes or locus specific probes, whenever required. The DNA probe was co-denatured at 75°C and hybridized overnight at 37°C in the thermobrite. The excess DNA probe was washed with 0.3% NP40 in 2XSSC buffer, and the signals were visualized using fluorescence microscopy. (All adult cases were included in postnatal category, for convenience of classification; this had no effect on the final results)

Results

Our retrospective data of last five years of 20,000 unselected postnatal and prenatal cases detected 18 SMCs establishing a frequency of 0.09%.

Out of these 18 SMCs, total 14 (Cases P1-P14) were successfully characterised by CMA and validated by FISH whenever possible (Figures 1–4) and the remaining four (Cases P15-P18) (Figure 4) were small and heterochromatic, seen microscopically during karyotyping but had normal CMA report, therefore their origin could not be established even by multiple attempts of FISH. The 14 characterized SMCs consisted of eight acrocentric chromosomes (57.14%), five non acrocentric chromosomes (35.71%) and a complex SMC (7.14%) originating from two chromosomes, an acrocentric chromosome 13 and non-acrocentric chromosome 10 (Table 1). Their overview of the results: the karyotypes, inheritance, CMA and FISH results, indications of the test, the phenotype/outcome of the pregnancies are all summarized in Table 2.

Acrocentric SMCs (Cases P1-P8): (Figure 1: P1, P2, P3 & P4 & Figure 2: P5, P6, P7 & P8): Out of the fourteen characterized SMCs, eight (57.1%) (8/14) were acrocentric chromosomes: Seven (50%) (7/14) were der (15) and one (7.14%) (1/14) was der(22).

Out of seven der(15) SMCs, two (both prenatals) were bisatellited der(15)(q11.2q13.3) (Figure 1: Cases P1&P2) with duplication 10.1Mb (22,765,628-32,899,558) and 12.2Mb (20,190,548-32,408,319) respectively, both containing the *SNRPN* critical region detected by CMA and FISH, two (both prenatal) were small pericentric der(15): one had 2.2Mb duplication of cytoband 15q11.1q11.2 (20,481,702-22,698,581) without *SNRPN* gene shown by CMA and confirmed by interphase FISH (Figure 1A: P3) and one (prenatal) detected as duplication CEP(15) with two normal signals for *SNRPN* by metaphase FISH (Figure1A:P4) with normal array report.

The remaining three SMCs der (15) were familial (two postnatals, one prenatal) (Figure 2: P5,P6 & P7): The affected child (P5) was a 5 years old female with a 2.6Mb gain at 15q11.1q11.2(20,055,137-22,698,581) and 177Kb gain at 9p24.3(204,193-381,489). Her mother (P6) was a carrier of the SMC der(15) with a 2.6Mb gain at 15q11.1q11.2(20,055,137-22,698,581) and her amniotic fluid sample (P7) CMA result also showed a 2.6Mb gain at 15q11.1q11.2 (20,095,481-22,698,581) which had the same gene content of der(15) as the mother (P6) with no duplication of chromosome 9p24.3 which was found in the sibling (P5). Metaphase FISH using DNA probe CEP15/*SNRPN*/PML showed duplication of CEP15 in all three P5, P6 and P7.

The eighth acrocentric SMC (postnatal) was der (22) (Figure 2: P8), characterized by CMA as 2.1Mb gain of cytoband 22q11.1q11.21. FISH for der (22) was not done, as the DNA probe was not available in the laboratory at that time.

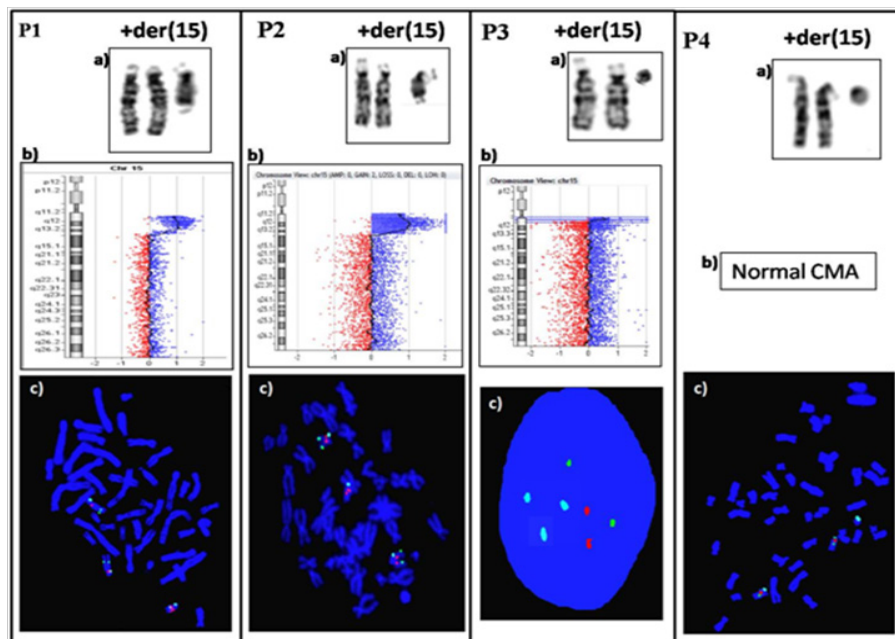


Figure 1 Cases P1, P2, P3,P4:

- a) Partial karyotypes of acrocentric chromosome 15 and SMC der(15)
- b) CMA images of dup 15q11.2q13.3 (P1), dup15q11.1q13.3(P2) and dup15q11.1q11.2(P3)
- c) FISH PROBE USED :CEP15/Spectrum Aqua, SNRPN/Spectrum Orange, PML/Spectrum Green:

P1 & P2: three signals for duplication CEP15 (aqua) and three signals for duplication SNRPN (orange) and two normal signals of PML (green).

