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ARTICLE

Phenotypic manifestations of copy number variation in chromosome 16p13.11

Sandesh C Sreenath Nagamani[1,8], Ayelet Erez[1,8], Patricia Bader[2], Seema R Lalani[1], Daryl A Scott[1], Fernando Scaglia[1], Sharon E Plon[3], Chun-Hui Tsai[4], Tyler Reimschisel[5], Elizabeth Roeder[6], Amy D Malphrus[7], Patricia A Eng[1], Patricia M Hixson[1], Sung-Hae L Kang[1], Pawel Stankiewicz[1], Ankita Patel[1] and Sau Wai Cheung*[,1]

The widespread clinical utilization of array comparative genome hybridization, has led to the unraveling of many new copy number variations (CNVs). Although some of these CNVs are clearly pathogenic, the phenotypic consequences of others, such as those in 16p13.11 remain unclear. Whereas deletions of 16p13.11 have been associated with multiple congenital anomalies, the relevance of duplications of the region is still being debated. We report detailed clinical and molecular characterization of 10 patients with duplication and 4 patients with deletion of 16p13.11. We found that patients with duplication of the region have varied clinical features including behavioral abnormalities, cognitive impairment, congenital heart defects and skeletal manifestations, such as hypermobility, craniosynostosis and polydactyly. These features were incompletely penetrant. Patients with deletion of the region presented with microcephaly, developmental delay and behavioral abnormalities as previously described. The CNVs were of varying sizes and were likely mediated by non-allelic homologous recombination between low copy repeats. Our findings expand the repertoire of clinical features observed in patients with CNV in 16p13.11 and strengthen the hypothesis that this is a dosage sensitive region with clinical relevance.
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INTRODUCTION

Structural variation of the human genome results from genomic rearrangements including deletions, duplications, insertions and inversions. All of these genomic rearrangements, except for inversions, result in copy number variation (CNV) or deviation from the normal number of copies for a given genomic segment. The availability of genome–wide screening tools has unraveled the extent to which CNVs play a role in human genetic variation.[1,2] Although the widespread utilization of one such tool, array comparative genomic hybridization (aCGH) has lead to the discovery of many novel genomic disorders;[3–10] it has also revealed many CNVs whose clinical relevance is uncertain. The ascertainment of the clinical significance of CNVs is often complicated by marked clinical heterogeneity, incomplete penetrance and the identification of similar or identical rearrangements in 'seemingly' normal individuals.[11–14] One such CNV with a yet uncharacterized clinical phenotype is a rearrangement in chromosome 16p13.11.

Chromosome 16 is rich in intrachromosomal segmental duplications or low copy repeats (LCRs)[15,16] that mediate recurrent genomic rearrangements. Recurrent deletions and reciprocal duplications in 16p13.11 have been previously reported.[14,17] Whereas the deletions have been associated with epilepsy[18–20] multiple congenital anomalies and cognitive impairment,[14,17] duplications have been implicated in autism spectrum disorders, intellectual disability[10,17,21,22] and schizophrenia.[23,24] The phenotypes associated with CNVs of 16p13.11 are not consistent and both deletions and duplications of the region have been observed in 'phenotypically normal' individuals.[14,17] The inconsistencies in clinical presentations and the presence of the rearrangement in unaffected relatives may be due to factors, such as incomplete penetrance, variable expressivity, failure to recognize subtle manifestations or imprinting.[17] Owing to the widespread spectrum associated with CNVs of the region, it is imperative that further studies be undertaken to ascertain whether these CNVs are indeed causative of the phenotypes or happen to be found in patients with such phenotypes by mere coincidence.

Using a 'reverse' genomic approach,[25] we sought to further the knowledge of the possible phenotypic consequences of this CNV by comparing the observed clinical features in patients with known deletions and duplications of 16p13.11. The Medical Genetics Laboratories at Baylor College of Medicine (BCM) has performed over 14 000 aCGH for clinical evaluation of subjects with developmental delay, dysmorphic features and/or multiple congenital anomalies from June 2007 to January 2010. During this period, we identified 56 patients with duplication and 30 patients with deletion of 16p13.11. In this

