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RESEARCH NOTE

Chromosome 2q14.3 microdeletion encompassing *CNTNAP5* gene in a patient carrying a complex chromosomal rearrangement

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Abstract. We report a patient with loss of chromosome region 2q14.3 encompassing exon 1 of the gene *CNTNAP5*. The deletion occurred in association with a *de novo* complex chromosomal rearrangement, characterized by routine G-banding, fluorescence *in situ* hybridization and microarray analysis. The presented patient's phenotype is dominated by severe early childhood weight gain, severe speech delay and behavioural problems. To our knowledge, a few similar patients have been reported previously. *CNTNAP5* is a member of the neurexin gene family and is associated with autism spectrum disorder and potentially other behavioural and neurodevelopmental disorders. Recent data point to its possible role in obesity and/or metabolism. The phenotype of the herein presented pediatric patient corroborates *CNTNAP5*'s pathogenic role in human disease.

Keywords. *CNTNAP5* gene; chromosome 2q14.3; complex chromosomal rearrangement.

Introduction

Contactin-associated protein 5 (*CNTNAP5*) is a member of the neurexin gene family along with closely related genes *CNTNAP1-4*. This protein family is involved in functions of central nervous system such as excitation, conduction, cell adhesion and communication, and the formation of myelinated axons (Zou *et al.* 2017). Members of the gene family are associated with epilepsy, schizophrenia, Alzheimer's disease, multiple sclerosis, autoimmune encephalitis and autism spectrum disorder (ASD) (Pagnamenta *et al.* 2010; Zou *et al.* 2017). Very little is known about *CNTNAP5* specifically. Genomewide association studies have linked it to antipsychotic response (Allen and Bishop 2019), major depressive disorder (Zhang *et al.* 2020), and posterior cortical atrophy (Schott *et al.* 2016). In mice, null mutation of a *CNTNAP5* orthologue is associated with perinatal lethality, while heteroyzgosity does not seem to confer any abnormal phenotype (Weichenhan *et al.* 2008). A few cases of *CNTNAP5* deletion have been reported previously, and here we report a further individual with a comparable phenotype carrying a deletion of chromosome 2q14.3 encompassing exon 1 of *CNTNAP5*. The deletion in the present patient occurred in association with a *de novo* complex chromosomal rearrangement (CCR). *De novo* CCRs are associated with a variety of health issues, including intellectual disability (ID), developmental delay (DD), infertility/recurrent miscarriages, and multiple congenital anomalies (Zhang *et al.* 2009; Poot and Haaf 2015). While the presence of this complex abnormality confounds genotype–phenotype correlation, we discuss the plausible pathogenic effect the deletion involving *CNTNAP5* could have had in the case of the presented patient.

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Clinical report

The proband is a 6-year-old male child of a nonconsanguineous Caucasian couple who was referred to genetic counselling due to Prader–Willi-like phenotype at four years of age, when he weighed 29 kg (>99th age and sex matched percentile, +5.2 SD), his height was 106 cm (90–97th percentile) and his head circumference was 52 cm (75–90th percentile). His birth parameters were within normal ranges (2750 g weight, 46 cm length). As an infant, he had muscular hypotonia and feeding difficulties. His motor development was slow, but steady, while his speech development showed severe delay. At three years of age, he underwent endocrinologic and cardiologic evaluations due to tachycardia and sudden weight gain, which found no underlying organic cause. During early childhood he also displayed polyphagia, which is however no longer part of his phenotype. Neuropsychiatric exploration determined his IQ to be 72, and revealed more severely delayed expressive speech compared to receptive speech. His vocabulary currently consists of 5–10 words. He shows mild hyperactivity, his attention span and movement coordination are poor and has recurrent hand flapping. Brain MRI, EEG, BAEP and BERA tests showed normal results. Family history is negative for congenital anomalies; the mother's twin sister has borderline personality disorder. The father had been diagnosed with melanoma and was receiving interferon treatment at the time of conception.

Patient and parental karyotypes were determined by analysis of 20-20 Giemsa-stained metaphases from standard 72-h peripheral blood lymphocyte cultures. The G-banded karyogram (figure 1a) of the child revealed a CCR involving eight breakpoints of chromosomes 1, 2, 4, 5, 10 and 17. To elucidate the CCR, a 24XCyte multicolour FISH (mFISH) probe kit (MetaSystems GmBH, Altlussheim, Germany) was applied according to the manufacturers' guidelines. This should have been the optimal method, however, due to technical issues the quality of the mFISH proved to be limited. We followed it by applying whole chromosome paint (WCP) probes for all the affected chromosomes (see table 1 in electronic supplementary material), which confirmed their involvement. In the next step, the relatively small resolution of G-banding and WCP FISH probes prompted us to verify each rearrangement by additional locus specific FISH probes (all used probes are listed in table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). The rearrangements were deduced by thorough examination of the G-banded karyotype (figure 1a) and all of the FISH images combined.

