CLINICAL REPORT

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Interstitial Microdeletions Including the Chromosome Band 4q13.2 and the *UBA6* Gene as Possible Causes of Intellectual Disability and Behavior Disorder

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The few proximal 4q chromosomal aberrations identified in patients with neurodevelopmental phenotypes that have been published to date are variable in type, size and breakpoints and, therefore, encompass different chromosome bands and genes, making the establishment of genotype–phenotype correlations a challenging task. Here, microarray-based copy number analysis allowed us the detection of two novel and partially overlapping deletions in two unrelated families. In Family 1, a 4q13.1-q13.2 deletion of 3.84 Mb was identified in a mother with mild intellectual disability and in her two children, both with mild intellectual disability and attention deficit hyperactivity disorder. In Family 2, a de novo 4q13.2-q13.3 deletion of 6.81 Mb was detected in a female patient, born to unaffected parents, with a diagnosis of mild intellectual disability, behavioral disorder and facial dysmorphism. The shortest region of overlap between these two aberrations is located at chromosome 4q13.2 and includes 17 genes amongst of which we suggest *UBA6* (ubiquitin-like modifier-activating enzyme 6) as a strong candidate gene for these phenotypes.

Key words: 4q13.1-q13.2 deletion; 4q13.2-q13.3 deletion; 4q13.2 deletion; SNP microarray; copy number variation; intellectual disability; attention deficit hyperactivity disorder; *EPHA5*; *UBA6*

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INTRODUCTION

To date, few clinical descriptions of patients with neurodevelopmental phenotypes and proximal 4q chromosomal aberrations

Conflict of interest: none.

Abbreviations: ID, intellectual disability; ADHD, attention deficit hyperactivity disorder; SNP, single nucleotide polymorphism; IQ, intelligence quotient; WAIS III, Wechsler adult intelligence scale-III; MRI, magnetic resonance imaging; WISC IV, Wechsler intelligence scale for children; EEG, electroencephalogram; CNV, copy number variation; OMIM, online mendelian inheritance in man; PTK, protein tyrosine kinase.

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have been reported. All of them are variable in type (both copy number losses and gains as well as complex rearrangements), size (ranging from ~1,5 Mb to ~25 Mb) and breakpoints and encompass different chromosome bands (restricted to 4q13 or expanding into adjacent chromosome bands) and genes [Zollino et al., 1995; Nowaczyk et al., 1997; Shashi et al., 1999; Eggermann et al., 2005; Bonnet et al., 2006; Lipska et al., 2011; Assawamakin et al., 2012; Girirajan et al., 2013; Matoso et al., 2013; Shimada et al., 2013; Hemati et al., 2014; Utine et al., 2014]. So that, the interpretation of their clinical implications, the discovery of candidate genes and the establishment of genotype–phenotype correlations are challenging tasks that remain to be addressed.

Here, we report on two novel and partially overlapping chromosomal aberrations located in the proximal region of chromosome 4q in two unrelated families. In addition, we review the clinical manifestations associated with chromosomal aberrations restricted to 4q13 in the literature and compare them with those present in our patients.

MATERIALS AND METHODS

DNA samples from both the patients and their families were obtained from peripheral blood and genotyped with different versions of Affymetrix genome-wide high density SNP microarrays (Affymetrix, Santa Clara, CA): CytoScan High-Density SNP array (Patient 1), CytoScan 750 K SNP array (his mother and sister) and Cytogenetics Whole-Genome 2.7M SNP array (Family 2). Microarray-based copy number analysis in these two families was performed using the Chromosome Analysis Suite software version 1.2.2 (Affymetrix) and the results were presented on the human genome assembly hg19.

RESULTS

Clinical Reports

Patient 1 (Family 1). Patient 1 is a male first evaluated at the age of 9 years due to learning difficulties. He corresponds to the second pregnancy of a mother diagnosed with mild intellectual disability (ID) (total intelligence quotient (IQ) of 65, assessed by the Wechsler Adult Intelligence Scale-III (WAIS-III)), rheumatoid arthritis and mild mitral insufficiency. He was born after 36 weeks of gestation and delivered by urgent caesarean due to fetal distress related to preeclampsia. A previous pregnancy ended in abortion due to intrauterine fetal death. His 7 years old sister was also first referred for learning difficulties and has a diagnosis of mild ID (total IQ of 60, assessed by the Wechsler Intelligence Scale for Children (WISC-IV)) and attention deficit hyperactivity disorder (ADHD). His maternal aunt had a congenital heart defect and an aunt of his mother suffered ID. Apgar scores were 5, 8, 9 at 1, 5, 10 min, respectively. His birth weight was 1,920 g (less than 10th centile), length 45 cm (less than 10th centile), and head circumference 33 cm (25th centile). Brain and abdominal ultrasound scans were normal.