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [2]Parkview Hospitals, Fort Wayne, IN, USA; [3]Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; [4]Division of Clinical Genetics, Children's Hospital, Aurora, CO, USA; [5]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN, USA; [6]Department of Pediatrics, University of Texas Health Science Center, San Antonio, TX, USA; [7]Division of Pediatric Neurology, Baylor College of Medicine, Houston, TX, USA
*Correspondence: Dr SW Cheung, Department of Molecular and Human Genetics, Baylor College of Medicine, 1 Baylor Plaza, NAB 2015, Houston, TX 77030, USA.
Tel: +1 713 798 6555; Fax: +1 713 798 3157; E-mail: scheung@bcm.tmc.edu
[8]These authors contributed equally to this work.
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cohort of patients, we were able to procure detailed clinical information on 10 patients with duplication and 4 patients with deletion of the region. In this report, we describe the molecular characterization and the clinical consequences of deletion and duplication of the region and compare our results with those presently available in the literature.

MATERIALS AND METHODS

Human subjects

Identification of 16p13.11 deletions and duplications was made by clinical diagnostic testing using aCGH. Clinical information was obtained from health-care providers using a checklist to standardize the data collection. The protocol approved by the institutional review board for human subjects' research at BCM.

FISH analysis

FISH analyses with bacterial artificial chromosome clones were performed using standard procedures. The BAC clone of interest was grown in TB media with 20 mg/ml chloramphenicol. DNA was extracted from BAC clones (Eppendorf Plasmid Mini Prep Kit, Hamburg, Germany) and directly labeled with SpectrumOrangeTM dUTP by nick translation (Vysis, Downer Grove, IL, USA) according to the manufacturer's instructions.

Array comparative genomic hybridization

We performed aCGH analysis on the clinical microarray platforms routinely used in our institution. The microarrays were designed in the Medical Genetics Laboratory of BCM. Most of the patient samples were initially interrogated using V8.OLIGO (180K), with the exception of patients 4, 5, 12 and 13 that were analyzed on V7.OLIGO (105K) array and patient 2 whose sample was run on V6.5OLIGO (44K) array. In order to provide consistent and detailed analyses of the breakpoint region, all the above-mentioned cases were reanalyzed on V8.OLIGO, a custom-designed array with approximately 180 000 (60 mer) interrogating oligonucleotides (oligos), manufactured by Agilent Technologies, Inc. (Santa Clara, CA, USA). This array contains the 'best-performing' oligos selected from Agilent's online library (eArray; https://earray.chem.agilent.com/earray/) and has been further optimized using empiric data. This array is designed to provide interrogation of all known microdeletion and microduplication syndrome regions as well as pericentromeric and subtelomeric regions as previously described.[26] In addition, 1784 genes either known to cause or hypothesized as candidate genes for various clinical phenotypes have exonic coverage with an average of 4.2 probes per exon as well as introns >10 kb. The entire genome is covered with an average resolution of 30 kb, excluding LCRs and other repetitive sequences. Further details are available at (https://www.bcm.edu/geneticlabs/). The procedures for DNA digestion, labeling, hybridization and data analysis, were performed as previously described.[27]

RESULTS

aCGH

The minimal size of the duplications ranged from 1.16 to 2.56 Mb while that of the deletions ranged from 0.81 to 1.13 Mb (Figure 1, Table 1). The ascertainment of exact breakpoints is complicated by the multiple LCRs in the region (Figure 2); however, the CNVs can be categorized into five categories: (1) a recurrent $\sim$1.3 Mb duplication and the reciprocal deletion with telomeric breakpoints between genomic coordinates 14 650 000 and 14 900 000 (hg18) and centromeric breakpoints between 16 200 000 and 16 800 000, (2) a recurrent $\sim$1.16 Mb duplication with telomeric breakpoints between 14 650 000 and 15 100 000 and centromeric breakpoints at $\sim$16 200 000, (3) a larger nonrecurrent duplication with breakpoints between 14 669 916–14 876 354 and 16 832 012–16 832099, (4) atypical duplications with proximal breakpoints centromeric to 17 500 000 and (5) a recurrent $\sim$1.13 deletion with breakpoints between 15 000 000–15 129 000 and 16 200 000–6 800 000 (Figure 1). All the deletions and duplications were confirmed by FISH analyses. In the case of deletions, two were *de novo* events, one was maternally inherited and parental testing could not be performed on the fourth family. Five of the duplications observed were maternally inherited; two were paternally inherited while the pattern of inheritance in the others could not be further investigated (Table 1).

