Indian J Med Res 142, December 2015, pp 699-712
DOI:10.4103/0971-5916.174561

Recurrent benign copy number variants & issues in interpretation of variants of unknown significance identified by cytogenetic microarray in Indian patients with intellectual disability

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Received May 29, 2013

***Background & objectives*: Cytogenetic microarray (CMA) is now recommended as a first-tier clinical diagnostic test in cases with idiopathic intellectual disability and/or developmental delay (ID/DD). Along with clinically relevant variants, CMA platforms also identify variants of unknown significance (VUS). This study was done to look for utility and various issues in interpretation of copy number variants (CNVs) in Indian patients with ID/DD.**

***Methods*: The CMA was performed in 86 Indian patients with idiopathic ID/DD with or without dysmorphic features. CNV was reported if copy number gain was >400 kb in size and copy number loss was > 200 kb in size.**

***Results*: Pathogenic CNVs were found in 18 of 86 (20.9%) patients. One large (14 Mb size) *de novo* heterozygous copy number gain was found in one patient. VUS (total 31) were present in 17 of 86 (19.7%) patients. Five novel recurrent benign CNVs were also present in our patients.**

***Interpretation & conclusions*: Our findings highlight the difficulties in interpretation of CNVs identified by CMA. More Indian data on VUS and recurrent benign CNVs will be helpful in the interpretation of CMA in patients with ID/DD.**

Key words Cytogenetic microarray - idiopathic intellectual disability - recurrent CNV - VUS

Cytogenetic/cytogenomic/chromosomal microarray (CMA) has been recommended as a first-tier diagnostic test in the work-up of patients with intellectual disability (ID)/ developmental delay (DD)/ multiple congenital anomalies (MCA) and/or autistic spectrum disorders (ASDs)[1]. The diagnostic yield is estimated to be in the range of 15-20 per cent in cases with idiopathic ID/DD[2]. Along with causal pathogenic copy number variants (CNVs), CMA platforms also identify many other CNVs which are difficult to be categorized in benign or pathogenic variants. These variants are called as variants of unknown significance (VUS)[2-4]. These pose great dilemma in front of cytogeneticists as well as to clinicians in providing genetic counselling, prediction of risk of recurrence and providing prenatal diagnosis. In this study we describe various issues in

interpretation of CNVs identified in CMA analysis in Indian patients with idiopathic ID/DD and report normal variants in Indian patients.

Material & Methods

This study was conducted in the department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India, from May 2012 to April 2013. All those patients with idiopathic ID/DD with or without malformation or dysmorphic features were included whose relevant clinical details were available and the family agreed to participate in the study and consented to provide the sample. Cytogenetic analysis by G banded karyotype at 450-550 band level was normal in all patients. CMA was performed in 86 cases with ID/DD with or without dysmorphic features in whom clinical examination and appropriate investigations had not provided aetiological diagnosis. CMA was performed in parents wherever consent of the parents and their blood samples were available. The present study protocol was approved by the institute ethical committee of SGPGI, Lucknow.

CMA analysis: CMA was performed by the Cytogenetics 2.7M Array (Affymertix ®, USA, 71 cases) and HumanCytoSNP-12 (Illumina, USA, 15 cases). Analysis was done by Affymetrix® Chromosomal Analysis Suite and Genome studio software (Illumina) as per manufacturers' protocol. Cytogenetics 2.7M Array has density of 2.7 million markers covering the whole genome. It also includes 400,000 probes to detect single nucleotide polymorphisms (SNPs) to enable the detection of copy neutral changes (loss of heterozygosity, LOH). Illumina HumanCytoSNP 12 has 200,000 probes for SNP, providing genome coverage and 220,000 cytogenetic markers for 250 targeted genomic regions. Human genome version GRCh 37:Feb 2009 (hg 19) (*http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19*) was used in data annotation.