He reached unsupported sitting at the age of 10 months, started walking at 18 months and constructed his first sentences after the age of 2 years. At the beginning of school age, difficulties to follow the pace of work of his peers, very low motivation for school work, high distractibility, poor sustained attention and restless and impulsive behaviors were noted. After a family interview and according to the data reported by his teachers, at the age of 7 years he was diagnosed of ADHD, combined type, associated with a mild ID according to the WISC-IV test, which yielded the following results: Full Scale IQ: 63, Verbal Comprehension Index: 83, Perceptual Reasoning Index: 68, Processing Speed Index: 62, and Working Memory: 68. He showed a good social interaction ability and difficulties in fine motor skills. Physical examination revealed no significant dysmorphic features and only low-set ears, hypoplastic nares (Fig. 1A) and a transverse crease on the right hand were noted. His expressive and social skills were preserved and he showed a good self-control during the interview. His somatometric parameters (weight: 21 kg, height: 122 cm, head circumference: 50.5 cm) were in the 3rd percentile. Haemogram test, conventional biochemical profile, thyroid hormone, amino acids in blood and urine, brain MRI (Magnetic Resonance Imaging), and X-fragile studies were all normal.

Photographs of Patient 1, his sister and mother are shown in Figures 1A–C.

Patient 2 (Family 2). Patient 2 is an 11 years and 7 months old female, second daughter of healthy and non-consanguineous parents of European descent, born after uneventful pregnancy and delivery, with a birth weight of 2,960 g and referred to the Pediatric Neurology Service at the age of 3 years and 11 months because of psychomotor developmental delay. A maternal uncle had schizophrenia and a first cousin presented dyslexia. There was no clinical history of ID in her family.

From the first month of life, she had frequent regurgitation and vomits and an esophageal pH test showed severe gastroesophageal reflux with poor evolution under pharmacological treatment. So that, the patient required surgical intervention being Nissen fundoplication procedure performed at the age of 11 months; despite that, the feeding difficulties persisted for years, with frequent vomiting and anorexia.

She started walking at 18 months and her first words were emitted when she was 2 years old; at the age of 3.5 years, her vocabulary consisted of simple 2–3 word sentences and she always showed a good comprehensive ability and a good nonverbal compensation, with gestures, of her expressive limitations. At 4.5 years signs of nervous and excitable behavior at school, as well as difficulties in school adaptation and in relationship with her peers were noted. Although she was sociable and affable, often incurred in disruptive behavior; showed intrusive in peer interactions, did not adapt to the rules and had behaviors that caused peer rejection, sometimes with obsessive interest in a particular classmate. Her learning progress was extremely deficient leading to the need for special education support at school with teachers specialized in therapeutic education and speech and hearing. At the age of 5 years, her intellectual ability, assessed by Wechsler Preschool and Primary Scale of Intelligence, showed a total IQ of 61, performance IQ of 67, and verbal IQ of 66. Raven's Progressive Matrices test scored less than the 5th percentile.

Physical examination at the time of the initial evaluation shown a good general appearance and her somatometric parameters were below 3rd centile for weight (12 kg) and height (90 cm) showing a