Clinical features

Phenotype associated with duplication of 16p13.11. Patients with duplication of 16p13.11 had variable presentations that could be broadly categorized into four clinical patterns: (1) predominantly skeletal features with craniosynostosis, polydactyly and joint hypermobility, (2) cardiac and aortic malformations, (3) cognitive impairment and (4) behavioral abnormalities (Table 2). Four patients had the skeletal anomalies, isolated polydactyly, isolated craniosynostosis, polydactyly with craniosynostosis and dolicocephaly with arachnodactyly. Two patients presented with cardiovascular malformations involving the right and left ventricular outflow tracts. One of these patients presented with tetralogy of Fallot, whereas the other had transposition of great vessels with aortic coarctation. Five patients demonstrated developmental delay involving motor and language faculties. Six patients had varying behavioral abnormalities including attention deficit hyperactivity disorder, aggression and disruptive temperament. Two patients had difficulties in social interactions that were compatible with autistic spectrum disorders.

Phenotype associated with deletion of 16p13.11. All four patients with 16p13.11 deletion presented with developmental delay (Table 3). The developmental delay involved motor skills in one patient, predominantly language skills in two patients and the last patient had significant delays in motor and speech milestones as well as aggressive behavior, suicidal ideations and impairments in social interaction. Interestingly, three patients had microcephaly and one of them had polymicrogyria. Craniofacial dysmorphisms were mild with no characteristic facial gestalt. Other organ system involvement was generally absent excepting for the development of Wilms' tumor in one patient.

DISCUSSION

We describe 10 patients with duplication and 4 patients with deletion of 16p13.11. The sizes of the rearrangements were variable. As there was clustering of breakpoints in the regions of LCRs, it is likely that they were mediated by non-allelic homologous recombination (NAHR). Many genomic disorders mediated by NAHR such as Prader Willi[28] and Smith–Magenis syndrome,[29] have variable sizes of deletions because of recombination between alternate LCRs.

Patients with duplication of 16p13.11 in our series had variable phenotypes that included skeletal manifestations, or cardiac malformations in addition to the cognitive impairment and behavioral abnormalities that have been previously described.[14,17] With respect to neuro-cognitive phenotype, five patients had developmental delay while six patients had behavioral abnormalities, including attention deficit hyperactivity disorder, aggression and disruptive temperament. Two patients had difficulties in social interactions that were suggestive of autistic spectrum disorder. Duplication of 16p13.11 has been previously reported to be associated with learning difficulties, speech delay and behavioral abnormalities including hyperactivity.[14,17] As the duplication is present in 'phenotypically normal' parents of patients as well as in the general population, it was hypothesized that this could be a 'benign variant'.[14] However, the duplications of 16p13.11 are significantly enriched in children with cognitive impairment and accounted for $\sim$1% of cases in an unselected cohort of children