The above mentioned multiple FISH (summarized in figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/) and M-FISH (figure 2 in electronic supplementary material at http://www.ias.ac.in/jgenet/) analyses revealed (i) a reciprocal translocation between chromosomes 1 and 2, t(1;2)(p22;q14.3); and (ii) a six-break rearrangement involving chromosome regions 4q21.1, 5p15.3, 10p11.2, 10q24.1, 17p13.2 and 17q25.2 (of which only the translocation between 10p11.2 and 17p13.2 is reciprocal). The CCR is shown on figure 1b.

Array comparative genomic hybridization (array CGH) was then completed on the Affymetrix/Applied Biosystems CytoScan HD microarray system (Thermo Fisher Scientific, Waltham, USA) and analysed using the chromosome analysis suite (ChAS; ThermoFisher Scientific, Applied Biosystems) software package. Array CGH unveiled a 1.424 Mb large deletion of chromosome 2q14.3 which likely originated from the reciprocal t(1;2) translocation (figure 3a in electronic supplementary material). The deletion is flanked by base pairs 123,431,180-124,854,926 [GRCh37/hg19] and involves exon 1 of *CNTNAP5* gene (NM_130773). Array result was confirmed by FISH (figure 3b in electronic supplementary material) using a 138.6 kb custom design single probe (targeting region chr2:124,711,463-124,850,067; Agilent Technologies, Santa Clara, USA); and was apparently *de novo*, as the parents were proven to have normal karyotypes and normal FISH patterns with the *CNTNAP5* gene specific probe.

Result and discussion

Single gene variants of *CNTNAP5* listed in ClinVar are classified as variants of unknown significance (VUS). Recently, the gene has also been implicated in metabolism/obesity. In a pilot study, whole blood gene expression profiles in 11 obese subjects with type 2 diabetes mellitus (T2DM) were examined before and after bariatric surgery, 200 genes with significantly altered expression were identified. *CNTNAP5* expression was found to be significantly correlated with changes in HbA1c content following surgery (Berisha *et al.* 2011). Several animal GWAS studies have linked the gene with morphological traits (Rahmatalla *et al.* 2018; Chen *et al.* 2020). One study found that an SNP of the chromosome 2 region harbouring *CNTNAP5* was significantly associated with increase in bicostal diameter in goats. As the gene product contains epidermal growth factor repeats, the authors suggested that *CNTNAP5* might participate in the central regulation of growth; similarly to the brain derived neurotropic factor (BDNF), which has been shown to have an effect on body growth (Rahmatalla *et al.* 2018).

A study linking *CNTNAP5* with ASD involved a family where the index patients (two brothers) had ASD and reading impairment, their sister and mother had isolated reading impairment, and their father showed autistic traits. Two CNVs were discovered in the family: a microdeletion of chromosome 7 leading to a *DOCK4–IMMP2L* fusion transcript, was present in the mother, and was transmitted to all three of her children. A second microdeletion of chromosome 2q14.3 was discovered in the two ASD boys and their father, but not in their dyslexic sister. The latter deletion caused the loss of exons 4–11 of *CNTNAP5* leading to

