CLINICAL REPORT

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Female Patient with Autistic Disorder, Intellectual Disability, and Co-Morbid Anxiety Disorder: Expanding the Phenotype Associated with the Recurrent 3q13.2–q13.31 Microdeletion

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In recent years, the advent of comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays and its use as a first genetic test for the diagnosis of patients with neurodevelopmental phenotypes has allowed the identification of novel submicroscopic chromosomal abnormalities (namely, copy number variants or CNVs), imperceptible by conventional cytogenetic techniques. The 3q13.31 microdeletion syndrome (OMIM #615433) has been defined as a genomic disorder mainly characterized by developmental delay, postnatal overgrowth, hypotonia, genital abnormalities in males, and characteristic craniofacial features. Although the 3q13.31 CNVs are variable in size, a 3.4 Mb recurrently altered region at 3q13.2–q13.31 has been recently described and non-allelic homologous recombination (NAHR) mediated by flanking human endogenous retrovirus (HERV-H) elements has been suggested as the mechanism of deletion formation. We expand the phenotypic spectrum associated with this recurrent deletion performing the clinical description of a 9-year-old female patient with autistic disorder, total absence of language, intellectual disability, anxiety disorder and disruptive, and compulsive eating behaviors. The array-based molecular karyotyping allowed the identification of a de novo recurrent 3q13.2–q13.31 deletion encompassing 25 genes. In addition, we compare her clinical phenotype with previous reports of patients with neurodevelopmental and behavioral disorders and proximal 3q microdeletions. Finally, we also review the candidate genes proposed so far for these phenotypes.

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Abbreviations: AD, autistic disorder; ADIR, autism diagnostic interview-revised; ADOS-G, autism diagnostic observation schedule-generic; ASD, autism spectrum disorder; BMI, body mass index; CGH, comparative genomic hybridization; CNV, copy number variation; HERVH, human endogenous retrovirus; ID, intellectual disability; IQ, intelligence quotient; K-SADS-PL, kiddie-SADS (schedule for affective disorders and schizophrenia for school-age children)—present and lifetime; MRI, magnetic resonance imaging; NAHR, non-allelic homologous recombination; SNP, single nucleotide polymorphism; SRO, shortest region of overlapping.

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INTRODUCTION

The long arm of chromosome 3 contains the region for the recently defined 3q13.31 microdeletion syndrome (OMIM #615433), a genomic disorder identified in patients with deletions ranging in size from approximately 580 kb to 22.4 Mb and mainly characterized by developmental delay, postnatal overgrowth, muscular hypotonia, genital abnormalities in males, and characteristic craniofacial features [Molin et al., 2012]. Very few clinical descriptions of patients with neurodevelopmental phenotypes and chromosome aberrations in the most proximal 3q region have been published to date, some detected by conventional cytogenetics [Jenkins et al., 1985; Okada et al., 1987; Genuardi et al., 1994; Mackie Ogilvie et al., 1998; Lawson-Yuen et al., 2006] and others, more recently, by high resolution molecular techniques [Simovich et al., 2008; Shimojima et al., 2009; Molin et al., 2012; Gimelli et al., 2013; Shuvarikov et al., 2013; Vuillaume et al., 2013; Wisniowiecka-Kowalnik et al., 2013; Lowther et al., 2014; Rasmussen et al., 2014]. Most of the breakpoints of these chromosomal aberrations are variable, although a recurrently deleted region at 3q13.2–q13.31 has been recently delineated. A mechanism of non-allelic homologous recombination (NAHR) between human endogenous retrovirus (HERV-H) elements has been suggested to explain its formation [Shuvarikov et al., 2013; Campbell et al., 2014]. The recurrent deletion includes the shortest region of overlapping (SRO) deletion defined by Molin et al. [2012] for the 3q13.31 microdeletion syndrome. This interval is located at 3q13.31, has a size of approximately 580 kb and contains five *RefSeq* genes, amongst of which *DRD3* and *ZBTB20* are potential candidates for neurodevelopmental and neuropsychiatric phenotypes, as well as for brain malformations and craniofacial abnormalities [Molin et al., 2012].

Here, the use of a genome-wide high resolution SNP array has allowed the identification of a proximal 3q copy number loss of 3.37 Mb equivalent to the recurrent 3q13.2–q13.31 microdeletion in a female patient with a phenotype mainly characterized by autistic disorder (AD), total absence of language, intellectual disability (ID), anxiety disorder and disruptive, and compulsive eating behaviors.

MATERIALS AND METHODS

DNA samples from both the patient and her parents were obtained from peripheral blood and genotyped with the Affymetrix CytoScan High-Density SNP array and the Affymetrix CytoScan 750 K SNP array (Affymetrix, Santa Clara, CA), respectively. Microarray-based copy number analysis was performed using the Chromosome Analysis Suite version 1.2.2 (Affymetrix) and the results were presented on the human genome assembly hg19 (Figs. 1A and 2A).