P3 & P4: three signals for dup CEP15 (aqua) and two normal signals of SNRPN (orange) and PML (green).

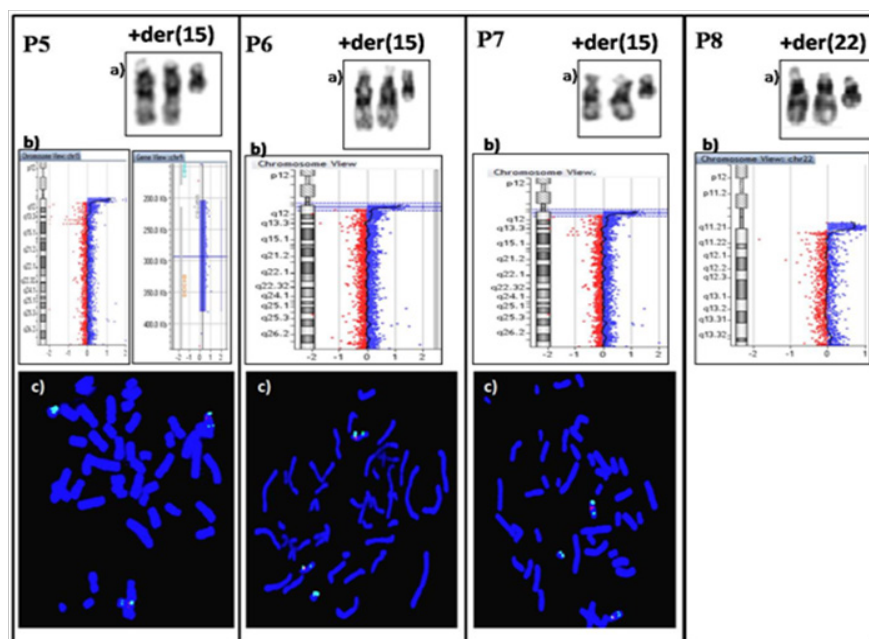


Figure 2 Cases P5,P6,P7:

- a) Partial karyotypes of acrocentric chromosome 15 and SMC der(15)
- b) CMA images of dup15q11.1q11.2(P5,P6,P7) and dup 9p24.3(P5)
- c) FISH PROBE USED :CEP15/Spectrum Aqua, SNRPN/Spectrum Orange,PML/Spectrum Green

P5, P6 & P7: three signals for dup CEP15(aqua) and two normal signals of SNRPN(orange) and PML (green)

Case P8

- a) Partial karyotypes of acrocentric chromosome 22 and SMC der(22)
- b) CMA images of dup(22q11.1q11.21)

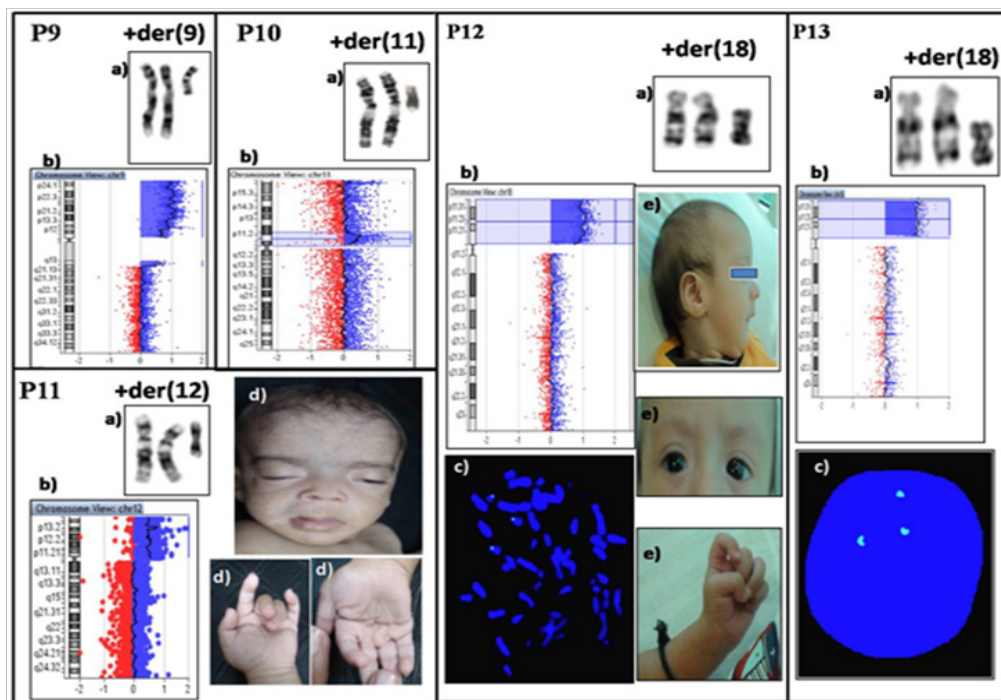


Figure 3 Cases P9,P10,P11,P12,P13:

- a) Partial karyotypes of non-acrocentric chromosome 9, 11,12,18,18, with their SMCs
- b) CMA images of dup 9, 11,12,18,18 respectively
- c) FISH PROBE USED :CEP18/Spectrum Aqua:

P12 & P13: three signals for dup CEP18(aqua)

- a) Clinical features associated with i12p in patient P11 : facial and hand dysmorphism
- b) Clinical features associated with Trisomy 18p in patient P12: dolichocephaly, telecanthus, antverted nares, small chin, clenched fingers

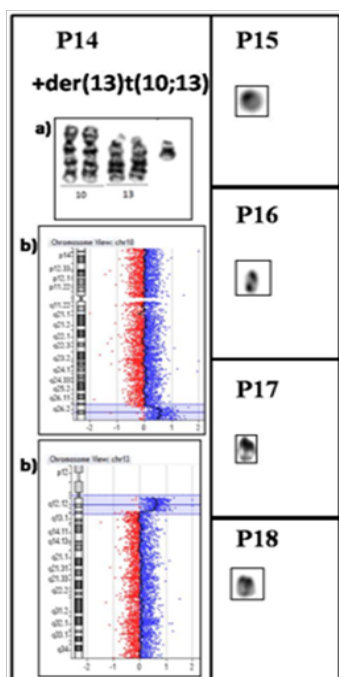


Figure 4 Cases P14:

- a) Partial karyotypes of chromosomes 10 & 13 and complex SMC derived from $t(10;13)(q26,q12.3)mat$
- b) CMA images of dup (10) and dup(13)

Cases P15, P16, P17, P18: Four very small heterochromatic SMCs which had normal array reports.

Non-acrocentric SMCs (Cases P9-P13) (Figure 3: P9, P10, P11, P12 & P13):

Five characterized SMCs were non-acrocentrics: one of each originated from der (9) (p24.3q21.11) (postnatal), 11p12p11.12 (prenatal), one was i(12p) (postnatal) and two were der(18p) (postnatal). The array results showed a 46.8Mb gain of 9p24.3p11.2 (115,981-46,933,102) and 5.3 Mb gain of 9q12q21.11 (65,632,517-71,016,040) (Figure 3:P9), a gain of 7.8Mb at 11p12p11.12 (42,922,228-50,768,675)(Figure 2:P10), a 34.6Mb gain of 12p13.33p11.1(162848-34827047) (Figure 3:P11), a 15.4MB gain of 18p11.32p11.21 (64,847-15,165,737) (Figure 3:P12) and 13.9Mb duplication of 18p11.32p11.21(148,963-14,081,887) (Figure 3:P13). FISH test was not performed for SMCs der (9), der (11), der(12) as the DNA probes were not available. The remaining two non-acrocentric SMCs of der (18) were validated using DNA probes CEP18.

Complex SMC (Case P14) (Figure 4: P14):

One novel complex SMC had originated from chromosome 10q and 13q (prenatal). This array result showed a 10.1 Mb gain at 10q26.13q26.3 (125266022-135434178) and a 7.9 Mb gain of 13q11q12.13 (19463637-27376648). This SMC was inherited from the mother who was a balanced translocation carrier with karyotype 46,XX,t (10;13)(q26;q12.3).

The remaining four SMCs (Cases P15-P18) (Figure 4:P15,P16,P17 & P18)

These were small and heterochromatic, seen microscopically during karyotyping but had normal CMA report

Mosaicism: Out of eighteen SMCs, seven (38.88%) had mosaicism and five of the mosaic SMCs analyzed in this study belonged to the prenatal category.

Inheritance: The origin of three SMCs was maternal and twelve were de novo and the parents of three individuals were unavailable for the study.

Discussion

SMCs are usually found as unexpected chromosomal abnormalities during karyotyping referred for various indications for chromosomal analysis. Knowing the gene content and the clinical outcome of each SMC is important for better understanding of its phenotypic consequence and recurrence especially in prenatal samples so that this can be conveyed to the parents during genetic counselling.^{8,9,10}

In the present retrospective study of 20,000 samples referred over the last five years, the overall frequency of SMC found was 0.09% (18/20,000) which was in concordance with the previous reports.^{2,11}

Approximately 66.7% of SMCs are reported as *de novo* and 30% as familial.² In our study 66.66% (12/18) were *de novo* in their origin and 16.66% (3/18) had maternal origin. However, in 16.66% (3/18) samples, the inheritance was not established as parental samples were not available. Most SMCs are *de novo* in origin, and this may lead to anxiety for parents during prenatal diagnosis.

Out of eighteen SMCs, we successfully characterized fourteen SMCs, but the remaining four had normal array results as they were very small and may have carried only heterochromatic material, which may have been harmless. According to our results of fourteen characterized SMCs, we formed three groups of SMCs: Acrocentrics, non-acrocentrics and complex SMCs (Table 1).