Copy number variants (CNVs): CNVs were reported only if copy number gain was >400 kb in size and copy number loss was more than >200 kb in size. CNVs were classified into benign/non-pathogenic, pathogenic/clinically relevant variants (which are associated with known microdeletion/microduplication syndrome and/or associated with clinical phenotype or large *de novo* variants with genes associated with phenotypes like autism, epilepsy, intellectual disability or other significant neurological dysfunction) and VUS (genomic variants which have not been previously reported in normal individuals and insufficient information regarding clinical significance)[4]. This delineation was made after looking into published literature and curetted databases[5]. The size of CNV, its gene content and its *de novo* or inherited status were also taken into consideration. VUS were further divided into possibly benign [inherited from either clinically normal parent and/or not reported in Database of Genomic Variants (DGV)[6], no relevant Online Mendelian Inheritance in Men (OMIM) phenotype[7], no relevant genes or a particular CNV was present in multiple patients in recurrent manner], possibly pathogenic (if it was *de novo* or OMIM loci associated with DD/ID/ASDs/ other central nervous system disorders like ataxia and epilepsy) and possibly VUS (no definite central nervous system associated genes or phenotype and/or one or more genes associated with basic cell function, *i.e.* embryogenesis, cell migration) according to available evidence of published literature and databases[3,4]. Patients harbouring at least two large CNVs (>5 Mb) were designated to have double segment imbalances. Subtelomeric copy number gains or losses were further validated by multiplex ligation dependent probe amplification (MLPA) test[8].

Results

A total of 86 patients with idiopathic DD/ID with or without malformation/dysmorphism were included in the study. Of these, nine (10.5%) were less than one year of age, 43 (50%) were between age 1 and 5 yr while 34 (39.5%) were more than 5 yr of age. Forty one (47.6%) patients were males while 45 (52.3%) were females.

Pathogenic CNVs: Pathogenic variants were found in 18 patients giving a yield of 20.9 per cent. Of these, 14 patients (13 deletions, 1 duplication) had variants which were already associated with known microdeletion/microduplication syndromes. The details of these patients are presented in Table I. Three of these 18 patients had double segment imbalances indicating the possibility of inherited/ *de novo* chromosomal rearrangement. Of these three, one family (in extended pedigree) had three children affected with global developmental delay with facial dysmorphism suggesting a familial balanced chromosomal translocation. Details of cases with double segment imbalances are presented in Table II. One patient had *de novo* heterozygous copy number gain of 14 Mb size. This patient was a 22 yr old male born in non-consanguineous family with no significant family history. The clinical features included short

Table I. Pathogenic variants seen in patients with idiopathic DD/ID (n=14) with known pathogenic gains/losses

S. No.	Age/sex	Clinical features	Deletion(del)/ duplication (dup)	Chromosomal band	Size of variant	Start and end nucleotide
1	12 yr/F	GDD, chorea	del	1p21.2-21.3	2.3 Mb	97,335,217-99,725,000
2	1 yr/F	GDD, facial dysmorphism, hypotonia	del	1p21.3	13.8 Mb	96768706-110605890
3	2 months/F	Mild GDD, facial dysmorphism, complex congenital heart disease	del	1p36	319 kb	248817-568426
4	1 yr/F	GDD, facial dysmorphism, post axial polydactyly	del	1p36	6.1 Mb	772944-6970121
5	3 yr/F	GDD, hemiparesis hemiconvulsion epilepsy syndrome (onset during infancy), facial dysmorphism, post axial polydactyly	del	1q44	1.8 Mb	244744522-246608189
6	1 yr/M	GDD, facial dysmorphism	del	6q11.1-14.1	20 Mb	57809085-82387124
7	11 yr/F	GDD, facial dysmorphism	del	7q11.2	428 kb	74139624-74568522
8	17 yr/M	GDD, post axial polydactyly in lower limbs	del	7q14.1	1.42 Mb (harbouring *GLI3 gene*)	39615502-43036979
9	1 yr/F	Failure to thrive, GDD, laryngomalacia	del	16p11.2	545 kb	29559989-30105430
10	3.5 yr/M	GDD, facial dysmorphism	del	16p11.2	206 kb	32303961-32510742
11	6.5 yr/M	GDD, short stature, micropenis	del	17p11.2	3.3 Mb	16926291-20244180
12	3 yr/F	GDD, facial dysmorphism, post axial polydactyly	del	22q11.2	3 Mb	17118296-20125656
13	5 months/M	GDD, microcephaly, lissencephaly	dup	Xq28	728 kb	152625374-153353398
14	1 yr/M	GDD, trigonocephaly, low set ears, prominent tragus, inguinal hernia	dup	15q25.3-q26.3	12.8 Mb	87453826-100319800