RESULTS

Clinical Report

The patient, a 9-year-old female, is the eldest of two children of a non-consanguineous couple of European descent. At birth, her mother was 36 years old and her father was 35. Family medical history included two maternal great-aunts with idiopathic ID and a maternal cousin with infantile cerebral palsy due to delivery problems.

She was born after an uneventful pregnancy without remarkable prenatal/perinatal data. Amniocentesis revealed a 46,XX karyotype. Delivery was programmed (due to low fetal weight in the last weeks of gestation) and prolonged. Apgar scores were 7–9 at 1–5 min, respectively. Her birth weight was 2,670 g (17th centile). Generalized hypotonia was present at birth.

Physical examination at the age of 4 years and 8 months showed neither dysmorphic features nor organomegaly or pigmentary abnormalities. Her weight was 16.4 kg (10th centile) and head circumference was 52 cm. Audiometry, hemogram test, acylcarnitine profile in blood, plasme and urine amino acids, and fatty acids in urine were all normal. However, brain MRI revealed a small sized corpus callosum suggesting a partial absence of splenium.

Motor skills were delayed and reported as follows: walking started at the age of 15–18 months, with subsequent gross motor clumsiness. At the age of 3 years, she presented stereotyped movements (flapping).

Her social and cognitive development was presumed to be completely normal until she was 18 months of age. As a remarkable feature, parents mentioned that, as a baby, she refused physical contact with others, except with her mother.

In the language domain, there was no babbling, her first words were at the age of approximately 12 months and her vocabulary only consisted of a few basic words. Language development was clearly delayed, experiencing a complete lost of language at some point around the age of 3 years. At that point, she also avoided eye contact, was described as a withdrawn girl and showed lack of interest in her peers.

No protodeclarative pointing was developed but, occasionally, she was able to use gestures for requests. A standardized assessment through the Portage Guide, held at the age of 3 years and 5 months, reported a level of social and cognitive skills equivalent to 7–12 months. At the beginning of her fourth year of life, symptoms of inflexibility and repetitive behaviors (e.g., excessive routines and rituals, difficulty in dealing with minor changes, stereotypies and complex mannerisms, adherence to fetish objects), unusual sensory interest, and a especial sensitivity to loud noises, became more evident. She also manifested some disruptive behaviors: uncontrolled tantrums and a tendency to bite objects (especially when she was excessively nervous or concentrated). She showed a clear tendency to isolation and indifference towards others, rejecting physical contact (except from her parents).

At the age of 8 years and 8 months, the patient underwent a clinical and neurocognitive assessment. Clinical evaluation included the administration of the Autism Diagnostic Interview-Revised (ADI-R) [Lord et al., 1994], the Autism Diagnostic Observation Schedule-Generic (ADOS-G) [Lord et al., 2000] and a semi-structured diagnostic interview, the Kiddie-SADS (Schedule for Affective Disorders and Schizophrenia for School-Age Children)-Present and Lifetime (K-SADS-PL) [1996].

Scores on the ADI-R were the following: Social interaction: 30 (cutoff score: 10); Communication and language: 12 (cutoff score: 7); Restricted and repetitive behaviors: 6 (cutoff score: 3). These scores, along with those obtained through the standardized observation (ADOS-G), confirmed a diagnosis of Autistic Disorder. In addition,