Out of 14 SMCs characterized, 57.14% (8/14) were acrocentric, 35.71% (5/14) were non acrocentric and 7.14% (1/14) were complex in nature.

SMCs of acrocentric origin (Cases P1 to P8)

Acrocentric Derivative der(15) (cases P1-P7): SMCs derived from chromosome 15 are the most frequent marker chromosomes identified.² This region is highly susceptible to genomic alterations, resulting in deletions, duplications, and the formation of SMCs. There are three well-known syndromes associated with this region: 15q11q13 duplication, 15q11-13 deletion or Prader Willi syndrome (PWS)/ Angelman syndrome (AS), and 15q13.3 duplication syndrome. In our study, 50% (7/14) of the characterized SMCs had originated from chromosome 15. This was in concordance with the other reports in literature.¹¹⁻¹³

(Cases P1, P2): bisatellited der(15)(q11.2q13.3) (both prenatal)

P1 was a 37 years old pregnant woman who had biochemical screen test positive with advanced maternal age whereas **P2** was a 32 years old pregnant woman with 5.76mm increased nuchal translucency on ultrasound. Karyotype report of amniotic fluid samples at 16 weeks of gestation showed bisatellited SMC in both samples. CMA results for

molecular characterization revealed 10.1Mb (22,765,628-32,899,558) and 12.2Mb (20,190,548-32,408,319) gains respectively on cytoband 15q11.2q13.3. The genes involved included *TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1*, *MKRN3*, *MAGEL2*, *NDN*, *C15orf2*, *SNRPN*, *PAR5*, *IPW*, *PARI*, *PAR4*, *UB E3A*, *ATP10A*, *GABRB3*, *GABRA5*, *GABRG3*, *OCA2*, *HERC2*, *APBA2*, *NDNL2*, *TJPI1*, *TRPM1*, *KLF13*, *OTUD7 A*, *CHRNA7*, *WHAMML1*, *GOLGA8IP*, *HERC2P2*, *HERC2P7*, *GOLGA8E*, *PWRN2*, *PWRN1*, *SNURF*, *SNORD1 07*, *PARSN*, *SNORD64*, *SNORD108*, *SNORD109B*, *SNORD109A*, *SNORD1161*, ... These duplications in both SMCs included the important gene *SNRPN*. The results were validated by metaphase FISH using Vysis DNA probe CEP15/SNRPN/PML.

Table 1 Distribution of SMCs according to their origin from acrocentric/non acrocentric chromosomes

SMC (N=18)	Prenatal (n=09)	Postnatal (n=09)
Characterized (n=14)		
Acrocentric (n=8) 57.14 %	Chr#15 (n=5) 35.71%	Chr# 15 (n=2) 14.28% Chr# 22 (n=1) 7.14%
Non-acrocentric (n=5) 35.71%	Chr#11 (n=1) 7.14%	Chr#12p (n=1) 7.14% Chr#18p (n=2) 14.28% Chr#9 (n=1) 7.14%
Complex (n=1) 7.14%	t(10;13) (n=1) 7.14%	
Unknown (n=4)	- (n=2) 14.28%	- (n=2) 14.28%

This can cause 15q11q13 duplication syndrome.¹¹ Individuals with 15q duplication syndrome commonly have variable degree of developmental delay, learning disabilities, autism spectrum disorders, anxiety, hyperactivity and epilepsy (OMIM-608636). Some genes such as *NIPA1*, *GABRB3*, *CHRNA7* and *UBE3A* have been associated with neurologic and neuropsychiatric disorders.¹⁴ The parents of the fetuses opted to terminate the pregnancies after genetic counselling.

(Cases P3, P4) - Small pericentric der (15) (Both prenatal)

P3 was a 29 years old pregnant woman with absent nasal bone on ultrasound analysis and **P4** was a 37 years old woman who had biochemical screen triple test positive. Amniotic fluid karyotype report of both revealed a small SMC with mosaicism (**P3** had 64% metaphases with SMC and **P4** had 56% metaphases with SMC. The second cell line in both was apparently normal).

CMA result of **P3** showed 2.2Mb gain at 15q11.1q11.2 (20,481,702-22,698,581). The genes involved were *BCL8*, *HERC2P3*,

GOLGA6L6, GOLGA8C, POTE8, NF1P1, LOC646214, CXADRP2, LOC727924, OR4M2, OR4N4, OR4N3P, REREP3. Genomic duplication 15q11.1q11.2 has been reported as benign in ISCA database (nssv1603246; chr15:20423115-22652121). *SNRPN* was not

present in this region.

CMA result of **P4** was a normal array report although the karyotype had a very small heterochromatic marker.