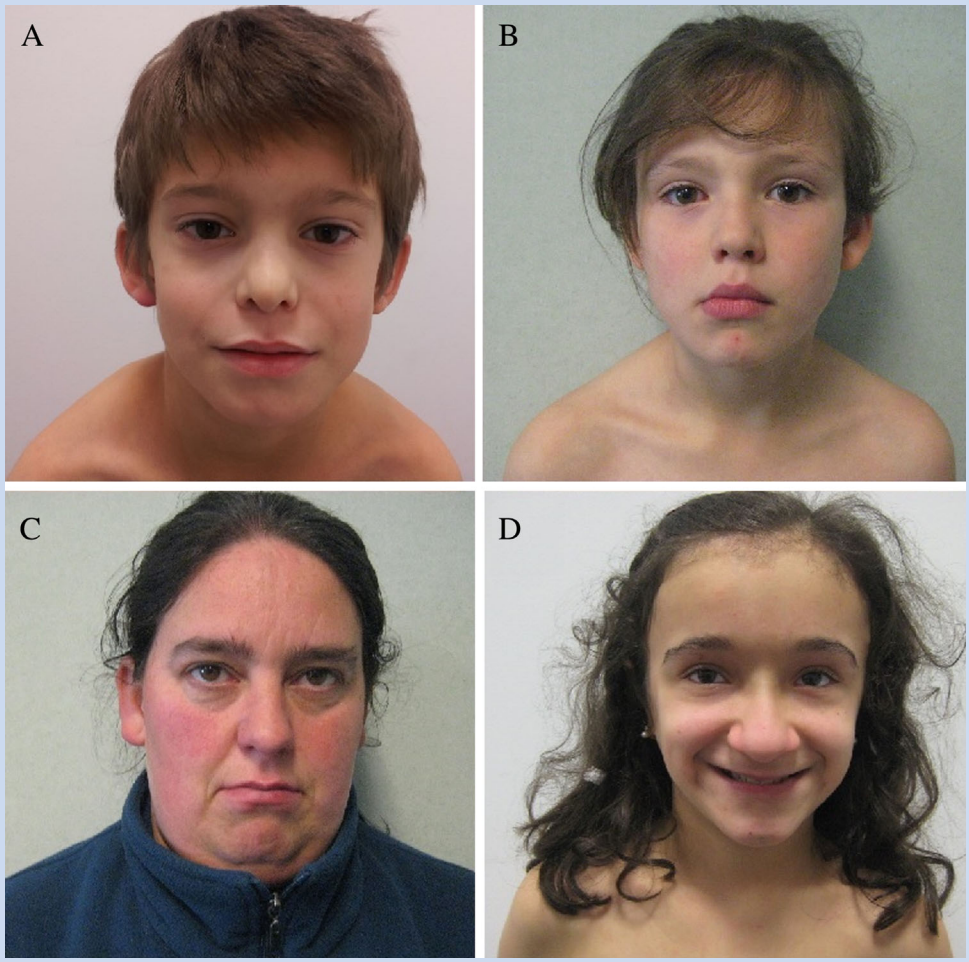

FIG. 1. A: Patient 1 facial image showing no significant dysmorphic features with the exception of low-set ears and hypoplastic nares. B: Facial photograph of Patients 1 sister showing no obvious dysmorphic features. C: Facial photograph of Patients 1 mother showing no obvious dysmorphic features. D: Patient 2 facial photograph showing mild facial dysmorphism: a triangular craniofacial profile, a broad forehead, narrow and slightly upslanted palpebral fissures, a broad nasal tip, and a prominent narrow chin. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

relative macrocephaly (head circumference 52.5 cm, slightly above 50th centile). Mild and non-distinctive dysmorphic features were noted, including a triangular craniofacial profile with a broad forehead and narrow and slightly upslanted palpebral fissures, a broad nasal tip and a prominent narrow chin with a dimple in its medial part (Fig. 1D). Additional explorations performed at that time, including ocular fundus exam, hearing assessment by auditory evoked potentials, brain MRI, EEG (electroencephalogram), cardiological examination, complete bone series and karyotype were all normal.

To date, at the age of 11 years and 7 months, her academic achievement is very low; she repeated two years and needs curricular adaptation at school. Her verbal communication skills are also low and generally reciprocal conversation must be addressed by an adult. She has not yet acquired reading lexical resources, cannot sum or subtract and is sociable and extroverted but has many adaptation difficulties in her peer group.

Molecular Analysis

Microarray-based copy number analysis in Patient 1 showed a 3.84 Mb aberration indicating arr 4q13.1q13.2 (65,736,024-69,582,428)X1 (build19) (Fig. 2A). The same finding was also identified in his sister and mother (Fig. 2A). Patient 2 showed a 6.81 Mb aberration indicating arr 4q13.2q13.3 (68,207,272-75,021,494)X1 (build19) (Fig. 2B). This aberration was not detected in her parents suggesting de novo origin. No additional rare exonic copy number variant (CNV) was detected in both patients.

DISCUSSION

To the best of our knowledge, the proximal 4q chromosomal aberrations identified in patients with neurodevelopmental phenotypes are rare, non recurrent, heterogeneous in size (usually affecting several megabases of sequence) and breakpoints and, in