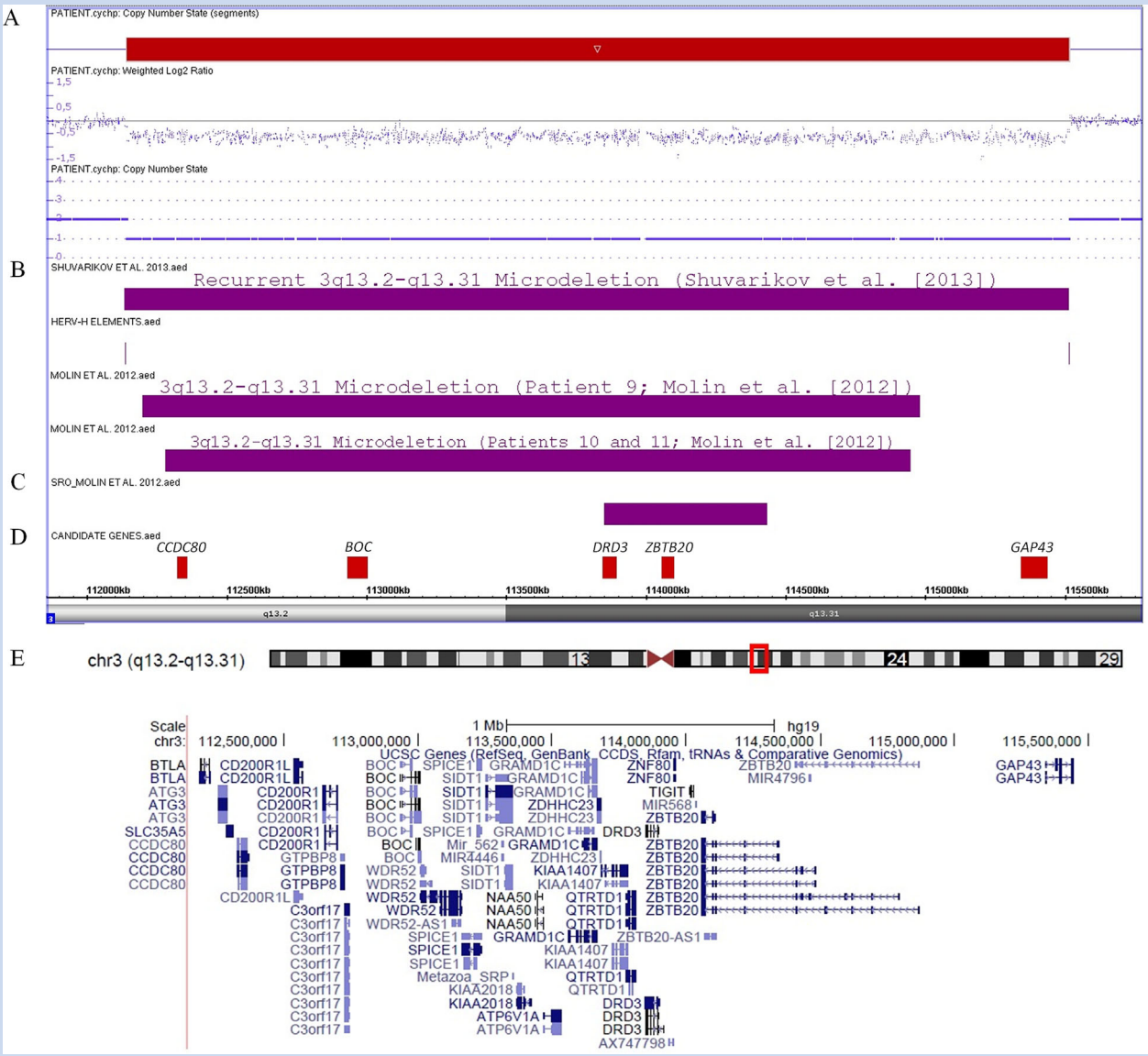

FIG. 1. A: Microarray-based copy number analysis performed with the Affymetrix CytoScan High Density array and visualized using the Affymetrix Chromosome Analysis Suite version 1.2.2. Image of the 3.37 Mb deletion at 3q13.2–q13.31 (arr 3q13.2–q13.31(112,144,081–115,514,432)X1 (build 19)) of our patient. B: Schematic overview showing the recurrent 3q13.2–q13.31 microdeletion [Shuvarikov et al., 2013], three of the deletions previously reported by Molin et al. [2012] that may represent this recurrent CNV (they had approximately the same size, location and gene content) [Molin et al., 2012] and the flanking HERV-H elements [Shuvarikov et al., 2013]. C: The shortest region of deletion overlap defined for the 3q13.31 microdeletion syndrome [Molin et al., 2012]. D: Candidate genes to explain the clinical features found in patients with the recurrent 3q13.2–q13.31 microdeletion. E: UCSC genes within this region, as plotted in the UCSC Genome Browser (hg19). The UCSC Genome LiftOver Tool was used for converting genome coordinates between hg18 and hg19 assemblies, where necessary. SRO: shortest region of overlapping. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

the semi-structured psychiatric interview K-SADS-PL reported a co-morbid diagnosis of Anxiety Disorder Not Otherwise Specified.

Neurodevelopmental assessment included cognitive and linguistic abilities. In order to assess cognitive abilities, a non-verbal intelligence test, The Leiter International Performance Scale-Revised [Roid and Miller, 1997], was applied. A non-verbal IQ of 65 was obtained, which corresponds to a developmental age of 3 years. Given the lack of oral expressive language, on the linguistic domain only receptive vocabulary was evaluated through the Peabody Picture Vocabulary Test-III [Dunn et al., 2006]. Results showed a similar level of delay regarding the patient's chronological age, reaching a Verbal Mental Age of 3 years and 2 months.

Therefore, these data pointed to a homogeneous neurocognitive profile, characterized by similar levels of functioning in different developmental areas.