Table 2 Overview of Results: Their karyotypes, inheritance, CMA and FISH results, Indication of the test and the phenotypes

Case #	Age/ Sex Pre/ Postnatal	Karyotype	inheritance	CMA	Size (gain)	FISH	Clinical features / indication of study	Clinical significance
a) ACROCENTRIC SMCs								
P1	37Y/F Prenatal	47, _ _ , + mar [100%]	De novo	arr [hg19] 15q11.2q13.3(22,765,628-32,899,558) x3	10.1 Mb	+mar.ish der(15) (D15Z1+ ,D15S10+,PML-)	Biochemical test positive	Pathogenic 15q11-q13 duplication syndrome (OMIM#608636)
P2	32Y/F Prenatal	47, _ _ , + mar [100%]	De novo	arr [hg19] 15q11.1q13.3(20,190,548-32,408,319) x3	12.2 Mb	+mar.ish der(15) (D15Z1+ ,D15S10+,PML-)	NT 5.76mm increased	Pathogenic 15q11-q13 duplication syndrome (OMIM#608636)
P3	29Y/F Prenatal	mos 47, _ _ , + mar [64%] /46 _ _ [32%]	De novo	arr [hg19] 15q11.1q11.2(20,481,702-22,698,581) x3	2.2 Mb	+mar.ish der(15)(D15Z1+, D15S10- ,PML-)	Absent NB	Benign
P4	37Y/F Prenatal	mos 47, _ _ , + mar[56%] /46, _ _ [44%]	De novo	arr [hg19] (1-22)x2, (_ _)	-	+mar.ish der(15)(D15Z1+, D15S10- ,PML-)	TT positive	Benign
P5	5Y/F Postnatal	47, XX, + mar [100%]	Maternal	arr[hg19] 9p24.3(204,193-381,489)x3, 15q11.1q11.2(20,055,137-22,698,581) x3	177 kb 2.6 Mb	Not done +mar.ish der(15)(D15Z1+, D15S10- ,PML-)	GDD, dysmorphism	dup9p24.3 likely pathogenic Dup 15q11.1q11.2- likely benign
P6	38Y/F Postnatal	47, XX, + mar [100%]	Not done	arr[hg19] 15q11.1q11.2(20,055,137-22,698,581) x3	2.6 Mb	+mar.ish der(15)(D15Z1+, D15S10- ,PML-)	Mother of P5. H/o one abortion	Benign
P7	38Y/F Prenatal	47, XX, + mar [100%]	Maternal	arr[hg19] 15q11.1q11.2(20,095,481-22,698,581) x3	2.6 Mb	+mar.ish der(15)(D15Z1+, D15S10- ,PML-)	Fetus of P6	Benign
P8	34Y/M Postnatal	47, XY, + mar [100%]	Not done	arr[hg19] 22q11.1q11.21(16,504,318-18,628,078) x3	2.1 Mb	Not done	Delayed milestone, difficulty in studies, h/o anorectal malformation (corrected surgically) h/o febrile seizures,	Pathogenic Cat Eye Syndrome (OMIM: 115470).

Table Continued

Case #	Age/ Sex	Karyotype	inheritance	CMA	Size	FISH	Clinical features / indication of study	Clinical significance
b) NON-ACROCENTRIC SMCs								
P9	1Y/M	47,XY,+mar [100%]	De novo	arr[hg19]	46.8Mb	Not done		Pathogenic
	Postnatal			9p24.3p11.2(115,98146,933,102)x3, 9q12q21.11(65,632,517-71,016,040)x3	5.3Mb		IUGR, Dysmorphism- upslant ,Corpus callosum agenesis	Duplication 9p24.3p11.2 DECIPHER Patient ID 292636
P10	36Y/F	mos 47,_,_ + mar [74%] /46,_,_ [26%]	De novo	arr [hg19]		Not done	Biochemical test positive ,AMA	Pathogenic DECIPHER Patient ID: 255428, 291037
	Prenatal			11p12p11.12(42,922,228-50,768,675) x3	7.8Mb			
P11	5 Mo/F	mos 47,XX,+mar [54%] /46,XX[36%] in skin	De novo	arr[hg19]		Not done	DD, growth failure, dysmorphism	Pathogenic Pallister-killian syndrome OMIM : 601803
	Postnatal			12p13.33p11.1(162848_34827047)x3	34.6Mb			
P12	4 Mo/M	47,XY,+ mar[100%]	De novo	arr[hg19]			Minor aspiration event and jaundice at birth, DD, dysmorphism, dolichocephaly, telecanthus, antiverted nares, small chin, club foot, bilateral undescended testis with both testis localized in the inguinal canal.	Pathogenic Trisomy 18p (ORPHA:1715)
	Postnatal			18p11.32p11.21(64,847-15,165,737)x3,	15.4Mb	+mar.ish der(18)(D18Z1+)		
P13	7Y/F	47,XX,+ mar[100%]	De novo	arr[hg19]			DD	Pathogenic
	Postnatal			18p11.32p11.21(148,963-14,081,887) x3	13.9Mb	+mar.ish der(18)(D18Z1+)		Trisomy 18p (ORPHA:1715)
c) COMPLEX SMC								
P14	29Y/F	mos 47,_,_ _,+mar[100%]	Maternal	arr[hg19]		Not done	Mother balanced translocation carrier 46,XX,t(10;13) (q26;q12.3)	Pathogenic
	Prenatal			10q26.1 3q26.3(125266022_135434178)x3, 13q11q12.13(19463637_27376648)x3	10.1Mb 7.9Mb			

Amniotic fluid samples of both **P3** and **P4** were validated by FISH with Vysis DNA probe for *SNRPN* which showed three aqua signals for CEP (15), two normal orange signals for the *SNRPN/15q11.2* probe and two green signals for *PML/15q22*. Therefore both **P3** and **P4** were likely benign. The karyotypes of both the parents of **P3** and **P4** were normal. Such small heterochromatic pericentric SMCs are usually asymptomatic or benign specially when found in mosaicism¹⁵ and also when there is no secondary copy number variation reported by CMA.¹⁶ The parents decided to continue their pregnancies, after genetic counselling.