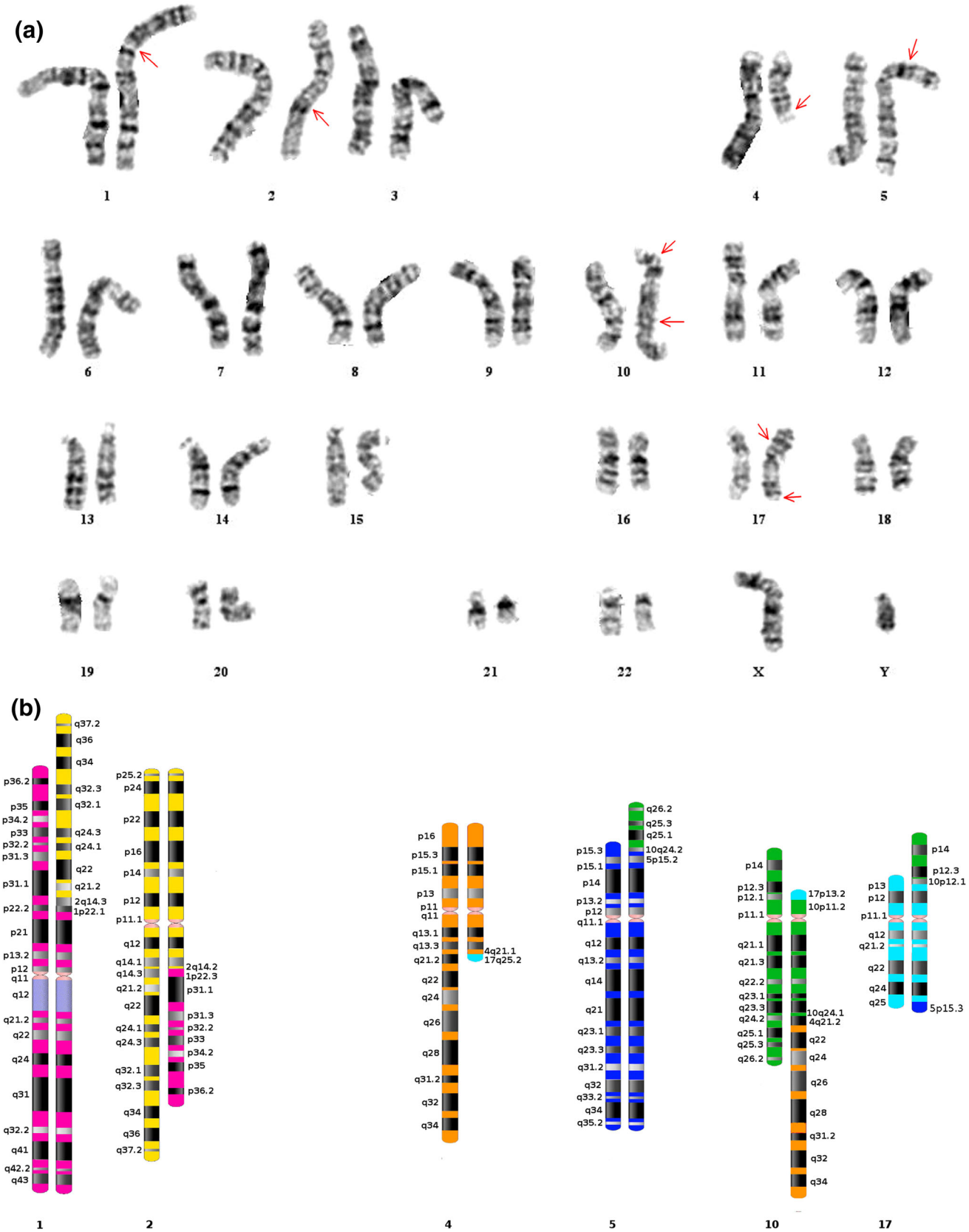

Figure 1. Karyogram and schematic representation of the complex chromosomal rearrangement. (a) The proband's karyogram. Red arrows point to the breakpoints involved in the rearrangements. (b) Ideograms representing the reciprocal translocation between chromosomes 1 and 2; and the complex rearrangement (chromosomes 4, 5, 10 and 17).

Table 1. Reported patients with 2q14.3 deletions.

Patient	Reference genome	Start breakpoint	End breakpoint	*CNTNAP5* deletion	Size (Mb)	Other CNV/CCR	Growth	NDD
Proband	hg19	123,431,180	124,854,926	Exon 1	1.424	CCR	Obesity	Language DD, short attention span, hyperactvity
Patients with overlapping deletions								
Ballarati	hg18 (hg19)*	123,795,826 (124,079,356)	126,817,471 (127,101,001)	Entire gene	3.022	CCR	Childhood growth parameters normal, adult BMI 30.1 kg/m^2	Global DD, ID, echolalia, behavioral perseverations
DECIPHER 265900	hg19	123,973,518	124,574,649	Not involved	0.601	7p11.2 microdup 15q25.1 microdup	No phenotypic information	
DECIPHER 331373	hg19	124,194,249	124,430,468	Not involved	0.236	15q26.3 microdel (*IGF1R* gene)	Short stature	Not listed
DECIPHER 356381	hg19	121,394,787	124,203,637	Not involved	2.809	None	IUGR	Speech and language DD
Other patients with a deletion encompassing *CNTNAP5* gene								
Pagnamenta	hg18 (hg19)*	124,836,663 (125,120,193)	125,063,827 (125,347,357)	Exons 4–11	0.227	7q microdel	Proband: birth weight 4200 g Brother: birth weight 3950 g	Proband: ASD, language DD Brother: ASD, language DD, stereotypical hand and finger movements, dyslexia
Ludington	hg19	125,204,264	126,042,867	Exons 6–24	0.838	None	Normal parameters	Microcephaly, thin corpus callosum, ID
DECIPHER 1563	hg19	125,177,191	129,483,105	Exons 5–24	4.306	None	Not listed	ID, ASD, short attention span, hyperactivity
DECIPHER 259675	hg19	125,362,271	125,476,133	Exons 12–13	0.114	None	No phenotypic information	
DECIPHER 274695	hg19	125,175,897	126,960,035	Exons 5–24	1.784	2q14.3 microdel 15q13.3 microdup	Not listed	ID, short attention span, seizures
DECIPHER 373490	hg19	125,572,166	127,083,103	Exons 20–24	1.511	None	No phenotypic information	