To date, the patient presents severe gross and fine motor clumsiness. She is still described as a very anxious and withdrawn girl with very limited response to social interaction. She prefers to spend most of her time at home and experiences high levels of stress under any unexpected event. Over time, she has improved some specific aspects of her social behavior (e.g., eye contact, emotional control, intentional communication with her mother). Restricted interests are now focused on watching cartoons on TV. Comprehension of spoken language is still very limited, although the production of speech sounds have recently increased. Severe sleep disturbances and compulsive eating behaviors are present. At the time of this report, her weight is at the 90th centile.

Clinical features of our patient are summarized in Table I.

Molecular Analysis

Microarray-based copy number analysis performed with the CytoScan High-Density SNP array (Affymetrix) allowed the detection of a heterozygous loss of 3.37 Mb at 3q13.2–q13.31 (arr 3q13.2–q13.31(112,144,081–115,514,432)X1 (build 19)) (Figs. 1A and 2A), encompassing 25 genes (*BTLA*, *ATG3*, *SLC35A5*, *CCDC80*, *CD200R1L*, *CD200R1*, *GTPBP8*, *C3orf17*, *BOC*, *WDR52*, *SPICE1*, *SIDT1*, *KIAA2018*, *NAA50*, *ATP6V1A*, *GRAMD1C*, *ZDHHC23*, *KIAA1407*, *QTRTD1*, *DRD3*, *ZNF80*, *TIGIT*, *MIR568*, *ZBTB20*, and *GAP43*) (Fig. 1E). The CytoScan High-Density SNP array includes, in this region, a total of 3,116 markers with a median intermarker distance of 1.08 kb. No additional rare exonic CNV was detected in her genome. The genetic analysis of the patient's parents, performed with the Affymetrix CytoScan 750 K SNP array, verified that they do not carry the deletion (data not shown).

DISCUSSION

Recently, Molin et al. [2012] have defined the 3q13.31 microdeletion syndrome, a genomic disorder identified in patients with deletions ranging in size from approximately 580 kb to 22.4 Mb and located at chromosome 3q12.3–q21.3 [Molin et al., 2012]. Since its delineation, few clinical reports of patients with neurodevelopmental and neuropsychiatric phenotypes and proximal 3q deletions have been published [Gimelli et al., 2013; Shuvarikov et al., 2013; Vuillaume et al., 2013; Wisniowiecka-Kowalnik et al., 2013; Lowther et al., 2014; Rasmussen et al., 2014] (Fig. 2B). Despite the heterogeneity in size and breakpoints and the phenotypic variability, most patients share common clinical features, including cognitive and motor delays, language problems, postnatal overgrowth/obesity, muscular hypotonia, genital abnormalities in males and characteristic craniofacial features (mainly short *philtrum*, epicanthal folds, hypertelorism, downslanting palpebral fissures, ptosis, broad, and bulbous nasal tip, high palate and protruding lips) [Simovich et al., 2008; Shimojima et al., 2009; Molin et al., 2012; Shuvarikov et al., 2013; Vuillaume et al., 2013; Wisniowiecka-Kowalnik et al., 2013; Lowther et al., 2014]. Also frequently, patients with 3q13.31 microdeletions presented cranial and brain abnormalities (including agenesis of the corpus callosum, ventriculomegaly, holoprosencephaly alobar, among others) [Molin et al., 2012; Shuvarikov et al., 2013; Vuillaume et al., 2013; Wisniowiecka-Kowalnik et al., 2013; Lowther et al., 2014], episodes of seizures or epilepsy [Shimojima et al., 2009; Molin et al., 2012; Shuvarikov et al., 2013; Vuillaume et al., 2013], skeletal malformations (including scoliosis, lordosis, thoracic kyphosis, and abnormalities in hands and feet) [Shimojima et al., 2009; Molin et al., 2012; Vuillaume et al., 2013; Lowther et al., 2014], and ophthalmological anomalies (such as myopia, strabismus or nystagmus) [Shimojima et al., 2009; Molin et al., 2013; Shuvarikov et al., 2013; Lowther et al., 2014]. Less commonly reported manifestations in 3q13.31 microdeletion carriers include additional congenital defects, such as renal and cardiac abnormalities [Molin et al., 2012; Shuvarikov et al., 2013]. Behavioral disorders are common, so that patients with attention deficit disorder and/or autism/autism spectrum disorders (ASDs) [Molin et al., 2012; Shuvarikov et al., 2013; Wisniowiecka-Kowalnik et al., 2013; Rasmussen et al., 2014], stereotypic behaviors, sensory issues, and hostility [Shuvarikov et al., 2013] have also been described. Finally, a recent clinical description of an adult patient provided evidence that schizophrenia could also be part of the range of the phenotypic manifestations associated to the 3q13.31 microdeletion syndrome [Lowther et al., 2014].