GDD, global developmental delay

stature, facial dysmorphism (maxillary hypoplasia) and brachydactyly (Figure). The patient was talkative and had friendly personality. This region was harbouring >75 genes [arr10q21.1q22.1(59168091-73319571) X3]. No gene was definitely associated with mental retardation/ developmental disability or other related disorders (UCSC genome browser hg19 version *http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19*). Important genes in this region include *NEUROG3* (transcription factor involved in neurogenesis) and *TFAM* (polymorphism has been reported in Alzheimer's disease and parkinsonism). Other genes were involved in various basic cellular functions including contact, motility, mRNA transport and metabolism. In DECIPHER (*https://decipher.sanger.ac.uk*) a few entries have been described in overlapping region associated with mental retardation. On the basis of large size and *de novo* nature, this CNV was interpreted as pathogenic.

VUS: Twenty five (29%) patients did not have any CNV detected by CMA. On the other hand, in 26

Table II. Double segment imbalances in three patients with global developmental delay

S. No	Age/gender	Clinical features	Involved chromosomal regions	CMA report (GRCh37/hg19 genome browser)
1	7 yr/M	Global developmental delay, facial dysmorphism, brachydactyly, congenital heart disease, mother had 6 first trimester abortion	3p26.3-p24.1 (26.8 Mb gain), 18p11.32-11.21(14.4 Mb loss)	arr3p26.3p24.1(81668-26977225)X3, 18p11.32p11.21(60739-14540632)X1
2	3 months/ F	Global developmental delay, facial dysmorphism, corpus callosal agenesis, 2 first cousins also had developmental delay	7q36.1 (9.3 Mb loss),11q24.1-25 (13 Mb gain)	arr7q36.1q36.3(49770238-159118443) X1, 11q24.1-25(121769912-134926021) X3
3	1 yr/M	Global developmental delay, hypotonia, mild cerebral atrophy	9p24.3-p23 (10.8Mb loss) 20q (12.1 Mb gain)	arr9p24.3p23(209111-11073967)X1, 20q13.2q13.33(50724046-62917655) X3

Source: http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19

(30.2%) patients all CNVs (total 41 CNVs, 13 losses, 28 gains) detected were interpreted as benign. Size of these benign CNVs was ranging in size from 226 kb to 3.3 Mb. Seventeen of 68 (25%) patients had one or more VUS (total 31) giving and average of 1.8 VUS per case.VUS, which were present in patients harbouring definitely pathogenic variants, were not included in this list. Almost half (9/17) of the patients were having multiple VUS. Maximum number of VUS in a single patient was four. Four out of 31 VUS (7.7%) were interpreted as possibly benign (2 gains and 2 losses, size range 233-1115 kb, Table III). Eleven CNVs (35.2% of all VUS), seen in 10 patients were interpreted as possibly VUS (all gains, size range is 422-2399 kb, Table IV). Sixteen CNVs (51.6% of all VUS) in 10 patients (1-2 per case) were interpreted as VUS, possibly pathogenic (6 losses, 10 gains, size range 206- 2284 kb, Table V).

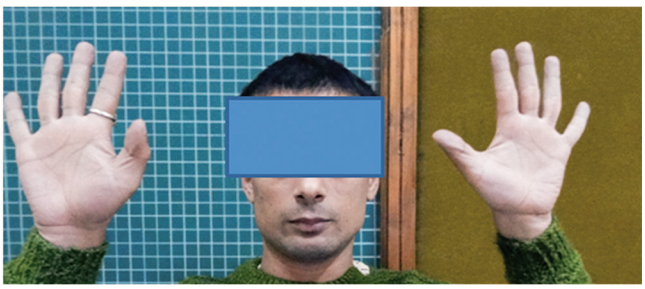

Figure. Photograph of patient, having *de novo* heterozygous 14 Mb gain on 10q21.1-22.1. Facial dysmorphism included maxillary hypoplasia and downslanting palpebral fissures. Hands showing brachydactyly.