(Cases P5, P6, P7) -Familial SMCs der(15) (two postnatal and one prenatal)

Patient (**P5**) was a 5 years old female child with global developmental delay and dysmorphism. Routine whole blood culture with subsequent karyotyping showed presence of SMC. Molecular characterization by CMA

showed 2.6Mb gain at 15q11.1q11.2(20,055,137-22,698,581), the genes involved were *GOLGA6L6*, *GOLGA8C*, *POTEB*, *NF1P1*, *LOC646214*, *CXADRP2*, *LOC727924*, *OR4M2*, *OR4N4*, *OR4N3P*, *R EREP3* (same as in **P3,P4**) but she also had 177Kb gain at 9p24.3(204,193-381,489), the genes involved were *DOCK8* and *C9orf66*. Diseases associated with *DOCK8* include Autosomal Recessive and Autosomal Dominant Non-Syndromic Intellectual Disability (www.genecard.org). The karyotype of the phenotypically normal mother (**P6**) was 47,XX,+mar and the father apparently had a normal karyotype 46,XY. Molecular characterization of the SMC by CMA confirmed a gain of 2.6Mb size at 15q11.1q11.2(20,055,137-22,698,581) in the mother with no additional secondary CNV and the genes involved on der(15) were same as in her daughter (**P5**) and other benign cases (**P3, P4**). So the SMC in the child (**P5**) was maternally inherited. The family consulted our genetic clinic for prenatal counselling during the next pregnancy. At 16 weeks of gestation, amniocentesis was done. Karyotyping and CMA were done simultaneously using the amniotic fluid sample (**P7**) at 16 weeks of gestation. Karyotype report of the foetus (**P7**) showed presence of the SMC. The CMA result showed a 2.6Mb gain at 15q11.1q11.2 (20,095,481-22,698,581) which had the same gene content of der(15) was same as the mother (**P6**) with no duplication of chromosome 9p24.3 as found in the sibling (**P5**). Metaphase FISH using DNA probe CEP15/SNRPN/PML showed duplication of CEP15 in all three **P5, P6** and **P7**.

Genomic duplication 15q11.1q11.2 has been reported as benign in ISCA database (nssv1603246; chr15:20423115-22652121). It is well documented in previous studies that the familial SMCs have no impact on phenotypes. In their previous affected child the phenotype may have been due to 177kb gain at 9p24.3 which was not present in any of the parents or the fetus. Duplication 9p24.3 has been reported to be associated with multiple neurodevelopmental disorders.¹⁷ *DOCK8* gene in this region, has been associated with intellectual disability. The parents were counselled that the SMC der(15) in the foetus (**P7**) may be benign. However, the pregnancy was terminated as the parents were anxious and did not want to take any chance.

(Case P8)-Acrocentric Derivative der(22) (postnatal)

The proband (**P8**) was 34 years old adult male with delayed milestone, difficulty in studies, and history of febrile seizures and anorectal malformation which was corrected by surgery

later in life. He was brought by his parents for premarital counselling. His karyotype report was 47,XY,+mar. Molecular characterization of the SMC showed 2.1 Mb duplication of cytoband 22q11.1q11.21 (16,504,318-18,628,078). The genes involved were *XKR3*, *IL17RA*, *CECR2*, *SLC25A18*, *ATP6V1E1*, *BID*, *MICAL3*, *PEX26*, *TUBA8*, *CCT8L2*, *psiTPTE22*, *HSFY1P1*, *GA B4*, *CECR7*, *CECR6*, *CECR5*, *CECR4*, *CECR1*, *BCL2L13*, *MIR3198*, *MIR648*, *FLJ41941*. Genomic duplication on cytoband 22q11.1q11.21 has been associated with global developmental delay, abnormal facial shape, growth

delay etc. (Patient DECIPHER ID: 288878). The multiple candidate genes *CECR2*, *CECR7*, *CECR6*, *CECR5*, *CECR4*, and *CECR1* are associated with Cat Eye Syndrome (CES) (OMIM: 115470). The characteristic features of CES are coloboma of iris, preauricular tag and pits, anal abnormalities, cardiac defects, renal atresia and ectopia, variable developmental delay and intellectual disability. The variability of clinical features in CES range from near normal to severe malformations.¹⁸⁻²⁰ The recurrence risk for offspring of an affected parent may be close to 50%. This SMC proved to be syndromic and hence clinically pathogenic.

SMCs of Non-acrocentric origin (Cases P9 to P13)

Most non-acrocentric SMCs are found to be pathogenic as seen in the present study.

(Case P9):Derivative der(9) (postnatal)

The proband (**P9**), a one year old male child had IUGR, dysmorphism, upslant squint, hypertelorism, and agenesis of corpus callosum. His blood karyotype report was 47,XY,+mar. Molecular characterization of the SMC by CMA showed of 46.8Mb gain of 9p24.3p11.2 (115,981-46,933,102) and 5.3 Mb gain of 9q12q21.11 (65,632,517-71,016,040). There were multiple genes involved. Parental karyotypes were normal, which established that the inheritance was de novo. No other copy number variations (CNVs) were detected in the patient or his parents.