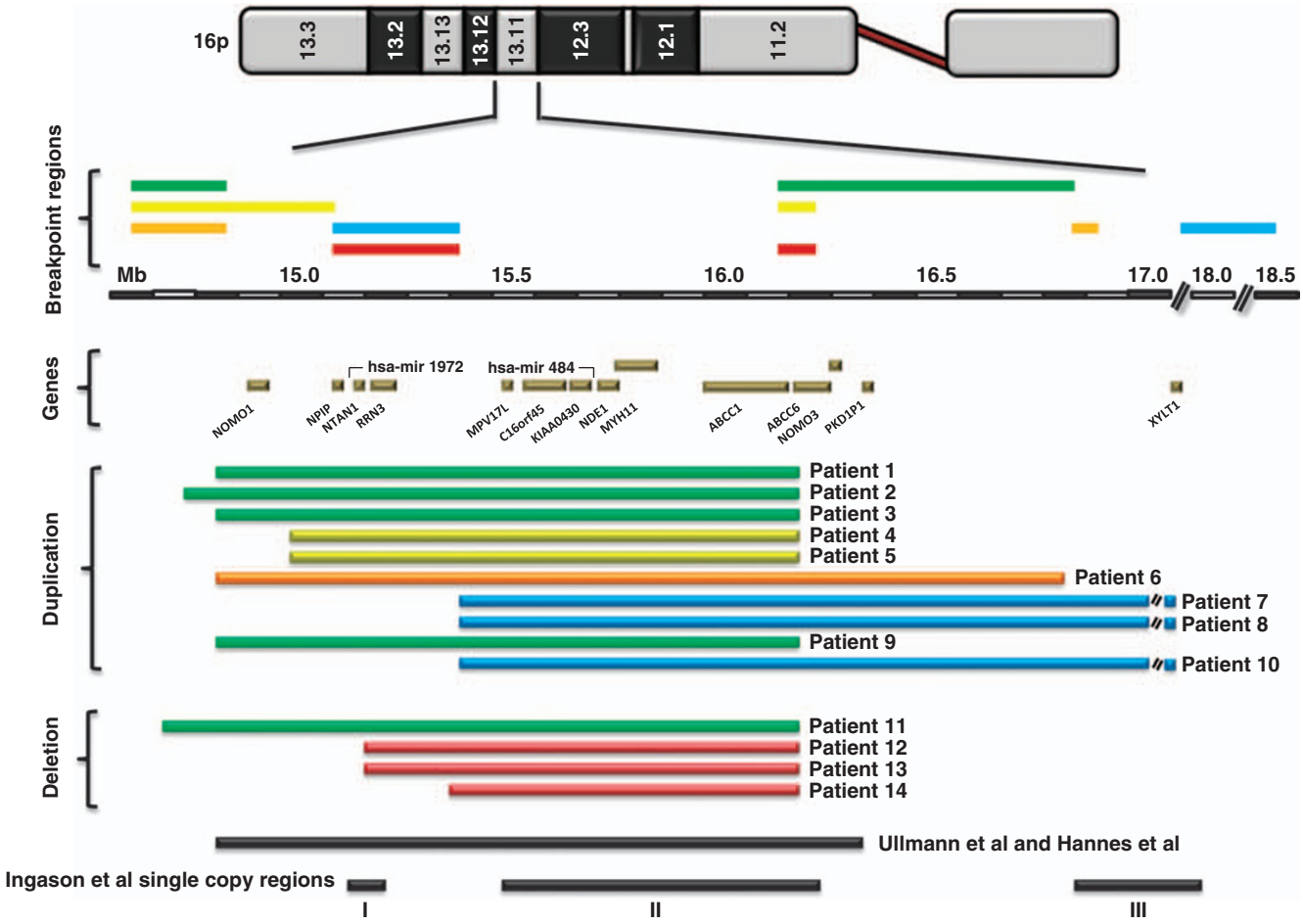

Figure 1 Size, extent and genomic content of deletions and duplications in our cohort of patients. The similar colored bars represent the minimal possible recurrent rearrangements likely mediated by the same LCRs/NAHR. The region is very rich in LCRs and these are not represented on the array. The possible breakpoint regions are represented in the upper panel. The previously mapped recurrent rearrangement by Ullmann *et al*[17] and Hannes *et al*[14], as well as the single copy regions devoid of repetitive elements termed intervals I, II and III by Ignason *et al* are shown at the bottom in black. Patients 1, 2, 3, 6 and 9 have duplication while patients 11–14 have deletion in the previously mapped recurrent rearrangement region. Patients 7, 8 and 10 have larger 'atypical duplications' encompassing intervals II and III.