Mb, megabase; CNV, copy number variation; CCR, complex chromosomal rearrangement; NDD, neurodevelopmental disorders; DD, developmental delay; ID, intellectual disability; IUGR, intrauterine growth restriction; ASD, autism spectrum disorder; *genome coordinates were converted using the 'LiftOver' tool of the UCSC Genome Browser (http://genome.ucsc.edu/).

transcripts that were predicted to result in a frameshift leading to a premature stop codon. Exons and intron–exon boundaries of *CNTNAP5* were subsequently sequenced in 143 ASD family probands and two nonsynonymous variations, cosegregating with ASD and absent in control subjects, were found. One variation (V1168I) was also detected in a further ASD cohort. The authors concluded that the two CNVs acted in tandem to create the phenotypic traits of the index family: exonic deletions of *DOCK4* were suggested to be aetiological factors in dyslexia, meanwhile rare variants of *CNTNAP5* were proposed as potential ASD risk factors (Pagnamenta *et al.* 2010).

To our knowledge, two cases with 2q14.3 deletion involving *CNTNAP5* gene have been described in the literature previously (Ballarati *et al.* 2009; Ludington *et al.* 2020). The Database of Genomic Variation and Phenotype in Humans using Ensembl Resources (DECIPHER) (Firth *et al.* 2009) lists three additional deletions (filtered by size: under 5 Mb) that overlap with the presented patient's deletion, and four further deletions (under 5 Mb) that encompass *CNTNAP5* (figure 3c in electronic supplementary material). The patients have overlapping neurodevelopmental and behavioural phenotypes: poor attention span, stereotypies, speech delay, intellectual disability (table 1). Of the cases with detailed phenotypic information, one further instance of overweight has been reported. In contrast to the presented Hungarian boy, who had Prader–Willi-like weight gain as a toddler, the patient reported by Ballarati *et al.* had normal growth parameters until adulthood, and his obesity is comparatively less severe (Ballarati *et al.* 2009). Considering the previously mentioned pilot studies that suggest a role for *CNTNAP5* in diabetes and body growth, further research in this area has potential value for human disease. Increased weight monitoring might be advisable for 2q13.4 deletion patients.

Interestingly, the Ballarati *et al.* patient carried the *CNTNAP5* deletion in association with a CCR as well. His karyotype involved a reciprocal translocation between chromosomes 1 and 15, an insertion of a chromosome 2 segment into chromosome 1, and three submicroscopic deletions on chromosome 2. The 2q14.3 deletion (chr2:123,795,826-126,817,471x1) is found near the proximal insertion breakpoint and is ∼3 Mb large. Given that *CNTNAP2*, another member of the neurexin gene family, is associated with delay in the age at first words in ASD patients (Alarcon *et al.* 2008), the authors speculated that *CNTNAP5* deletion may be an aetiological factor in the delay of first spoken words in their patient (Ballarati *et al.* 2009).

In both the patient presented here, the patient discussed by Ballarati et al (2009), as well as other patients carrying CCRs, exact genotype–phenotype correlation poses a considerable challenge. Studies investigating the molecular signature of CCRs suggest that these complex events are likely mediated by microhomology-mediated replication-based mechanisms: Fork stalling and template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR) (Pellestor *et al.* 2011). However, cases with no identifiable microhomologies point to the possibility of other mechanisms (for example serial nonhomologous end joining) also being involved in CCR formation (Zhang *et al.* 2009). Chromothripsis has also been proposed as a possible alternative (Poot and Haaf 2015). Many CCRs (∼70%) are found in healthy individuals, but when they do confer a phenotype, the underlying pathogenic effect can be attributed to several factors: breakpoints can affect one or multiple genes leading to gene dosage-dependent changes; CCRs can disrupt the greater genomic architecture, which can alter gene expression through different pathways; and they can result in mixed mutation mechanisms, e.g., recessive pathogenic variant unmasking, etc. (Zhang *et al.* 2009; Pellestor et al. 2011; Poot and Haaf 2015). Due to this complexity, further assessment of the presented patient's CCR would require extensive mapping of the breakpoints at the nucleotide level, and even then, genotype–phenotype correlation would still be confounded by the need to separately evaluate each breakpoint, as well as their potentially additive consequences.

In summary, we present a boy with a deletion of 2q14.3, whose clinical presentation seems to corroborate previous suppositions regarding this chromosome region and *CNTNAP5* gene. While the majority of his symptoms seem to be attributable to his deletion and/or the haploinsufficiency of *CNTNAP5*, we cannot disregard the possible pathogenic/modifying effect of his CCR as a whole. Further clinical and molecular genetic studies and the establishment of comprehensive, CCR-specific databases in the near future should facilitate the complex interpretation and genetic counselling of CCRs.

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