Although these 3q13.31 CNVs are heterogeneous in size, location, and gene content (Fig. 2B and C), Shuvarikov et al. [2013] have recently identified nine patients with a recurrent deletion of about 3.4 Mb located at 3q13.2–q13.31 and encompassing 28 genes [Shuvarikov et al., 2013]. Molecular analysis of the breakpoints of these deletions allowed to identify directly oriented HERV-H elements, of approximately 5 kb and with more than 95% DNA sequence identity. Based on these findings, the authors have proposed that these HERV-H elements could be the substrate for NAHR, the potential underlying mechanism for this recurrent aberration [Shuvarikov et al., 2013; Campbell et al., 2014]. Our patient has a 3.37 Mb deletion at 3q13.2–q13.31, located at this recurrently altered region (Figs. 1A and 2A) and three of the deletions previously reported by Molin et al. [2013] (patients 9–11) may also represent this recurrent deletion since they had approximately the same size, location and gene content (Fig. 1B). In addition, Karavitakis et al. [2014] have recently described a male patient with dysmorphic features and multiple congenital anomalies and a 3q13.2–q13.31 microduplication that may represent the reciprocal CNV [Karavitakis et al., 2014] (Fig. 2B).

The most relevant clinical features of our patient are summarized in Table I, which also reviews the phenotype of previous patients with the recurrent 3q13.2–q13.31 deletion [Molin et al., 2012; Shuvarikov et al., 2013]. This summary shows that this recurrent deletion is mainly expressed with ID/developmental delay/cognitive delay, language and motor delay, muscular hypotonia, and behavior problems. Consistent with these descriptions, our patient's phenotype includes ID, severe gross and fine motor clumsiness, absence of language, and muscular hypotonia at birth. Regarding her behavior, she presents a combination of features not previously reported for the 3q13.2–q13.31 deletion in a single individual. She has AD and a co-morbid diagnosis of anxiety disorder, manifested disruptive behaviors (i.e., uncontrolled tantrums) at primary school and, at the time of this report, also

TABLE I. A: Summary of the Most Relevant Clinical Features Present in Our Patient and in Previous Reports With the Recurrent 3q13.2–q13.31 Microdeletion. B: Summary of the Inheritance Pattern in Previously Reported Patients With the Recurrent 3q13.2–q13.31 Microdeletion and Complete Family Genetic Study, Including Our Patient. Modified From Molin et al. [2012] and Shuvarikov et al. [2013]

(A) Clinical characteristics of patients with the recurrent 3q13.2–q13.31 deletion*	Molin et al. [2012] n = 3 (2 M, 1 F)***	Shuvarikov et al. [2013] n = 9 (4 M, 5 F)	Total (n)	Total (%)	Present patient n = 1 (1 F)
ID/cognitive delay/developmental delay	3/3	8/9	11/12	91.67%	Yes
Language/speech delay	2/3	9/9	11/12	91.67%	Yes
Motor delay	3/3	9/9	12/12	100.00%	Yes
Growth anomalies/obesity/overgrowth	1/3	2/9	3/12	25.00%	No
Behavior problems	1/3 (0/3 Autism)	7/9 (3/9 Autism)	8/12 (3/12 Autism)	66.67% (25.00% Autism)	AD, anxiety disorder, disruptive behavior, compulsive eating behavior, sleep disturbances
Muscular hypotonia	0/1	9/9	9/10	90.00%	Yes
Seizures/epilepsy/EEG anomalies	0/3	4/9	4/12	33.33%	No
Brain abnormalities	NS	4/7	4/7	57.14%	Small corpus callosum, possible absence of splenium
Ophthalmologic abnormalities					
Strabismus/nystagmus	1/2	4/9	5/11	45.45%	No
Myopia/hyperopia	0/2	3/9	3/11	27.27%	No
Skeletal anomalies	0/3	5/9	5/12	41.67%	No
Genital abnormalities in males	1/2	2/4	3/6	50.00%	—
Renal abnormalities	0/3	1/9	1/12	8.33%	No
(B) Inheritance pattern of patients with the recurrent 3q13.2–q13.31 deletion**	**Molin et al. [2012] n = 3 (2 M, 1 F)*****	**Shuvarikov et al. [2013] n = 9 (4 M, 5 F)**	**Total (n)**	**Total (%)**	**Present patient n = 1 (1 F)**
De novo	3/3	7/7	10/10	100.00%	Yes

[A] n, number of subjects; M, male; F, female; ID, intellectual disability; AD, autistic disorder; EEG, electroencephalogram; NS, not specified.
*Not all clinical features could be evaluated in all patients.
**Not all patients have a complete genetic study.
***Deletions in patients 9, 10, and 11 from Molin et al. [2012] may represent the recurrent 3q13.2–q13.31 microdeletion.
[B] n, number of subjects with complete family genetic test.