Table 1 Molecular mapping of CNVs in chromosome 16p13.11

Pt	*CNV*	*Telomeric break-point*	*Centromeric break-point*	*Min size (Mb)*	*CNV interval*	*Genes encompassed by the CNV*	*Inheritance*
1	Dup	14 669 916–14 876 354	16 215 893–16 831 960	1.33	I, II	*NOMO1–>ABCC6*	Maternal
2	Dup	14 656 335–14 656 349	16 199 736–16 831 960	1.54	I, II	*NOMO1–>ABCC6*	Maternal
3	Dup	14 669 916–14 876 354	16 191 972–16 194 115	1.31	I, II	*NOMO1–>ABCC6*	Unknown
4	Dup	14 669 916–15 034 210	16 194 224–16 199 648	1.16	I, II	*MPV17L–>ABCC6*	Paternal
5	Dup	14 669 916–15 034 210	16 194 224–16 199 648	1.16	I, II	*MPV17L–>ABCC6*	Paternal
6	Dup	14 669 916–14 876 354	16 832 012–16 832 099	1.31	I, II, III	*NOMO1–>ABCC6*	No CNV in mother
7	Dup	15 129 615–15 402 121	17 963 057–18 516 134	2.56	I, II, III	*MPV17L–>XYLT1*	Maternal
8	Dup	15 129 615–15 402 121	17 963 057–18 516 134	2.56	I, II, III	*MPV17L–>XYLT1*	Maternal
9	Dup	14 669 916–14 876 354	16 191 529–16 191 611	1.31	I, II	*NOMO1–>ABCC6*	Maternal
10	Dup	15 129 615–15 402 121	17 583 505–17 962 996	2.18	I, II, III	*MPV17L–>XYLT1*	Unknown
11	Del	14 876 354–14 669 916	16 191 736–16 831 960	1.52	I, II	*NOMO1–>ABCC6*	Unknown
12	Del	15 129 615–15 129 615	16 199 736–16 831 960	1.16	I, II	*NTAN1–>ABCC6*	*De novo*
13	Del	15 034 269–15 062 188	16 199 736–16 831 665	1.13	I, II	*NTAN1–>ABCC6*	*De novo*
14	Del	15 129 615–15 402 121	16 215 893–16 831 960	0.81	I, II	*MPV17L–>ABCC6*	Maternal

Abbreviations: Del, deletion; CNV, copy number variation; Dup, duplication; Pt, patient.
The data represent the location of breakpoints and the minimal possible size of the CNV as assessed by V8.OLIGO.

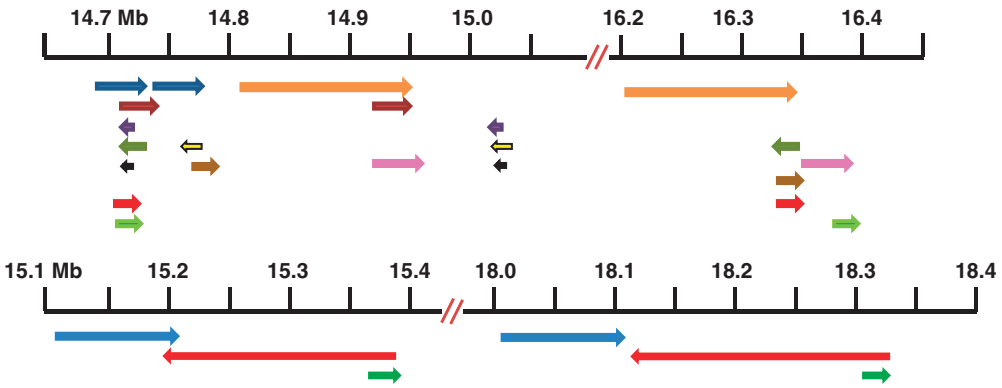

Figure 2 LCR structures in the proximal and distal breakpoint regions of the rearrangements in 16p13.11. Each of the colored arrows depict pairwise alignment with >98% homology. The upper panel depicts the LCRs that may have mediated the deletions in all four patients and the duplication in patients 1–6 and 9. Note that use of alternative LCRs can give rise to different size deletions. The lower panel depicts the LCRs in the breakpoint regions of the atypical duplications in patients 7, 8 and 10.

with unexplained intellectual impairment.[21] Interestingly, the duplications have also been associated with autism, cognitive impairment[17] and schizophrenia.[23,24,30] These data combined with the findings reported in this study suggest that the duplication of the region is indeed pathogenic though the penetrance is clearly incomplete. Although the small sample size and variable expressivity preclude the definitive correlation of the size and extent of the duplication with the neuro-cognitive phenotype, we observed that all patients with duplication extending centromeric to interval II (patients 6, 7, 8 and 10) had language or motor delays along with ADHD and behavioral abnormalities while only two of the five patients (patients 4 and 9) with proximal breakpoints within interval II presented with neuro-cognitive issues. This may be due to the fact that the other three patients (1, 2 and 3) with proximal breakpoints within interval II were younger than 2 years and the cognitive and language impairments may not have been apparent. The fact that region of 16p13.11 centromeric to interval II is gene poor and that significant neuropsychiatric manifestations have been previously documented with duplications involving intervals I and II imply that this is the critical region responsible for these features.[14,17,23] Ullman *et al*[17] had previously hypothesized that duplications that are paternally transmitted are benign while the maternal transmission leads to clinical manifestations. However, there are no known or predicted imprinted genes in the region (http://www.geneimprint.com/site/genes-by-species). Moreover, we observed clinical manifestations in patients with duplications inherited from both parents that imply imprinting does not significantly influence the phenotype.