Trisomy 9p is a rare chromosomal syndrome in which the duplication may involve a portion of the short arm (9p22), the entire short arm (9p) or the short arm and a portion of the long arm (9q11q13). Trisomy 9p syndrome has wide phenotypic variability, typically characterized by development delay, craniofacial dysmorphism, downslanting palpebral fissures, deep set eyes, hypertelorism, short wide neck, prominent nose, short philtrum, downturned corners of the mouth, low set ears, digital anomalies and short stature or growth deficiency before birth (intrauterine growth retardation) and variable degree of intellectual disability. Less frequently patients present with cardiopathy and renal, skeletal, and central nervous system malformations. The chromosome 9 is rich in segmental duplication, especially in pericentromeric region, and is therefore prone to illegitimate recombination, either intrachromosomal or interchromosomal, which predisposes it to rearrangements, frequently involving pericentromeric regions.^{21, 22}

The duplicated region consisted of a) cytoband 9p24.3 to 9p23 which is the potential ASD candidate locus; b) cytoband 9p22.3to 9p22.2 which has been identified as a critical region for the 9p duplication syndrome; c) cytoband 9p22.1 to 9p13.1 has been reported to be duplicated in a normal individual; and d) 9p13.1p11.2 has been reported to be benign.²³

(Case P10): Derivative der(11) (prenatal)

A prenatal amniotic fluid sample (P10) was taken from a high risk pregnancy of women with advanced maternal age (36years), triple test positive and history of recurrent deep vein thrombosis. She was a heterozygous carrier for Factor V Leiden. Karyotyping of amniocytes showed an aneuploidy in the form of a SMC. Molecular characterization of the SMC by CMA showed a novel duplication of 7.8Mb at 11p12p11.12 (42,922,228-50,768,675). The genes involved were: *API5, ALKBH3, EXT2, ALX4, CD82, SYT13, CHST1, SLC35C1, CRY2, MAPK8IP1, PEX16, GYLTL1B, MDK, CHRM4, ARHGAP1, F2, CKAP5, LRP4, ARFGAP2, PACSIN3, DDB2, ACP2, MADD, MYBPC3, SPI1, SLC39A13, PSMC3, RAPSIN, NDUFS3, NUP160, PTPRJ, FOLH1, LOC399881, TTC17, MIR670, MIR1292, HSD17B12, LOC729799, C11orf96, ACCSL, ACCS, TSPAN18TP53I1I, , LOC221122, PRDM11, DKFZp779M0652, C11orf94, PHF21A, CREB3L1, DGKZ, AMBRA1, MIR31601, MIR31602, HARB1, ATG13, ZNF408, SNORD67, C11orf49, NR1H3, CELF1, PTPMT1, KBTBD4, FAM180B, CIQTNF4, MTCH2, AGBL2, FNBP4, OR4B1, OR4X2, OR4X1, OR4S1, OR4C3, OR4C45, OR4A47, LOC440040, OR4C13, , OR4C12, LOC441601, LOC646813.*

Genomic duplications on 11p12p11.12 are associated with hypertelorism, intellectual disability, global developmental delay (DECIPHER Patient ID: 255428, 291037). Karyotyping of both parents was apparently normal which established the origin to be *de novo*. However, *F2* gene duplicated in the SMC region is associated with Factor V Leiden pathway and the mother was a heterozygous carrier of Factor V Leiden. This requires further study. The parents were counselled about this clinically significant aneuploidy and decision was left to the couple.

(Case P11): Derivative der(12) (postnatal)

Case P11 was a five months old female with developmental delay, facial and hand dysmorphism and growth failure. Her blood karyotype was mos 47,XX,+mar[2]/46,XX[48]. CMA result showed a 34.6Mb duplication of 12p13.33p11.1(162848-34827047). Her skin biopsy was cultured and the karyotype was mos47,XX,+i(12p)[27]/46,XX[23]. Genomic duplication of 12p has been associated with Pallister-Killian syndrome (OMIM: 601803). The phenotype associated with this syndrome is macrocephaly, abnormal muscle tone, characteristic facial features, developmental delay and intellectual disability.

(Cases P12 and P13): Derivative der(18) (postnatal)

The proband (P12) was a four months old male child who had developmental delay, dolichocephaly, telecanthus, antverted nares, small chin, clenched fingers, club foot, bilateral undescended testis. He had minor aspiration event and jaundice at birth. Another patient, seven years old female child (P13) had developmental delay. Blood sample of both (P12 and P13) were subjected to CMA as a first-tier test, which showed 15.4MB duplication of 18p11.32p11.21 (64,847-15,165,737) and 13.9Mb duplication of 18p11.32p11.21(148,963-14,081,887) respectively. Karyotyping showed the presence of SMC in all metaphases (100%). Validation by Vysis DNA FISH probe CEP 18/ Spectrum aqua in both cases showed three aqua signals. Trisomy 18p is a rare but well documented syndrome, with prevalence reported as 1:1000, 000 (ORPHA1715). The phenotypic features include cognitive impairment, psychomotor delay, dysmorphic features, atrial septal defect and a club foot.²⁴