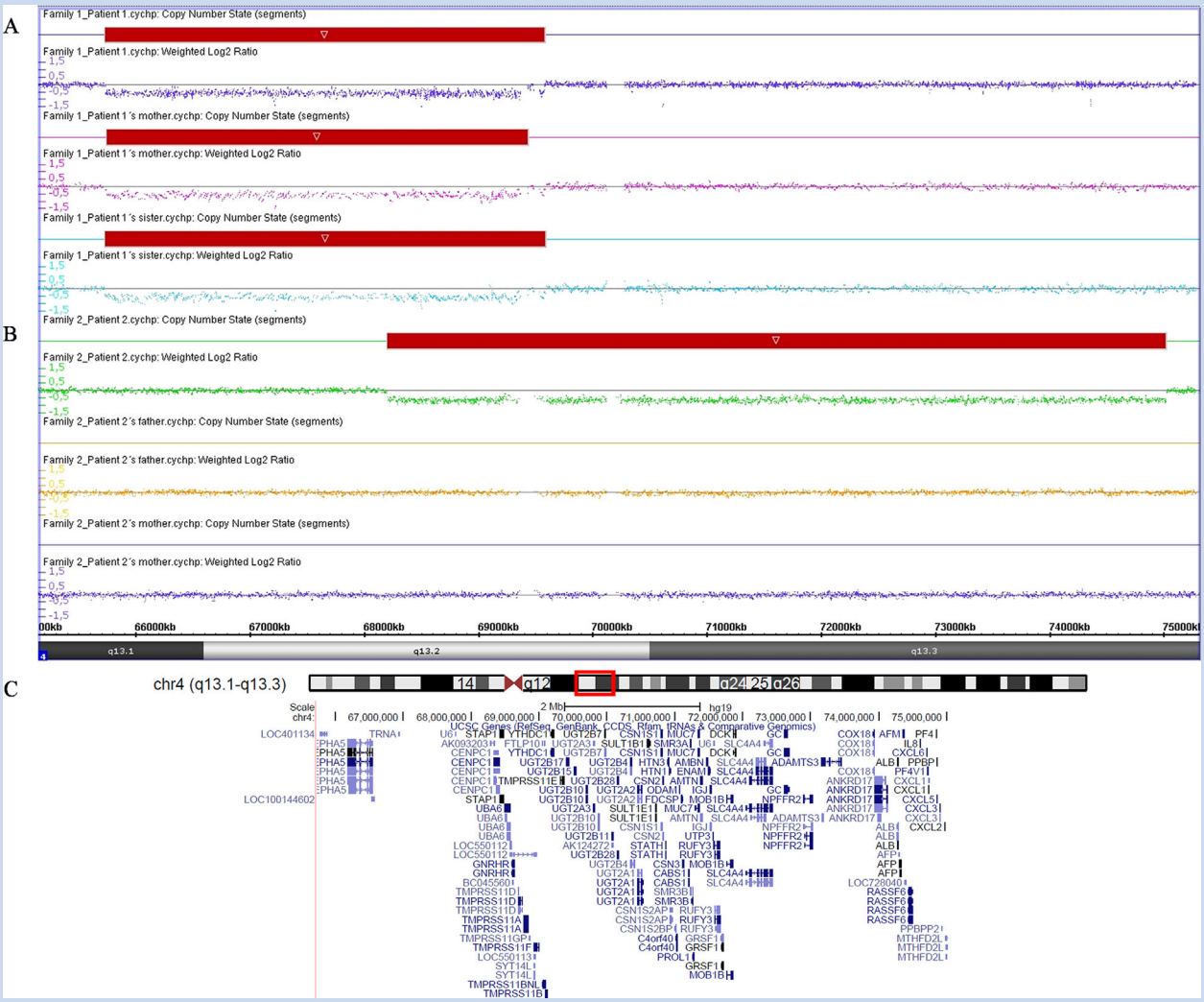

FIG. 2. Microarray-based copy number analysis performed with the Affymetrix Chromosome Analysis Suite version 1.2.2. A: Image of the 3.84 Mb deletion at 4q13.1-q13.2 (arr 4q13.1-q13.2 (65,736,024-69,582,428)X1 (build 19)) in Patient 1 and his family. B: Image of the 6.81 Mb 4q13.2-q13.3 region (arr 4q13.2-q13.3 (68,207,272-75,021,494)X1 (build19)) in Patient 2 (deleted) and her parents (non deleted). C: UCSC genes within the region 4q13.1-q13.3 deleted between our both patients, as plotted in the UCSC Genome Browser (hg19). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

consequence, involve different chromosome bands and a high number of genes [Zollino et al., 1995; Nowaczyk et al., 1997; Shashi et al., 1999; Eggermann et al., 2005; Bonnet et al., 2006; Lipska et al., 2011; Assawamakin et al., 2012; Girirajan et al., 2013; Matoso et al., 2013; Shimada et al., 2013; Hemati et al., 2014; Utine et al., 2014]. Amongst these alterations we can distinguish chromosomal aberrations restricted to 4q13 chromosome bands [Girirajan et al., 2013; Matoso et al., 2013; Shimada et al., 2013] (Table I), as those of our patients, from larger alterations involving additional chromosome bands [Zollino et al., 1995; Nowaczyk et al., 1997; Shashi et al., 1999; Eggermann et al., 2005; Bonnet et al., 2006; Lipska et al., 2011; Assawamakin et al., 2012; Hemati et al., 2014; Utine et al., 2014]. Figure 3 is an schematic overview of the proximal 4q region showing the SNP array results in our patients, previously reported chromosomal alterations restricted to 4q13 chromosome bands and OMIM-morbidity and candidate genes implicated in this interval (Fig. 3; Table I). Larger deletions, with partial or total overlap with those identified in our patients, include a 4q13.2-q21.22 deletion [Eggermann et al., 2005], a large deletion of approximately 18 Mb detected at 4q13.1q21.21 [Utine et al., 2014], as well as non- recurrent interstitial deletions with breakpoints in 4q12 and expanding different chromosome bands [Hemati et al., 2014]. Earlier reports have also identified large alterations affecting the proximal 4q region, but no detailed definition at a molecular level was provided since they were detected with conventional techniques [Zollino et al., 1995; Nowaczyk et al., 1997; Shashi et al., 1999] and will not be discussed in this paper.