Of the 15 patients with single definite pathogenic variant, nine were also having possibly pathogenic VUS or possibly VUS at unrelated parts of genomes. Three patients had single VUS. Rest of them were harbouring 2-5 VUS. One of the three patients with double segment imbalances had VUS at different chromosomal region (1.7 Mb loss at 10q21.1) apart from two primary gains/losses.

Recurrent benign CNVs: Five CNVs including 4 gains and 1 loss (size range 301-927 Kb, Table VI) were present as recurrent benign CNVs in our patients. The size of each CNV was much larger than those variants which were reported in DGV (hg19) (Database of Genomic Variant; *http:projects.tcag.ca/variation/*).

LOH regions: We analyzed LOH regions in 36 patients in whom CMA was performed by Affymetrix2.7 M array and no definite pathogenic variant was identified. Laboratory cut-off for analysing these LOH regions was kept as 5Mb and X chromosome was not included in the analysis. This 5Mb cut-off was decided on the basis of study done by Sund *et al*[9]. Of these 36 patients, two were born by consanguineous parentage and in another patient there was history of similarly affected sibling but there was no consanguinity. In consanguineous (between first cousins) families, the number of LOH regions (>5Mb size) was 3 and 12, respectively. Total region of homozygosity was 91 and 235 Mb, respectively (3.1 and 8.1% of total autosomes). In 34 non-consanguineous families, 27 (84%) had no significant LOH regions. Three patients had single LOH region (5-6Mb) on an autosome. In four families

Table III. Possibly benign variants of unknown significance

S. No	Age/gender	Clinical features	Type of CNV	Position	Start nucleotide	End nucleotide	Size in kb	Genes (GRCh37/hg19 genome browser)
1.	4.5 yr/F	DD and mild facial dysmorphism	Loss	16p13.11	16523266	16756507	233	-
2	2 yr/F	DD	Loss	Xq21.1	82946790	83230011	283	*CYLC1*
3	2 yr/F	DD	Gain	Xp22.33	836976	1952789	1115	*CRLF2, CSF2RA, IL3RA, SLC25A6, ASMTL-AS, ASMTL, P2RY8, AKAP17A, ASMT*
4	5 yr/M	DD, behavioural abnormality	Gain	6q27	170093128	170638018	544	*WDR27, C6orf120, PHF10, TCTE3, C6orf70, NCRNA00242, C6orf208, LOC154449, DLL1, FAM120B*

(11.7% among non-consanguineous families), 2-24 LOH regions (32-188 Mb) were found, which were corresponding to 1.1 - 6.5 per cent of total autosomes.

Discussion

The diagnostic yield of CMA in our patients with idiopathic ID/DD was 20.9 per cent which was in accordance with other studies showing the diagnostic contribution of CMA in the range of 15-20 per cent[10,11]. Of the 18 pathogenic variants, five were located in subtelomeric region. These subtelomeric gains/losses can be identified by MLPA using probe set for subtelomeric regions. Also MLPA can be used to diagnose cases with known microdeletion and microduplication syndromes. At present MLPA probe set for common microdeletion contains probes for 21 regions. In a study done at our centre the diagnostic yield of MLPA using subtelomeric and common microdeletion probe set in patients with idiopathic developmental delay was 9.3 per cent[8]. MLPA can be acceptable substitute to CMA in those families who can not afford CMA.

We also found one novel pathogenic copy number gain of 14 Mb size in one patient with DD and facial dysmorphism. Though not described in literature, various genes in this region are involved in basic cellular metabolism including neurogenesis. There were three patients with double segment imbalances. In these patients, possibilities can be interchromosomal exchange of segments representing the possibility of chromosomal imbalance or separate chromosomal events[12]. The risk of recurrence in the former case will be up to 50 per cent if inherited in comparison to <1 per cent in the later events as most of these pathogenic variants are *de novo* in origin. In all these cases karyotype of patients/parents or fluorescent *in-situ* hybridization analysis will be essential for accurate risk prediction of recurrence in family.