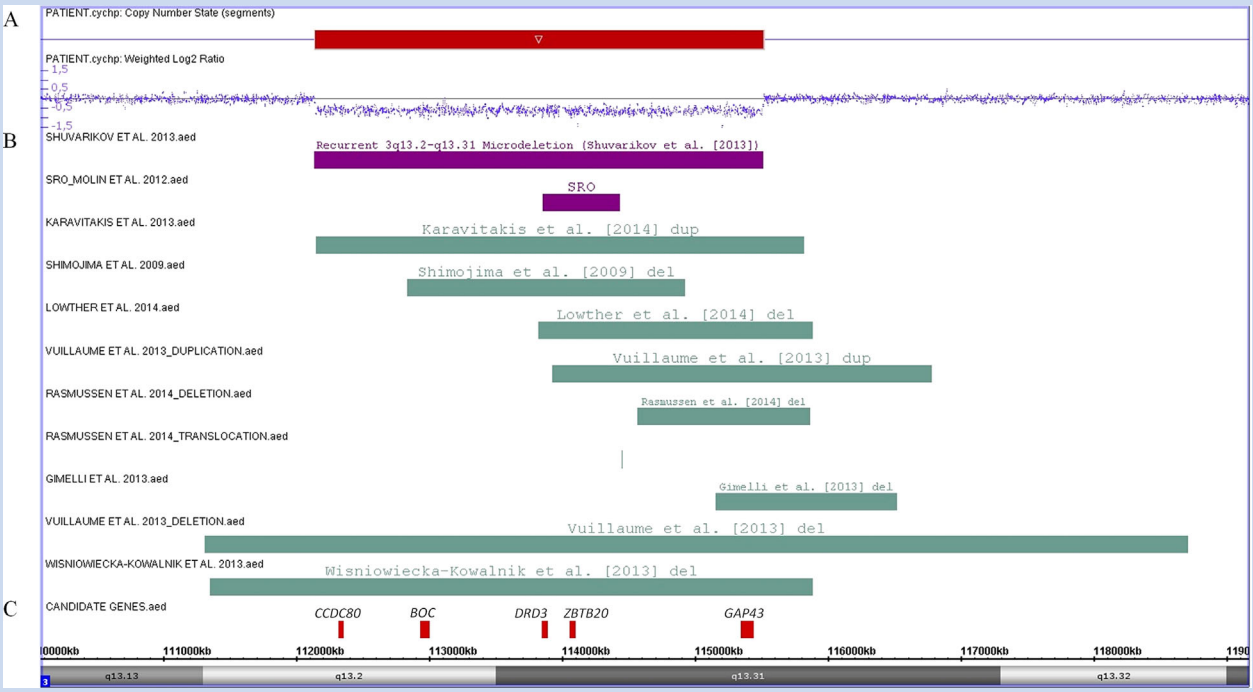

FIG. 2. Schematic overview of the proximal 3q13.13–q13.32 region showing: A: the 3q13.2–q13.31 interval deleted in our patient, B: the recurrent 3q13.2–q13.31 microdeletion described by Shuvarikov et al. [2014], the SRO defined by Molin et al. [2012], previously reported 3q13 chromosomal alterations overlapping with that of our patient and C: candidate genes [Shimojima et al., 2009; Molin et al., 2012; Gimelli et al., 2013; Shuvarikov et al., 2013; Vuillaume et al., 2013; Wisniowiecka-Kowalnik et al., 2013; Karavitakis et al., 2014; Lowther et al., 2014; Rasmussen et al., 2014]. The UCSC Genome LiftOver Tool was used for converting genome coordinates between hg18 and hg19 assemblies, where necessary. SRO: shortest region of overlapping; del: deletion; dup: duplication. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

suffered compulsive eating behaviors and severe sleep disturbances. Although, less frequently reported, congenital defects (such as brain, ophthalmological, and skeletal anomalies), epilepsy/seizures, and growth anomalies (obesity or overgrowth) have also been found in patients with the recurrent 3q13.2–q13.31 deletion. Our patient did not present any of these manifestations, with the exception of a small sized corpus callosum (Table I).