TABLE I. 4q13-Restricted Alterations. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

	Overlapping Genes with Family 1 (Patient 1, his mother and sister)	Overlapping Genes with Family 2 (Patient 2)	Candidate genes
Girirajan et al. [2013] 4q13.1 duplication (chr. 4: 63,125,710 - 64,711,798 bp; hg19) ~ 1.6 Mb 0 genes (breakpoint near *EPHA5*) *De novo*	None (breakpoint near *EPHA5*)	None (breakpoint near *EPHA5*)	Breakpoint near *EPHA5*: autism [Girirajan et al., 2013] and ADHD [Matoso et al., 2013]
Matoso et al. [2013] 4q13.1-q13.3 duplication (chr. 4: 62,800,754 - 71,464,035 bp; hg19) ~ 8.6 Mb 50 genes Maternally inherited	***LOC401134, EPHA5, LOC100144602, CENPC1, STAP1, UBA6, LOC550112, GNRHR, TMPRSS11D, TMPRSS11A, LOC644759, TMPRSS11F, SYT14L, FTLP10, TMPRSS11BNL, TMPRSS11B, YTHDC1, TMPRSS11E, UGT2B17, UGT2B15***	***CENPC1, STAP1, UBA6, LOC550112, GNRHR, TMPRSS11D, TMPRSS11A, LOC644759, TMPRSS11F, SYT14L, FTLP10, TMPRSS11BNL, TMPRSS11B, YTHDC1, TMPRSS11E, UGT2B17, UGT2B15,*** *UGT2B10, UGT2A3, UGT2B7, UGT2B11, UGT2B28, UGT2B4, UGT2A1, UGT2A2, SULT1B1, SULT1E1, CSN1S1, CSN2, STATH, HTN3, HTN1, CSN1S2AP, CSN1S2BP, C4orf40, ODAM, C4orf7, CSN3, C4orf35, SMR3A, SMR3B, PROL1, MUC7, AMTN*	*EPHA5*: autism [Girirajan et al., 2013] and ADHD [Matoso et al., 2013]. ***UBA6***: cognitive and behavior phenotypes (*) [Lee et al., 2013; Lee et al., 2015]
Shimada et al. [2013] 4q13.2-q13.3 deletion (**) (chr. 4: 67,006,250 - 71,068,535 bp; hg19) ~ 4.1 Mb 37 genes *De novo*	***CENPC1, STAP1, UBA6, LOC550112, GNRHR, TMPRSS11D, TMPRSS11A, LOC644759, TMPRSS11F, SYT14L, FTLP10, TMPRSS11BNL, TMPRSS11B, YTHDC1, TMPRSS11E, UGT2B17, UGT2B15***	***CENPC1, STAP1, UBA6, LOC550112, GNRHR, TMPRSS11D, TMPRSS11A, LOC644759, TMPRSS11F, SYT14L, FTLP10, TMPRSS11BNL, TMPRSS11B, YTHDC1, TMPRSS11E, UGT2B17, UGT2B15,*** *UGT2B10, UGT2A3, UGT2B7, UGT2B11, UGT2B28, UGT2B4, UGT2A1, UGT2A2, SULT1B1, SULT1E1, CSN1S1, CSN2, STATH, HTN3, HTN1, CSN1S2AP, CSN1S2BP, C4orf40, ODAM*	Breakpoint near *EPHA5*: autism [Girirajan et al., 2013] and ADHD [Matoso et al., 2013]. ***UBA6***: cognitive and behavior phenotypes (*) [Lee et al., 2013; Lee et al., 2015]

RefSeq and candidate genes, genomic coordinates, size, number of genes and inheritance pattern of 4q13-restricted chromosomal aberrations reported in the literature. [Girirajan et al., 2013; Matoso et al., 2013; Shimada et al., 2013]. The UCSC Genome LiftOver Tool was used for converting genome coordinates from hg18 to hg19 assemblies, where necessary. The genes overlapping with those of our both families are shown in bold. OMIM morbidity genes are shown in green. Candidate genes are shown in red. (*) Reduced size, brain morphological defects, learning and memory defects, and behavior phenotypes in mice [Lee et al., 2013, 2015]. (**)Patient with a Saethre-Chotzen-like phenotype, severe ID and autism with microdeletions of 4.1 Mb (4q13.2-q13.3) and 5.5 Mb (7p15.3-p21.1) [Shimada et al., 2013].