Complex SMCs originated from more than one chromosome (Case P14) (prenatal)

A 29 years old female (P14) with history of recurrent pregnancy loss, was referred for prenatal diagnosis. Karyotyping of the blood sample of the couple showed that she was a balanced translocation carrier with karyotype 46,XX,t(10:13)(q26;q12.3) and the karyotype of her husband was apparently normal. Her prenatal sample (P14) of amniotic fluid was taken for prenatal diagnosis at 16 weeks of gestation. The karyotype report showed the presence of a SMC which required molecular characterization by CMA. CMA results showed a duplication of 10.1Mb at 10q26.13q26.3 (125266022-135434178) and 7.9Mb at 13q11q12.13 (19463637-27376648).

Overlapping genomic duplications of cytoband 10q26.13q26.3 (125792404-129167091, 130670683-135377532) have been reported as pathogenic variations in patients with global developmental delay in ISCA database (nssv582217, nssv582218). Overlapping genomic duplication of 3.0 Mb on cytoband 13q11q12.13 (20411535-23478894) has been reported as a pathogenic variation in a patient with global developmental delay in ISCA database (nssv578643). Therefore, the patient and her husband were counselled about the prognosis and they opted to terminate the pregnancy.

To the best of our knowledge there have been no reports of complex SMCs originating from chromosomes 10 and 13 which has been inherited from the maternal balanced translocation t(10;13). The SMC must have originated from chromosome disjunction at a ratio of 3:1. Another such complex most commonly detected SMC is der(22) in Emanuel Syndrome (OMIM # 609029), commonly derived from a carrier parental with balanced t(11;22) which is the most frequent translocation seen in human.

Four very small SMCs with normal CMA results (cases P15-18)(two prenatal & two postnatal)

Case P15 and P16 were two prenatal cases with 35 years and 28 years as maternal age. P15 had nasal bone absent and P16 had fetal ascites detected at level II ultrasound scan. Culture and subsequent karyotyping results of amniotic fluid sample at 16 weeks of gestation showed 16% and 24% SMC respectively and CMA results of both P15 and P16 were normal.

Case P17 was a 31years old female with congenital renal anomaly, dextrocardia and extra cervical rib. Karyotype report was 47,XX,+mar. A small heterochromatic SMC was seen in all metaphases (100%) from the lymphocyte cultures. CMA showed normal result.

Case P18 was a two years old male child. He had developmental delay and dysmorphism. CMA result was normal. Karyotype report was: mos 47,XY,+mar[7]/46,XY[43]. There was presence of low-level mosaicism as only 14% metaphases showed presence of small heterochromatic SMC.

All four SMCs could not be characterized by CMA and could not be identified even by multiple attempts of FISH as they were either only centromeric, heterochromatic or had very low level mosaicism. More than half of SMC carriers present with low level mosaicism.¹⁵

Limitations of CMA: Microarray platforms usually do not have SNPs or probes for detection of the heterochromatic regions of the genome. Low level mosaicism is not detected by CMA. Balanced translocations are also not detected by CMA, therefore parental studies for SMCs and mosaicism should be carried out by karyotyping.

It has been suggested that most SMCs are the result of a multiple-step mechanism, initiated by maternal meiotic non-disjunction followed by postzygotic anaphase lagging of the supernumerary chromosome and its subsequent chromothripsis.²⁵

The pathological consequence of the SMC and its risk of recurrence are related to its size, gene content, whether heterochromatic or euchromatic, mosaic or non-mosaic and/or uniparental disomy.^{15,26} A small SMC may just be heterochromatic with no pathological consequence and another SMC can be pathogenic or causative for specific syndrome called the SMC related syndromes e.g. Cat Eye syndrome, Pallister Killian syndrome, etc., therefore molecular characterization of the SMC is important.⁷

Combined classical cytogenetic techniques such as Karyotyping, FISH and CMA are used by most scientists to decide the origin, detect the euchromatin or heterochromatin and level of mosaicism in order to render precise genetic counseling. Elucidating the role of these genes in development and differentiation during embryogenesis will decide whether the SMC is pathogenic or benign. Preimplantation genetic diagnosis (PGD) is often offered to prospective parents to identify SMC chromosome, for selection of normal and balanced embryos for transfer during IVF.

The SMC is not only a structurally abnormal chromosome but also an aneuploidy. The risk of recurrence of any aneuploidy is 1%. SMC-related known syndromes such as 15q11q13 duplication (n=2), Cat Eye syndrome, Trisomy 9p syndrome, i(12)p Pallister Killian syndrome and the rare Trisomy 18p syndrome(n=2) are all pathogenic. The presence of phenotypic abnormality in a prenatal sample would therefore vary depending on the molecular characterization of the SMC. Accumulation of more cases may help to close the gap of knowledge to some extent to help in disease management of postnatal cases and genetic counselling of prenatal cases.

Conclusion

The study emphasizes the usefulness of CMA in identification and characterization of the additional genetic material of the SMC which can be correlated with the phenotypes of the postnatal patient for future management and also the clinical significance conveyed to the parents when the SMC is found in prenatal samples.

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Conflicts of interest

No conflict of interest to declare.

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