Interestingly, 60 per cent patients who were having at least one definite pathogenic variant were also having clinically important CNVs at other genomic location. These VUS in patients may contribute towards modulation of clinical features leading to phenotypic differences of the patients. In a study conducted by Girirajan *et al*[13], in 32,587 children with developmental delay, prevalence of second additional genetic variant was 10 per cent. They have hypothesized that these CNVs may be responsible for phenotypic variations in microdeletion/microduplication syndromes.

In this study, we found 31 VUS in 17 patients with no definitely pathogenic variants. Pyatt *et al*[10] in their study on 1998 samples found 563 abnormalities in 490 patients. The size range of these VUS was 33 kb to 2.9 Mb. Similar to this study, frequency of duplication variants were much more than deletion (66 vs 33% in

Table IV. Variants of unknown significance (Possibly VUS).

S. No.	Age/ gender	Clinical details	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
1	2 yr/M	DD* and facial dysmorphism	Gain	5q13.2	68993838	70291604	948	*SMN2, SMN1, SMN2, SMN1, SMN2, SMN2, SERF1A, SERF1A, SERF1B, SERF1A, LOC653188*	Loci for juvenile myoclonic epilepsy
2	5 yr/F	DD	Gain	16p12.3	18231098	18793717	562	*MIR3180-3, MIR3180-1, MIR3180-2, MIR3179-1, MIR3179-3, MIR3179-2, NOMO2, ABCC6P1*	NOMO2:protein complex that participates in the nodal signalling pathway during vertebrate development,
3	2 yr/F	DD	Gain	2p25.3	1400685	2088034	687	*TPO, PXDN, MYT1L*	Genes related to thyroid hormone synthesis, hemocytes derived from head mesoderm at a very early stage of differentiation, extracellular matrix consolidation, phagocytosis, and defense. DECIPHER-170 kb duplication known to be associated with mental retardation
4	3 yr/F	DD, facial dysmorphism	Gain	11p11.12	48639002	49083041	444	-	OMIM phenotype of Potocki-Shaffer syndrome (#601224) associated with mental retardation, craniodysostosis, multiple exostosis)
5	5 yr/M	DD, behavioural abnormality	Gain	18p23	75573945	76100423	526	None	no gene but OMIM phenotype of 18q- syndrome
6	10 months/ M	DD	Gain	18q23	75572083	76090868	518	None	Not very well reported in DGV, no genes, OMIM phenotype of 18q deletion syndrome, 1 poorly characterised entry in DECIPHER associated with MR with size of 55 Mb

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S. No.	Age/ gender	Clinical details	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
7	8.5 yr/M	DD and gynecomastia	Gain	13q31.3	92773468	94174869	1401	*GPC5, GPC6*	GPC5: control of cell division and growth regulation, GPC6 : cell surface coreceptor for growth factors, extracellular matrix proteins, proteases and anti-proteases.
8	42 yr/F	DD	Loss	5p15.33	34489	2434069	2399	*PLEKHG4B, LRRC14B, CCDC127, SDHA, PDCD6, AHRR, LOC100310782, C5orf55, EXOC3, LOC25845, SLC9A3, CEP72, TPPP, ZDHHC11, BRD9, TRIP13, NKD2, SLC12A7, SLC6A19, SLC6A18, TERT, CLPTM1L, SLC6A3, LPCAT1, SDHAP3, LOC728613, MIR4277, MRPL36, NDUFS6, IRX4*	In DECIPHER, overlapping regions reported as having speech delay
9	3 yr/M	DD with seizures with facial dysmorphism.	Loss	8p23.1	7219734	7425632	205	*DEFB107B, FAM90A7, SPAG11B, DEFB103A, DEFB105B, DEFB105A, SPAG11B, DEFB106A, DEFB106B, SPAG11B,*	Overlapping regions in DECIPHER associated with MR, OMIM loci for generalized epilepsy with febrile seizures
10	23 yr/M	DD	Loss	Xq28	154586793	154888046	301	*VAMP7, SPRY3, VAMP7, IL9R, IL9R*	In the region of Xq28 but not involving candidate genes like *ABCD1*, and *BCAP31*, genes function : The encoded protein localizes to late endosomes and lysosomes and is involved in the fusion of transport vesicles to their target membranes.
11	2 yr/F	DD	Loss	Xq13.3	75295849	75851194	555	*CXorf26, MAGEE1*	Unknown,signaling role in brain, muscle, andperipheral nerve. Loci linked to mental retardation.