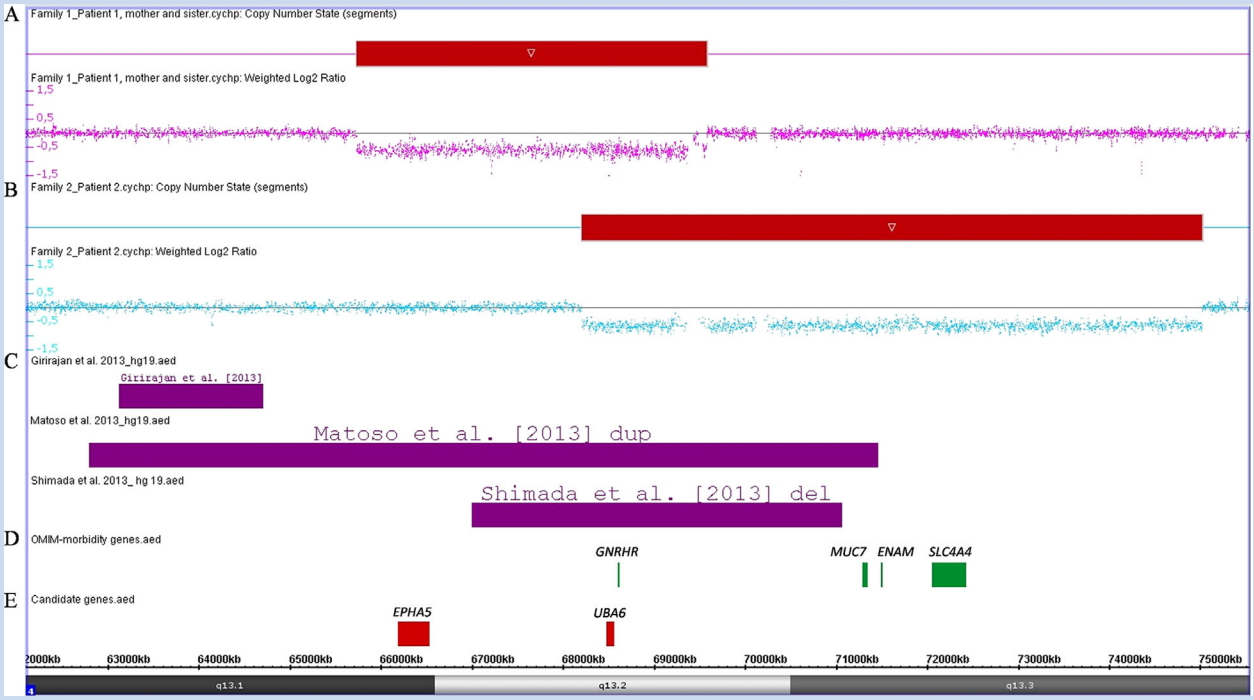

FIG. 3. Schematic overview of the proximal 4q region showing (A,B) the SNP array results in our two families, (C) previous reports of individuals with 4q13-restricted chromosomal aberrations [Girirajan et al., 2013; Matoso et al., 2013; Shimada et al., 2013] and (D) OMIM-morbidity, and (E) candidate genes located within this region. The UCSC Genome LiftOver Tool was used for converting genome coordinates between hg18 and hg19 assemblies, where necessary. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

The shortest region of overlap between our both deletions is located at 4q13.2 and include 17 known genes (Fig. 2; Table II). Patient 1 has a 3.84 Mb deletion at 4q13.1-q13.2 also identified in his intellectually affected sister and mother (Fig. 2A). Patient 2, with no clinical history of ID in her family, has a de novo 6.81 Mb deletion at 4q13.2-q13.3 (Fig. 2B).

Matoso et al. [2013] have recently described a family with a maternally inherited insertional translocation der(21) ins(21;4)(q21;q13.1q13.3) that results in an ~8.6 Mb duplication at 4q13.1-q13.3 in two affected siblings. One of the affected children was a female with learning disability, speech disorder, and ADHD. Her brother, with the same chromosomal abnormality, showed behavioral problems and ADHD [Matoso et al., 2013] (Table III). According to these authors, a gene that deserves special attention in the 4q13.1-q13.3 interval duplicated in their patients is *EPHA5* (Ephrin receptor, OMIM 600004). This gene encodes for the Ephrin type-A receptor 5, a member of the EPH/ELK subfamily of receptor PTKs (Protein Tyrosine Kinases), involved in neurogenesis, mainly expressed in the nervous system [Olivieri and Miescher, 1999] and proposed by these authors as a potential candidate gene to explain the phenotype of their patients (ADHD and learning difficulties) [Matoso et al., 2013]. In line with these findings, Girirajan et al. [2013] have recently detected a de novo 4q13.1 duplication of ~1.6 Mb of size near the *EPHA5* gene in a male patient with autism [Girirajan et al., 2013] (Tables I and III) and noted that a previous genome-wide association study had already identified a significant association between a marker near *EPHA5* and autism [Weiss et al., 2009]. Bonnet et al. [2006] also reported a patient with a severe phenotype, characterized by developmental delay, tall stature and obesity, involving a mosaic supernumerary ring chromosome with a larger duplication of the proximal bands 4q11–q13.2, where *EPHA5* is located. In line with the potential implication of *EPHA5* in ADHD, this patient also presented attention deficit [Bonnet et al., 2006]. Shimada et al. [2013] have recently reported a male patient with autism and interpersonal difficulties, harboring a 4q13.2-q13.3 microdeletion with its proximal breakpoint near to *EPHA5* site [Shimada et al., 2013] (Tables I and III). However, any genotype–phenotype correlation in this patient is difficult due to the concurrence of microdeletions at 4q13.2-q13.3 and 7p15.3-p21.1 and the complexity of his phenotype: a Saethre-Chotzen-like phenotype, severe ID and autism. Finally, a large de novo deletion of approximately 18 Mb was detected at 4q13.1q21.21 [Utine et al., 2014] and even though the breakpoints were not provided in the original paper (and we cannot assure *EPHA5* is included), the patient also presented behavior problems (hyperactivity, disinhibition and social interaction problems). At this point, it should be mentioned that *EPHA5* is deleted in our Patient 1 who, apart from mild ID, was also diagnosed with ADHD, combined type. Thus, our finding is consistent with the conclusions of Matoso et al. [2013] and could be suggested that the altered dose of *EPHA5* (and not only the duplication) may have a potential role in cognitive and behavioral disorders. However, *EPHA5* is not altered in our Patient 2, who also manifests mild ID and behavior problems. So that, we hypothesize that additional or alternative factors (including genes in the non overlapping interval deleted in our Patient 2 and/or in the minimal region of overlap in our two families) might contribute to these phenotypes.

