An Interstitial 17q11.2 de novo Deletion Involving the *CDK5R1* Gene in a High-Functioning Autistic Patient

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Established Facts

- The *CDK5R1* gene has a pivotal role in neurodevelopment and postnatally in spatial learning and long-term plasticity.
- *CDK5R1* has been proposed as a candidate gene for intellectual disability in the *NF1* microdeletion syndrome and in idiopathic mental retardation.
- However, in vitro functional studies on *CDK5R1* gene variants have shown controversial results relative to a possible role of *CDK5R1* in intellectual disability.

Novel Insights

- This is the first report that associates a *CDK5R1* heterozygous deletion with autism spectrum disorder.
- The reported genotype-phenotype association in our patient is strongly supported by the CreER-p35 conditional knockout mouse model and by expression data.
- Our data show for the first time that loss of one copy of the *CDK5R1* gene is not correlated with intellectual disability.

Keywords

CDK5R1 · Haploinsufficiency · High-functioning autism · Intellectual disability

Abstract

We describe a 32-year-old male patient diagnosed with high-functioning autism spectrum disorder carrying a de novo 196-kb interstitial deletion at chromosome 17q11.2. The deletion was detected by array CGH (180K Agilent) and

confirmed by quantitative PCR on genomic DNA. The deleted region spans the entire *PSMD11* and *CDK5R1* genes and partially the *MYO1D* gene. The *CDK5R1* gene encodes for a regulatory subunit of the cyclin-dependent kinase 5 responsible for its brain-specific activation. This gene has been previously associated with intellectual disability in humans. A reduction in *CDK5R1* transcript was detected, consistent with the genomic deletion. Based on the functional role of *CDK5R1*, this gene appears as the best candidate to explain the clinical phenotype of our patient, whose neuropsychological profile has more resemblance with some of the higher brain function anomalies recently described in the CreER-p35 conditional knockout mouse model than previously described patients with intellectual disability.

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Autism spectrum disorder (ASD) is a genetically heterogeneous neurodevelopmental condition characterized by repetitive and stereotyped behaviors, restricted interests, and social and communication deficits [American Psychiatric Association, 2013]. Its clinical phenotype is extremely variable, ranging from lack of expressive language in comorbidity with severe intellectual disability, to social deficits and narrowed interests in high-functioning individuals with fluent expressive language and normal IQ. The majority of ASD forms are currently considered a multifactorial disorder with a strong genetic component, in which rare and common variants with variable levels of penetrance interact among themselves and with environmental factors [Persico and Napolioni, 2013]. New genome-wide technologies, such as CGH and SNP arrays as well as NGS, have allowed the identification of many candidate ASD genes in large cohorts. The Simons Foundation website (<https://www.simonsfoundation.org>), one of the most exhaustive publicly available web resources concerning autism genetic research, currently lists 813 autism candidate genes ranked into 7 main categories based on different levels of evidence, supporting the role of each gene in autism pathogenesis. We hereby report on a young man diagnosed with high-functioning ASD due to social skill deficits and anxiety, who underwent a thorough medical assessment including genetic testing.

Clinical Report

A 32-year-old man was referred to our department due to social skills deficit and an anxiety disorder. He is the first child of healthy unrelated parents and was born at 42 weeks of gestation by normal

Table 1. Neuropsychological test results

Cognitive test	Score or level of performance
WAIS-IV	
Verbal IQ	94 (normal)
Visuo-perceptive reasoning IQ	121 (normal)
Working memory	77 (poor)
Visuo-motor speed	86 (normal)
Full scale IQ	95 (normal)
Executive functions	
Trail making test	Normal
Wisconsin card sorting test/set-shifting ability	Normal
Tower of London	Normal
Elithorn perceptual maze test	Normal
Phonological fluency	Poor
Memory	
Short-term verbal memory	Poor
Verbal episodic memory	Normal
Verbal learning	Normal
Visuo-spatial memory	Normal
Attention	
Sustained visual attention	Normal
Visuo-constructional abilities	
Rey complex figure	Normal
Arithmetical abilities	
Mental calculus	Normal
Mathematical reasoning	Normal
ADOS-2	
Language and communication	3
Social interaction	9

ADOS-2, Autism Diagnostic Observation Schedule; WAIS, Wechsler Adult Intelligence Scale.