DD, developmental delay; DECIPHER, database of genomic variation and phenotype in humans using ensemobl resources (*https://decipher.sanger.ac.uk/*); OMIM, mendelian inheritance in man (*http://omim.org/*); DGV, database of genomic variants (*http://dgv.tcag.ca/dgv/app/home*); UCSC, genome browser (*https://genome.ucsc.edu/*)

Table V. Possibly pathogenic variants of unknown significance.

Age/gender	Clinical features	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
19 yr/M	DD	Loss	19q12	32469826	32678763	208	None	No genes, within loci of OMIM phenotype of 19q13.3 microdeletion syndrome
10 yr/F	DD with autistic features	Loss	1q21.2	148905145	149348106	442	*LOC645166, LOC388692*	Loci for spinocerebellar ataxia, asperger syndrome
2 yr/M	Global DD	Loss	1q22	155065768	156347462	1281	*EFNA1, RAG1AP1, DPM3, KRTCAP2, TRIM46, MUC1, MIR92B, THBS3, MTX1, GBAP1, GBA, FAM189B, SCAMP3, CLK2, HCN3, PKLR, FDPS, C1orf104, RUSC1, ASH1L, MIR555, POU5F1P4, LOC645676, MSTO1, YY1AP1, DAP3, MSTO2P, GON4L, SYT11, RIT1, KIAA0907, SNORA42, SCARNA4, RXFP4, ARHGEF2, SSR2, UBQLN4, ROBLD3, RAB25, MEX3A, LMNA, SEMA4A, SLC25A44, PMF1, BGLAP, PAQR6, SMG5, TMEM79, C1orf85, VHLL, CCT3, C1orf182, RHBG*	2-3 entries in DECIPHER with MR, larger deletion, susceptibility loci for Asperger syndrome
		Loss	1q21.3	153966698	154916907	950	*NUP210L, TPM3, MIR190B, C1orf189, C1orf43, UBAP2L, HAX1, AQP10, ATP8B2, IL6R, SHE, TDRD10, UBE2Q1, CHRNB2, ADAR, KCNN3, PMVK, PBXIP1*	Genes for nocturnal frontal lobe epilepsy

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Age/Gender	Clinical features	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
		Loss	19q13.31	43292660	43499264	206	*LOC100289650, PSG10, PSG1, PSG6, PSG7*	OMIM phenotype of episodic ataxia, Loci for specific language impairment, benign infantile familial seizures, Autosomal recessive mental retardation type 7
		Gain	Xq27.1	138670515	139346041	675	*MCF2, ATP11C, MIR505, CXorf66*	Loci for familial susceptibility to migraine, spinocerebellar ataxia, Pettigrew syndrome (dandy walker malformation, seizures, basal ganglia disease) , X linked MR 11
42 yr/F	DD	Loss	2q11.2	97762137	98088225	326	*ANKRD36*	Loci for adult myoclonic epilepsy, autism susceptibility locus
		Gain	20q13.33	59101098	60163909	1062	*CDH4*	Loci associated with spinocerebellar ataxia, CDH4 gene implicated in brain segmentation and neuronal outgrowth.
8.5 yr/M	DD with gynecomastia	Gain	8p23.3	911085	1541761	630	*LOC286083, DLGAP2*	Loci for generalized epilepsy with febrile seizures, in DECIPHER-590 kb duplication, inherited variants also associated with MR and deafness
10 months/M	Global developmental delay	Gain	3p26.3	417661	841904	424	*CHL1*	Loci for 3p- syndrome and spinocerebellar ataxia, CHL1 gene - L1 gene family of neural cell adhesion molecules. It is a neural recognition molecule that may be involved in signal transduction pathways