In that sense, Assawamakin et al. [2012] have recently described a family with ID and a maternally derived complex structural variation resulting in partial trisomy 4q13.2-q22.1 with a complex translocation t(8;20) in two affected siblings with ID. Their mother, also with cognitive impairment, carried the same complex chromosomal aberration present in her children but in combination with a heterozygous 4q13.2-q22.1 deletion, resulting in no copy number change in this

TABLE II. SNP Array Results in Our Patients. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

	Number of overlapping genes	*RefSeq* Genes	OMIM Morbidity Genes	Candidate genes
FAMILY 1 **Patient 1, his mother and sister** 4q13.1-q13.2 deletion (chr. 4: 65,736,024 - 69,582,428 bp; hg19) ~ 3.8 Mb 20 genes Familiar	17	*LOC401134, EPHA5, LOC100144602,* ***CENPC1, STAP1, UBA6, LOC550112, GNRHR, TMPRSS11D, TMPRSS11A, LOC644759, TMPRSS11F, SYT14L, FTLP10, TMPRSS11BNL, TMPRSS11B, YTHDC1, TMPRSS11E, UGT2B17, UGT2B15***	***GNRHR***	*EPHA5:* autism [Girirajan et al., 2013] and ADHD [Matoso et al., 2013]. ***UBA6:*** cognitive and behavior phenotypes (*) [Lee et al., 2013; Lee et al., 2015]
FAMILY 2 **Patient 2** 4q13.2-q13.3 deletion (chr. 4: 68,207,272-75,021,494 bp; hg19) ~ 6.8 Mb 72 genes *De novo*	17	***CENPC1, STAP1, UBA6, LOC550112, GNRHR, TMPRSS11D, TMPRSS11A, LOC644759, TMPRSS11F, SYT14L, FTLP10, TMPRSS11BNL, TMPRSS11B, YTHDC1, TMPRSS11E, UGT2B17, UGT2B15,*** *UGT2B10, UGT2A3, UGT2B7, UGT2B11, UGT2B28, UGT2B4, UGT2A1, UGT2A2, SULT1B1, SULT1E1, CSN1S1, CSN2, STATH, HTN3, HTN1, CSN1S2AP, CSN1S2BP, C4orf40, ODAM, C4orf7, CSN3, C4orf35, SMR3A, SMR3B, PROL1, MUC7, AMTN, AMBN, ENAM, IGJ, UTP3, RUFY3, GRSF1, MOBKL1A, DCK, SLC4A4, GC, NPFFR2, ADAMTS3, COX18, ANKRD17, ALB, AFP, AFM, RASSF6, IL8, CXCL6, PF4V1, CXCL1, PF4, PPBP, CXCL5, CXCL3, PPBPL2, CXCL2*	***GNRHR****, MUC7, ENAM, SLC4A4*	***UBA6:*** cognitive and behavior phenotypes (*) [Lee et al., 2013; Lee et al., 2015]

RefSeq, OMIM-morbidity and candidate genes, genomic coordinates, size, number of genes and inheritance pattern of the chromosomal aberrations in Family 1 (4q13.1-q13.2 deletion) and Family 2 (4q13.2-q13.3 deletion). The overlapping genes between both families are shown in bold. Candidate genes are shown in red. (*) Reduced size, brain morphological defects, learning and memory defects and behavior phenotypes in mice [Lee et al., 2013, 2015].