There are ~14 known protein coding genes in the 16p13.11 region. Two genes that may be relevant to the neuro-cognitive phenotype are *NDE1* (*nudE nuclear distribution gene E homolog 1*) and *NTAN1* (*N-terminal asparagine amidase*). *NDE1* encodes for a protein that localizes to the centrosome and interacts with other centrosome components as part of a multiprotein complex including LIS1 that regulates dynein function. *Nde1* null mice have abnormal cerebral cortices and microcephaly.[31] *NTAN1* encodes for aspargine specific N-terminal amidase, and mice lacking this enzyme show abnormalities in spontaneous activity, spatial memory and a socially conditioned exploratory phenotype.[32] Although the loss of copy number in these genes has resulted in neurological manifestations in animal models, the phenotypic consequences of gain of copy number is still unclear. Thus, the role of duplication of these genes in behavioral and cognitive impairments is at best speculative. However, it is not unusual for reciprocal deletions and duplications to present with overlapping phenotypes. For example, there are many shared behavioral features between patients with Rett syndrome, Smith–Magenis syndrome, Williams–Bueren syndrome and their respective reciprocal gains, *MECP2* duplication, Potocki–Lupski syndrome, and 7q11.2 duplication. The overlap of neurodevelopmental and psychiatric phenotypes that results from either loss or gain of the same proteins or RNA molecules supports an emerging theme that normal cognition and behavior depend on tight neuronal homeostatic control mechanisms.[33]

In contrast to previous reports of neurological consequences of duplication, the skeletal features such as craniosynostosis, polydactyly, syndatyly and cardiac malformations observed in our patients have not been reported. There are no genes in the duplicated interval with a known role in cardiac or musculoskeletal development. The reason for the observed cardiac and skeletal features may be due to the involvement of regulatory elements, including two known microRNAs (hsa-mir 1972 and hsa-mir 484), epistatic influences or interactions with other gene modifiers.

Patients with deletion of 16p13.11 presented with varying degrees of developmental delay, behavioral abnormalities and impairments in social interaction (Table 3). The variable expressivity of neuropsychiatric manifestations because of microdeletion at a locus may at least in part be explained by the presence of second rearrangement elsewhere in the genome. Girirajan *et al*[34] recently demonstrated that patients with deletions of 16p12.1 were more likely to carry additional large CNV when compared with matched controls and proposed a 'two-hit' model in which the 16p12.1 microdeletion as a single event predisposes to a neuropsychiatric event and exacerbates neurodevelopmental phenotypes in association with other large deletions or duplications. However, we did not detect any additional large CNV in any of our patients.

Microcephaly was observed in two patients as previously described.[14] As homozygous loss of *Nde1* leads to altered neuronal migration in animal models, it is possible that haploinsufficiency of *NDE1* contributes to the development of microcephaly in 16p13.11 deletion patients.

One of the patients in our cohort had seizures. Microdeletions of 16p13.11 is the most prevalent single genetic risk factor for overall seizure susceptibility identified to date[18,19] and haploinsufficiency of some of the genes in the deleted interval has been hypothesized as the causative factor.

In conclusion, our report furthers the knowledge of the phenotypic consequences of CNV of 16p13.11. While the duplications are