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Age/Gender	Clinical features	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
8 yr/M	Global developmental delay with MRI brain showing neuronal migration disorder.	Gain	Xp22.31	7815148	8392712	577	*PNPLA4, MIR651, VCX2*	Multiple DECIPHER enteries, loci for X linked MR . Genes a ffect both the stability and translation of mRNAs.
		Gain	20q13.33	60634798	62917655	2282	*TAF4, LSM14B, PSMA7, SS18L1, GTPBP5, HRH3, OSBPL2, ADRM1, LAMA5, RPS21, CABLES2, C20orf151, GATA5, C20orf200, C20orf166, MIR1-1, MIR133A2, SLCO4A1, LOC100127888, NTSR1, C20orf20, OGFR, COL9A3, TCFL5, DPH3P1, DIDO1, C20orf11, SLC17A9, BHLHE23, LOC63930, NCRNA00029, 13LOC100144597, HAR1B, HAR1A, MIR124-3, YTHDF1, BIRC7, MIR3196, NKAIN4, FLJ16779, ARFGAP1, MIR4326, COL20A1, CHRNA4, KCNQ2, EEF1A2, PPDPF, PTK6, SRMS, C20orf195, PRIC285, GMEB2, STMN3, RTEL1, TNFRSF6B, ARFRP1, ZGPAT, LIME1, SLC2A4RG, ZBTB46, C20orf135, TPD52L2, DNAJC5, MIR941-1, MIR941-3, MIR941-2, UCKL1, MIR1914, MIR647, UCKL1-AS, ZNF512B, SAMD10, PRPF6, NCRNA00176, SOX18, TCEA2, RGS19, OPRL1, C20orf201, NPBWR2, MYT1, PCMTD2*	Multiple DECIPHER entries with associated with MR, many genes, associated with early infantile epileptic encephalopathy, familial seizures

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Age/Gender	Clinical features	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
5 yr/M	DD with behavioural abnormality	Gain	19p13.3	245465	2529993	2284	*PPAP2C, MIER2, THEG, C2CD4C, SHC2, ODF3L2, MADCAM1, C19orf20, CDC34, GZMM, BSG, HCN2, POLRMT, FGF22, RNF126, FSTL3, PRSSL1, PALM, C19orf21, PTBP1, LPPR3, MIR3187, AZU1, PRTN3, ELANE, CFD, MED16, C19orf22, KISS1R, ARID3A, WDR18, GRIN3B, C19orf6, CNN2, ABCA7, HMHA1, POLR2E, GPX4, SBNO2, STK11, C19orf26, ATP5D, MIDN, C19orf23, CIRBP, C19orf24, EFNA2, MUM1, NDUFS7, GAMT, DAZAP1, RPS15, APC2, C19orf25, PCSK4, REEP6, ADAMTSL5, PLK5P, MEX3D, MBD3, UQCR11, TCF3, ONECUT3, ATP8B3, REXO1, MIR1909, LOC100288123, KLF16, FAM108A1, SCAMP4, ADAT3, CSNK1G2, C19orf34, BTBD2, MKNK2, MOBKL2A, IZUMO4, AP3D1, DOT1L, PLEKHJ1, MIR1227, SF3A2, AMH, MIR4321, JSRP1, OAZ1, C19orf35, LINGO3, LSM7, SPPL2B, TMPRSS9, TIMM13, LMNB2, GADD45B, GNG7*	Loci associated with familial febrile seizures, Spinocerebellar ataxia 26, susceptibility to migraine, multiple entries in DECIPHER with MR

Contd...