The SRO deletion defined by Molin et al. [2012] for the 3q13.31 microdeletion syndrome is within the recurrent 3q13.2–q13.31 deletion. This interval is located at chromosome band 3q13.31, has a size of approximately 580 kb and contains five *RefSeq* genes, including *DRD3* and *ZBTB20* as potential candidates genes for neurodevelopmental and neuropsychiatric phenotypes, as well as for brain malformations and craniofacial abnormalities [Molin et al., 2012] (Fig. 1C, 1D and 2B). *DRD3* (OMIM *126451) encodes the D3 subtype of the dopamine receptors and is expressed in the limbic areas of the brain known to be associated with cognitive, emotional, and endocrine functions. This gene is listed in the OMIM database as a morbidity gene for being a susceptibility locus for schizophrenia (OMIM #181500) and hereditary essential tremor 1 (OMIM #190300). In addition, several studies have provided evidence of association between common variants in *DRD3* and ASD [de Krom et al., 2009; Staal et al., 2012; Toma et al., 2013], so that it has been suggested as a candidate gene for both the neurodevelopmental and behavioral phenotypes [Shimojima et al., 2009; Molin et al., 2012; Shuvarikov et al., 2013; Vuillaume et al., 2013; Wisniowiecka-Kowalnik et al., 2013], as well as for the neuropsychiatric disorders [Lowther et al., 2014] associated with the 3q13.31 microdeletion syndrome. Additional clinical features that have been attributed to this gene include muscular hypotonia [Shimojima et al., 2009; Shuvarikov et al., 2013] and abnormal postnatal growth [Vuillaume et al., 2013]. *ZBTB20* (OMIM *606025) belongs to the POK (POZ and Kruppel) family of transcriptional repressors, is a cell fate determinant for hippocampal neurons [Nielsen et al., 2007; Xie et al., 2010] and it has been suggested that it might be implicated in development of the human archicortex [Nielsen et al., 2014]. Such findings have suggested a potential role for *ZBTB20* in the brain abnormalities frequently associated with proximal 3q microdeletions, such as agenesis of the corpus callosum [Molin et al., 2012; Shuvarikov et al., 2013], so that it might also be implicated in the small sized corpus callosum in our patient. Moreover, a de novo interruption of this gene, due to a chromosome 3 inversion, has been associated with a phenotype of pervasive developmental delay [Talkowski et al., 2012]; a de novo t(3;18) translocation only disrupting *ZBTB20* was identified in a patient with developmental delay, attention-deficit hyperactivity disorder, psychosis, Tourettés syndrome, autistic traits and dysmorphic facial features [Rasmussen et al., 2014] (Fig. 2B); and a de novo

1.3 Mb deletion, truncating *ZBTB20, GAP43* and *LSAMP* but not involving any other gene within the SRO defined by Molin et al. [2012] [Molin et al., 2012], was carried by a patient with developmental delay, autism and facial dysmorphism [Rasmussen et al., 2014] (Fig. 2B). In addition, heterozygous missense mutations in this gene have been recently described in individuals with Primrose syndrome (OMIM #259050), a disorder clinically related to the 3q13.31 microdeletion syndrome but with a more severe phenotype. Functional analysis revealed strongly reduced levels of DNA binding for all mutants compared to wild-type, suggesting a dominant-negative impact of these mutations on the wild-type allele in Primrose syndrome compared to the *ZBTB20* haploinsufficiency that likely underlies the 3q13.31 microdeletion syndrome [Cordeddu et al., 2014]. Finally, a recently reported epigenetic study has shown that hypermethylation in the coding region of *ZBTB20* is associated with major depressive disorder [Davies et al., 2014]. Overall, those findings provide evidence for the importance of this gene in neurodevelopmental, behavioral, and neuropsychiatric disorders, as it has been suggested by several authors [Molin et al., 2012; Vuillaume et al., 2013; Cordeddu et al., 2014; Lowther et al., 2014]. In that sense, Rasmussen et al. [2014] have explored the implication of the orthologous human genes previously shown to be directly repressed by Zbtb20 in the developing murine brain in neurodevelopmental disorders, confirming the binding of Zbtb20 to evolutionary conserved regions in murine hippocampal neurons for six of them: *Cntn4*, *Gad1*, *Nrxn1*, *Nrxn3*, *Scn2a*, and *Snap25* [Rasmussen et al., 2014]. The *ZBTB20* gene has also been involved in growth and glucose homeostasis and it has been shown that *Zbtb20* knockout mice exhibit postnatal growth delay, metabolic disorders and lethality [Sutherland et al., 2009]. Thus, it has been proposed to explain the overgrowth/obesity characteristic of some patients with 3q13.31 microdeletions [Molin et al., 2012; Vuillaume et al., 2013] and it has been suggested that its overexpression might be causing the undergrowth found in a patient with the 3q13.2–q13.31 microduplication [Karavitakis et al., 2014]. However, and as is reflected in Table I, this feature is not always present in patients with the recurrent 3q13.2–q13.31 deletion and additional factors might be favoring its expression. Our patient never had overweight/obesity and her current weight, although in the 90th centile, is within the normal range.