Table 2 Clinical features of patients with duplication of 16p13.11

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*	*Patient 6*	*Patient 7*	*Patient 8*	*Patient 9*	*Patient 10*
Age at evaluation	1 year 3 months	1 year 5 months	5 months	9 years	7 months	6 years	7 years 4 months	10 years 8 months	21 months	7 years 8 months
Sex	F	M	F	M	M	M	F	F	F	M
Reason for referral	Craniosynostosis, polydactyly	Polydactyly	CHD	Tall stature, ADHD, PDD	CHD	ADHD, autistic spectrum disorder, CI	CI , ADHD	PDD, CI, ADHD	Craniosynostosis, CI	Language delay, ADHD
Duplication interval	I+II	I+II	I+II	I+II	I+II	I+II	I+II+III	I+II+III	I+II	I+II+III
Head circumference (centile)	NA	75	NA	98	10	NA	50	50	2	50
Weight (centile)	<3	75	4	90	25	25	NA	NA	50	50
Height (centile)	<3	75	NA	>95	50	56	95	94	50	50
Dysmorphic features										
Epicanthic folds	+	+	NA	–	–	–	+	+	+	–
Low set ears	+	+	NA	–	–	–	–	+	+	–
Posterior rotated ears	+	+	NA	–	–	–	–	–	+	+
Nasal abnormalities	–	–	–	–	+	–	–	–	+	+
Mouth and palate							High arched palate	+	+	+
Cardiovascular system	–	–	TOF	–	TGA, VSD, ASD, aortic coarctation	–	–	–	–	–
Nervous system										
Speech delay	–	–	–	–	–	+	+	+	+	+
Motor delay	–	–	–	–	–	+	+	+	–	
Abnormal behavior	–	–	–	+	–	Very hyperactive	ADHD	ADHD, aggressive and disruptive	–	ADHD
Seizures	–	–	–	–	–	–	–	–	–	–
Hypotonia	–	–	–	–	–	–	–	–	–	–
Hypertonia	–	–	–	–	+	–	–	–	–	–
Structural brain abnormalities	–	–	–	–	–	–	NA	NA	NA	–
Hearing loss	–	–	NA	–	–	–	–	–		–
Skeletal system										
Hypermobility	–	–	NA	+	–	–	+	+	–	+
Craniosynostosis	Sagittal and metopic	–	NA	–	–	–	–	–	Metopic	–
Polydactyly/syndactyly	+	+	NA	–	–	–	–	–	+	–
Others	–	–	NA	Arachnodactyly	–	–	Pes Planus	Pes Planus	–	Pectus excavatum
Other organ system involvement	–	–	NA	–	Vocal cord palsy	–	NA	NA	–	–

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, atrial septal defect; CHD, congenital heart disease; CI, cognitive impairment; PDD, pervasive developmental delay; TGA, transposition of great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
+ Denotes the presence of a characteristic, – denotes its absence and NA implies datum was not available.

Table 3 Clinical features of patients with deletion of 16p13.11

	Patient 11	*Patient 12*	*Patient 13*	*Patient 14*	*Ullmann* et al	*Hannes* et al
Age at evaluation	9 months	4 years 10 months	19 months	9 years		
Sex	F					
Reason for referral	DD, WT	DD	DD, seizures	DD, hearing loss		
Deleted interval	I+II	I+II	I+II	I+II		
Head circumference (centile)	5	<3	97	<3	>90	Microcephaly
Weight (centile)	5	<3	74	50		
Height (centile)	NA	25	63	25		
Dysmorphic features	–	–	NA	+	+	+
Cardiovascular system	–	–	–	–	+	+
Nervous system						
Speech delay	–	+	+	+	+	+
Motor delay	+	–	–	+	+	+
Abnormal behavior	–	–	–	+	+	+
Seizures	–	–	–	–	NA	+
Hypotonia	+	–	–	+	+	+
Hypertonia	–	–	–	–		+
Structural brain abnormalities	–	–	Polymicrogyria		–	+
Hearing loss	–	NA	NA	+	+	NA
Skeletal system						
Hypermobility	NA	–	NA	+	+	NA
Craniosynostosis	NA	–	NA	–	–	NA
Polydactyly	NA	–	NA	–	–	NA
Others						Pectus excavatum
Other organ system involvement	WT	–	NA	Strabismus	–	Cleft palate, cleft lip, hydrocephalus

Abbreviations: DD, developmental delay; + denotes the presence of a characteristic; – denotes its absence; NA implies datum was not available.
The findings in our cohort have been compared with three previous studies that reported deletions of the region.

associated with cognitive impairment, behavioral abnormalities and possibly skeletal and cardiac manifestations, deletions manifest with microcephaly, cognitive impairment and seizures. The clinical spectrum associated with both duplications and deletions are quite variable and the manifestations are incompletely penetrant making genetic counseling of such families a challenging prospect.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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