Age/Gender	Clinical features	Type of CNV	Chromosome position	Start nucleotide	End nucleotide	Size of CNV (kb)	Genes (GRCh37/hg19 genome browser)	Remarks
2 yr/M	DD with microcephaly	Gain	19p13.3	3256520	4193494	936	*CELF5, NFIC, C19orf77, DOHH, FZR1, C19orf28, C19orf71, HMG20B, GIPC3, TBXA2R, C19orf29, PIP5K1C, TJP3, APBA3, MRPL54, RAX2, MATK, ZFR2, ATCAY, ITGB1BP3, DAPK3, MIR637, EEF2, SNORD37, PIAS4, ZBTB7A, MAP2K2, CREB3L3, SIRT6, ANKRD24*	ATCAY gene : Cayman type of cerebellar ataxia, hypotonia since birth, pshchomotor retardation, since birth. Autosomal recessive(omim)
23 yr/M	DD	Gain	16p11.2	33016127	33420084	403	*LOC729355, TP53TG3, LOC729355, TP53TG3*	Region of 16p11.2 duplication syndrome, autism susceptibility locus
1 yr/M	DD with facial dysmorphism	Gain	6q16.1	92296817	93008151	711	No genes	Spinocerebellar ataxia, schizophrenia susceptibility locus

DD, developmental delay; OMIM, mendelian inheritance in man (*http://omim.org/*); DECIPHER, database of genomic variation and phenotype in humans using ensemobl resources (*https://decipher.sanger.ac.uk/)*; MR, mental retardation; UCSC, genome browser (*https://genome.ucsc.edu/*)

Table VI. Recurrent benign copy number variants

Type of CNV	Chromosome position (GRCh37/hg19 genome browser)	Start nucleotide	End nucleotide	Size of the CNV (kb)	Number of patients having CNV
Gain	6q27	168879957	169369190	489	3
Gain	14q32.33	105466939	106033135	566	4
Loss	17q12	33357810	33658959	301	3
Gain	Xq21.3	90634737	91313584	678	4
Gain	Xq21.3	89241618	90168748	927	5

Source: *http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19*

our study and 63 vs 36% in their study). In the present study, of the 31 VUS, 27 CNVs had to be interpreted as either possibly pathogenic VUS or possibly VUS. The various reasons for these VUS can be different CMA platforms, unavailability of stringent guidelines for interpretation, wide variation in phenotype of a particular CNV, rapidly expanding databases of benign as well as pathogenic variants, genes of unknown function, non availability of family members for genetic testing and reduced penetrance of various pathogenic CNVs[3,10].

We reported five benign recurrent CNVs in our patients. The presence of these variants indicates towards the possibility of ethnic variation of benign variants. Also, there is some evidence that certain variants may predispose a particular population to abnormal phenotype and provide protection to other population[14,15].

The limitation of our study was small number of patients. Also parental CMA analysis could not be done in many cases with VUS, mainly because of unavailability of parents' samples. Initially *de novo* variants were thought to be more significant in terms of its pathogenicity and inherited benign variants were considered to be more benign. According to recent published literature[13], penetrance of such variants can range from 10-60 per cent. Girirajan *et al*[14] proposed two hit model for variability of phenotype in recurrent CNVs or for those inherited from either parent. We found 91-235 Mb regions of homozygosity in consanguineous families and 32-188 Mb region of homozygosity in 11.7 per cent of non-consanguineous families. Percentage of shared genome and patients with LOH regions were more than published literature. This may be due to inbreeding over many generations as there is custom of marrying amongst specific caste group. In a previous study, the detection rate of LOH regions was present in 4.2 per cent patients[14]. In that study, discrepancies between clinical documentation of parental consanguinity/illegal parental relationship were raised[14]. However, being at a clinical genetics centre we ourselves have taken detailed family history. Hence there is definite documentation of consanguinity.

In conclusion, this study of CMA from Indian patients with ID/DD with diagnostic yield of 20.9 per cent highlights the difficulty in interpretation of CNVs identified by CMA. Our study also highlights the importance of MLPA as an acceptable substitute of CMA for those families who cannot afford CMA due to cost constraints. There is a need for more Indian data about recurrent benign CNV in the population, as it will further help us in categorization of CNVs into benign vs VUS.

Acknowledgment

Authors thank the Indian Council of Medical Research for financial support, Dr Sameer Sawant from National Botanical Research Institute for allowing to use Microarray scanner at his centre and patients' families for their co-operation.

Conflicts of Interest: None.

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