delivery. Birthweight was 4,200 g. He was exclusively breastfed for 3 months; weaning was accomplished around the fourth month without major problems. Developmental milestones and physical growth were achieved normally. Babbling started at 6 months. Expressive language for single words started at 14 months and first sentences at 20 months. At about 18 months, psychomotor clumsiness was noted for the first time. The child fell down frequently while running; ocular motor deficits, balance problems, and difficulties going up and down the stairs were also reported. Behavioral disturbances including a tendency to isolate from his peers, poor communication, and separation anxiety from his mother were first noted when he started attending kindergarten. As a child, he would tend to obsessively play the same games over and over again. Pain hyposensitivity was also reported. During elementary school, separation anxiety was still present. Difficulties in homework performance, comprehension, and visual-motor coordination in handwriting as well as attention deficit and oppositional traits were observed by parents and teachers. Repetitive and stereotyped patterns of behavior (body and leg sway, toe running, opening and closing his fists), restricted interests and activities were first noted when he was a child and persisted into adulthood. He also displayed emotional self-regulation deficits with psychomotor agitation when frustrated. As an adult, he has achieved a satisfactory level of personal autonomy: he lives with his parents and works as a warehouseman. His EEG was normal, and no dys-

In addition, long-term visual memory was normal (Rey complex figure and Benton visual retention test). No deficits were found for arithmetic skills or in mental calculus. The patient was clinically diagnosed with a high-functioning ASD due to lack of social and emotional reciprocity, poor social abilities, impairment in spontaneous nonverbal communication, deficits in eye contact, poor facial expression, and reduced mimicry [confirmed by Autism Diagnostic Observation Schedule (ADOS-2) testing].

Materials and Methods

Peripheral blood was drawn from the patient and his parents into EDTA-containing tubes. Genomic DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA), and array CGH was performed using the SurePrint G3 Human CGH 4x180K Microarray kit (Agilent Technologies, Santa Clara, CA, USA). The deletion was confirmed by quantitative real-time PCR (qPCR) using Sybr Green methodology targeting an intronic region of the *CDK5R1* gene and normalized with RNase P gene. Real time experiments were performed using the 7900HT Fast System (Applied Biosystems, Foster City, CA, USA). RNA was extracted from blood drawn into Tempus tubes, using the TempusTM Spin RNA isolation reagent kit (P/N 4378926, Applied Biosystems) and retrotranscribed by the High Capacity RNA-to-cDNA kit (P/A 4387406, Applied Biosystems). RNA was extracted from peripheral blood mononuclear cells (PBMCs), retrotranscribed into cDNA and used to quantify *CDK5R1* mRNA levels by qPCR using Sybr Green methodology. The amount of mRNA was calculated using the $2^{-\Delta\Delta C_t}$ method and expression values were normalized to the internal control gene *GAPDH*. The calibrator sample included mRNA from 3 healthy human controls matched by sex and age. Primer sequences are available on request. Statistical comparisons were made using unpaired Student *t* test. *p* values of 0.01 or less were considered to represent a statistically significant difference.

Results

A 17q11.2 de novo deletion spanning approximately 196 kb was detected from oligomer A_16_P03233537 (hg19, 30,750,126 bp, first deleted) to oligomer A_16_P40794889 (30,946,150 bp, last deleted) (Fig. 1A, B). It involves the whole *PSMD11* (OMIM 604449) and *CDK5R1* (OMIM 603460) genes as well as the last 2 exons of the *MYO1D* gene (OMIM 606539) (Fig. 1A, B). The *PSMD11* gene encodes for a subunit of the PA700 complex, which belongs to the 26S proteasome involved in the degradation of cellular-ubiquitinated proteins [Vilchez et al., 2012]. The *MYO1D* gene encodes for an unconventional myosin involved in left-right patterning of visceral organs [Nagase et al., 1998]. The *CDK5R1* gene is specifically expressed in the central nervous system where it activates the cyclin-dependent kinase 5 encoded by the

CDK5 gene (OMIM 123831). Consistent with the loss of one allele, a decrease in *CDK5R1* mRNA level was detected by RT-qPCR in the proband's PBMCs (Fig. 1C) when compared to typically developing male control subjects of similar age. DECIPHER (<http://decipher.sanger.ac.uk/>) reports 10 patients with chromosome 17q11.2 deletions partially or fully overlapping the one detected in our proband. Only 3 of them (267258, 289944, and 317985) carry microdeletions limited to 17q11.2, though larger than the one detected in our proband as they range in size from 0.97 to 1.18 Mb (Fig. 1A). These 3 patients all display intellectual disability, hypotonia, and fatigue.