Another dosage sensitive gene, *CCDC80* (coiled-Coil Domain containing 80) (Fig. 1D and 2C), has been implicated in adipogenesis in mice [Tremblay et al., 2009]; hence, one could hypothesize that *CCDC80* could also be responsible for the mirror phenotype in the abnormal postnatal growth observed in patients with microdeletions (overgrowth) [Molin et al., 2012; Shuvarikov et al., 2013] and microduplications (undergrowth) [Karavitakis et al., 2014] at chromosome 3q13.2–q13.31. Furthermore, loss of the homologous gene *ccdc80-l1* induces motility issues in zebrafish [Brusegan et al., 2012]; so that, Karavitakis et al. [2014] have suggested its potential implication in the muscular hypotonia seen in patients with dose alterations at 3q13.2–q13.31 [Karavitakis et al., 2014], a phenotypic manifestation observed in our patient who also has only one copy of *CCDC80*.

Two additional recurrently altered genes that merit particular attention as candidates for the phenotype associated with the 3q13 proximal microdeletions are *BOC* and *GAP43*, both deleted in our patient (Fig. 1D and 2C). The *BOC* (OMIM *608708; Brother of *CDON*) gene is a cell surface receptor of the immunoglobulin (Ig)/fibronectin type III repeat family and has been involved in differentiation of myogenic cells [Kang et al., 2002, 2003]; so that, it might be an alternative or an additional candidate gene for the muscle hypotonia in our patient, as it has been previously suggested for patients with 3q13.2–q13.31 CNVs encompassing this gene [Shimojima et al., 2009; Shuvarikov et al., 2013; Karavitakis et al., 2014] (Fig. 2B and 2C). Haploinsufficiency of *BOC*, independently or in combination with haploinsufficiency of *ZBTB20* and/or *GAP43*, has also been proposed to explain the brain abnormalities (specifically the corpus callosum abnormalities) observed in patients with 3q13 deletions [Shuvarikov et al., 2013]. Finally, *GAP43* (OMIM *162060; Growth-Associated Protein 43) is implicated in brain and neuronal functions (including neurite outgrowth, neurotransmission, and synaptic plasticity) [Denny, 2006], has been related to learning delay and autistic-like behaviors in mice [Zaccaria et al., 2010] and autism in human [Allen-Brady et al., 2009] and has been suggested as a candidate to participate in various phenotypic manifestations of the 3q13.31 microduplication syndrome such as developmental delay [Molin et al., 2012; Vuillaume et al., 2013], hypotonia [Shuvarikov et al., 2013], brain abnormalities (agenesis of the corpus callosum) [Molin et al., 2012; Shuvarikov et al., 2013; Vuillaume et al., 2013], and schizophrenia [Lowther et al., 2014]. Recently, Gimelli et al. [2013] described a female patient with a 3q13.31 deletion restricted to *GAP43* (deleted in our patient) and *LSAMP* (not deleted in our patient), with a phenotype characterized by neuropsychiatric disorders and renal, vascular, and skeletal abnormalities [Gimelli et al., 2013]. Together, these findings suggest a key role for *GAP43* in neurodevelopmental and behavioral features, so that its haploinsufficiency could be participating in our patient's phenotype, mainly characterized by AD, anxiety disorder and ID.

In line with previous reports [Molin et al., 2012; Shuvarikov et al., 2013] (Table IB), the 3q13.2–q13.31 deletion was not carried by our patient's parents (data not shown).

In conclusion, the use of a genome-wide high density SNP array allowed us the detection of a new proximal 3q deletion, included within the 3q13.31 microdeletion syndrome region and whose breakpoints are located at the boundaries of the recently identified recurrent 3q13.2–q13.31 deletion. Based on this finding and as it has been recently proposed [Shuvarikov et al., 2013; Campbell et al., 2014], the potential mechanism underlying this deletion might be NAHR mediated by HERV-H elements. Our description of this new patient, with a phenotype characterized by autistic disorder, total absence of language, intellectual disability, anxiety disorder and disruptive, and compulsive eating behaviors, contributes to expand the phenotypic spectrum associated with the recurrent 3q13.2–q13.31 microdeletion. Finally, the most plausible candidate genes to explain the different phenotypic features associated to that deletion might include *DRD3*, *ZBTB20*, *CCDC80*, *BOC*, and *GAP43*. However, additional factors, such as genetic (e.g., recessive mutations in the remaining allele and/or in any other location in the genome), epigenetic and/or environment modifiers, cannot be excluded, and could be modifying the clinical manifestations of these patients and favoring the clinical heterogeneity associated to their phenotypes.

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