TABLE III. Clinical Manifestations of Our Patients and Previous Patients With 4q13-Restricted Alterations

	Intellectual disability / developmental delay	Language and/or Speech delays	Behavior disorders	Autism spectrum disorders	Feeding difficulties	Growth delay/problems	Facial dysmorphism	Microcephaly / Macrocephaly	Congenital cardiac defects
FAMILY 1 **P1 /sister / mother** 4q13.1-q13.2 deletion	mild ID /mild ID / mild ID	yes / yes / yes	ADHD / ADHD / -	no / no / no	no / no / no	no / no / no	mild / no /no	no / no / no	no / no / yes (mild mitral insufficiency)
FAMILY 2 **P2** 4q13.2-q13.3 deletion	mild ID	yes	yes	no	yes	short stature	mild	relative macrocephaly	no
Girirajan et al. [2013] 4q13.1 duplication	NA	NA	NA	autism	NA	NA	NA	NA / NA	NA
Matoso et al. [2013] P1 / P2 4q13.1-q13.3 duplication	yes / yes	yes / yes	ADHD / ADHD	no /no	NA / NA	no /no	no / mild	no /no	NA / NA
Shimada et al. [2013] 4q13.2-q13.3 deletion (*)	severe ID	yes	interpersonal problems	autism	NA	no	yes	no	no

Summary of the most relevant clinical features present in our patients and in previous reports of individuals with 4q13-restricted chromosomal alterations [Girirajan et al., 2013; Matoso et al., 2013; Shimada et al., 2013]. P1, Patient 1; P2, Patient 2; NA, not available. (*) Patient with a Saethre-Chotzen-like phenotype, severe ID and autism with microdeletions of 4.1 Mb (4q13.2-q13.3) and 5.5 Mb (7p15.3-p21.1) [Shimada et al., 2013].

region. The breakpoints of the chromosome 4 alterations in the mother affected 3 genes, including *SLC4A4* (deleted in our Patient 2) but, even though recessive mutations in this gene have been implicated in ID (OMIM #604278), the authors could not find a concordance between previous reports and her phenotype [Assawamakin et al., 2012] and although is deleted in our Patient 2, its implication in her phenotype remains unclear.

One additional gene, *UBA6* (ubiquitin-like modifier-activating enzyme 6), included in our minimal region of overlap and also altered in previously reported patients [Assawamakin et al., 2012; Matoso et al., 2013; Shimada et al., 2013; Utine et al., 2014] deserves special attention (Figs. 2 and 3; Tables I and II). This gene participates in the ubiquitin transfer cascade with a role in mouse embryonic development. Recently, Lee et al. [2013] have showed that *Uba6*-deficient mice have significantly reduced size and increased postnatal lethality compared with control mice. Furthermore, morphological defects in the hippocampus and amygdala and a reduced density of dendritic spines were detected in these knockout mice. These authors also presented evidence that *Uba6* deficiency generated learning and memory defects, abnormalities in social behavior and an increased metabolic activity consistent with hyperactivity in mice. They also argued that *Uba6* could be involved in the effects on behavioral phenotypes, potentially through the complex interaction of different targets of the Uba6-Use 1 pathway [Lee et al., 2013]. In a more recent and comprehensive analysis of the autism-like phenotype, Lee et al. [2015] also showed that mice lacking Uba6 displayed increased anxiety, decreased social interaction, and defective communication [Lee et al., 2015]. Although complementary studies and new findings in additional patients are needed, it is important to stress that the *UBA6* gene is duplicated in the patients with a diagnosis of ADHD and learning difficulties described by Matoso et al. [2013] and in the two siblings with ID reported by Assawamakin et al. [2012], one of them also with attention deficit, hyperactivity and poor eye contact [Assawamakin et al., 2012]. Additionally, this gene is deleted in the patient with severe ID and autistic behaviors documented by Shimada et al. [2013], in the patient with ID, hyperactivity, disinhibition and social interaction problems described by Utine et al. [2014] and, as it was previously mentioned, is included in the minimal critical region deleted in our patients (Tables I and II), all with ID and behavior disorders. According to this, *UBA6* could be a strong candidate gene for these cognitive and behavioral phenotypes.

In conclusion, the heterogeneity in type, size, breakpoints, chromosome bands and genes characteristic of the proximal 4q chromosomal aberrations described so far makes the establishment of genotype–phenotype correlations challenging. However, literature review and comparison of molecular and clinical findings of previous reports with those present in our patients (with shorter and partially overlapping 4q13-restricted deletions) helped us to narrow a critical region at 4q13.2 for neurodevelopmental and behavior phenotypes and allowed to propose the *UBA6* gene as a strong candidate for these disorders, specifically mild ID and ADHD. However, the involvement of other genes located at 4q13.1-q13.3, as *EPHA5* and *SLC4A4*, cannot be ruled out and functional studies and clinical reports like the one presented here may contribute to better understand genotype–phenotype correlations.

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