Discussion

The chromosome 17q11.2 microdeletion detected in our patient falls within the larger interval associated with the *NF1* microdeletion syndrome (OMIM 613675), but does not encompass the *NF1* gene, which is considered the main causative gene for the syndrome [Riva et al., 2000]. The 17q11.2 deletion syndrome is characterized by an excessive number of early-onset neurofibromas, intellectual disability, cardiovascular malformations, and variable dysmorphisms [Venturin et al., 2004], all absent in our proband. The CNV detected in our patient is a rare de novo deletion not listed in the Database of Genomic Variants among CNVs found in phenotypically normal individuals. It is relatively small in size, spanning only 196 kb, and no patient carrying similarly sized CNVs is listed in the DECIPHER database. This microdeletion encompasses 3 *RefSeq* genes, namely *PSMD11*, *CDK5R1*, and the last 2 exons of *MYO1D*. These genes are not currently present in the Simons Foundation website. *PSMD11* and *MYO1D* are ubiquitously expressed, whereas *CDK5R1* is highly expressed in the cerebral cortex and to a lesser extent in the mucosa of the esophagus, in the small intestine, the spleen and in PBMCs [GTEx Consortium, 2013]. Among these 3 genes, *CDK5R1* has a specific role in neurodevelopment and in higher brain functions thus appearing as the most interesting candidate to explain the patient's phenotype. The *CDK5R1* gene is listed in another important bioinformatic autism web-based resource called "AutismKB" (<http://autismkb.cbi.pku.edu.cn/index.php>), which lists the *CDK5R1* gene as a candidate gene for ASD. Evidences for its role in ASD include (1) one microarray gene expression study in children with ASD [Gregg et al., 2008] and (2) six linkage studies of which the most significant is by Sutcliffe et al. [2005]. The first study reports a dysregulation of the *CDK5R1* gene

expression in autistic children compared to age- and sex-matched typically developed controls, whereas linkage studies demonstrated a strong association of the 17q11.2 region with ASD.

The protein encoded by *CDK5R1*, known also as p35, is necessary for the activation of *CDK5*, the cyclin-dependent kinase 5, in the brain. The *CDK5/CDK5R1* complex is required for neurite outgrowth, cortical lamination, and dendritic spine morphogenesis. *Cdk5r* null mice display severe cortical lamination defects and suffer from seizures and adult mortality [Chae et al., 1997]. Abnormal neocortical development characterized by inverted layering and branched migration instead of classical linear radial migration was also reported in *Cdk5r* null mice [Gupta et al., 2003]. In addition to neurodevelopmental defects, null mice showed motor coordination deficits and impaired long-term synaptic plasticity [He et al., 2014]. Consistent with low expression of *CDK5* and *CDK5R1*, delayed responses to painful thermal stimulation in primary afferent nociceptive C fibers in the knockout mouse dorsal root ganglia were also reported [Pareek et al., 2006]. The CreER-*Cdk5r1* conditional knockout mice have defective spatial learning and memory along with decreased anxiety-like behavior [Mishiba et al., 2014]. The mouse phenotype results from a reduction in the spine density of pyramidal neurons, reduced sensitivity to synaptic input, and defective long-term depression induction in the hippocampus. In the light of this evidence, the 25% decrease in *CDK5R1* mRNA detected in the PBMCs of our patient, if paralleled by similar reductions in the central nervous system, may explain his peculiar neuropsychological profile characterized by moderate deficits in communication and social interaction, reduced pain sensitivity, and motor clumsiness. The *CDK5R1* gene has been proposed as a candidate gene for intellectual disability in the 17q11.2 deletion syndrome [Venturin et al., 2004]. This hypothesis finds support in a recent report by Osio et al. [2018] describing the 3 above-mentioned unrelated patients listed in the DECIPHER database, affected by intellectual disability, hypotonia, and fatigue, who carry overlapping de novo 17q11.2 microdeletions (Fig. 1A). The smallest region of overlap between these 3 patients contains 7 genes including *CDK5R1*, which is, considered by the authors, a good candidate for explaining the developmental delay. In mutation screenings involving patients with idiopathic intellectual disability, several variants in the 3'-UTR and in the coding region of the *CDK5R1* gene were found [Venturin et al., 2006; Moncini et al., 2016]. However, the functional significance of some of these mutations is controver-

sial. Some studies report altered gene expression levels and/or altered protein function [Moncini et al., 2016], whereas others found no significant differences in the biochemical and neurodevelopmental properties of the mutant and wild-type forms of *CDK5R1* [Takada et al., 2015].

In summary, our data suggest that the heterozygous deletion of the *CDK5R1* gene yields a decrease in *CDK5R1* mRNA level in our proband, compatible with phenotypic traits less severe than previously described intellectual disabilities. Our patient in fact displays high-functioning autism, short-term verbal memory deficits, difficulties in motor coordination, and pain hyposensitivity. Further research will have to address whether similar heterozygous deletions reduce *CDK5R1* gene expression to a similar or to a different extent in different patients, possibly supporting an epigenetic modulation at this locus as primarily responsible for clinical variability in penetrance. Alternatively, deletion size (i.e., other deleted loci) or epistatic interactions with genetic variants located in other chromosomes may contribute to interindividual differences in phenotypic severity.

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Statement of Ethics

Informed consent for the present study and image publication was obtained from the patient. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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