CLINICAL REPORT

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TBR1 is the Candidate Gene for Intellectual Disability in Patients With a 2q24.2 Interstitial Deletion

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Interstitial deletion of 2q24.2 is a rarely described cytogenetic aberration in patients with intellectual disability (ID). Previously reported genotype–phenotype correlation identified a minimum deleted region of 2.65 Mb including 15 genes. Recently, a patient with a de novo 2q24.2 microdeletion of 0.4 Mb encompassing only three genes was described. However, the precise relationship between most deleted genes and the clinical features remains unclear. Here we describe a 12-year-old male patient diagnosed with growth retardation and ID. He also showed microcephaly, right palpebral ptosis, scapular winging, and pectus excavatum. Single nucleotide polymorphisms (SNP) array analysis showed a de novo interstitial deletion of 0.122 Mb at 2q24.2 region harboring only *TBR1* (T-box, brain, 1; OMIM: 604616), which encodes a T-box family transcription factor expressed in post-mitotic projection neurons and functionally significant in embryologic corticogenesis. This is the first case of a deletion at 2q24.2 involving only *TBR1*. This finding narrows the smallest region of overlap (SRO) for deletions in this region and strengthens the previously suggested hypothesis that this gene is a strong candidate for the ID phenotype. The identification of *TBR1* as candidate for ID encourages further molecular studies to identify novel mutations to understand the pathogenic effects of its haploinsufficiency. Finally, this report provides a review on 10 2q24.2 microdeletion patients.

Key words: 2q24.2; *TBR1*; smallest region of overlap; SNP Arrays analysis

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INTRODUCTION

In recent years, nine comparable submicroscopic deletions within 2q24.2 have been described in patients with a variable clinical phenotype including intellectual disability (ID), short stature, microcephaly, and dysmorphic features, suggesting that haploinsufficiency of one or more genes in 2q24.2 might be responsible for the common phenotypic features in these patients [Krepischi et al., 2010; Takatsuki et al., 2010; Magri et al., 2011; Traylor et al., 2012]. In particular, we described [Palumbo et al., 2012b] a girl with ID and generalized hypotonia carrying a 7.5-Mb deletion in 2q24.1q24.2. Combining our data with phenotypic and genotypic data of patients from the literature, we were able to restrict the candidate region for the observed traits to a 2.65-Mb interval containing 15 genes. More recently, Burrage et al. [2013] narrowed the smallest region of overlap (SRO) reporting a patient showing ID and short stature with a mosaic deletion of 0.422 Mb in 2q24.2 encompassing only three genes: *TANK*, *PSDM14*, and *TBR1*. Interestingly, they proposed as candidate gene for the neurological phenotype *TBR1*, encoding for a transcription factor expressed in the brain with an important functional role in early cortical development. In this study, we report the molecular and clinical

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characterization of a 2q24.2 deletion patient with ID, growth delay and slight dysmorphisms. The deletion, identified by SNP-array analysis, encompassed only the *TBR1*. We propose *TBR1* as candidate for ID.

CLINICAL REPORT

The patient is the eldest of three children of healthy and non-consanguineous parents. He was referred to the genetics clinic for investigation regarding short stature and psychomotor disability. The boy was born at 41 weeks gestation after caesarean performed because of fetal distress. There was no family history of ID, congenital anomalies or neurological disorders. His birth parameters were: weight 2,870 g (<3rd centile); height 49 cm (10th centile); head circumference was not reported. At age 3 years, he was admitted to a children hospital. Standard karyotyping and fragile X testing were normal. The electroencephalogram (EEG) showed no paroxysmal anomalies while awake, but revealed rare sharp waves and spike on central and vertex region during sleep. Brain magnetic resonance imaging (MRI) did not identify structural brain anomalies, ophthalmic evaluation showed moderate astigmatism, and audiometric examination was normal. A physical examination at the age 12 years revealed mild facial dysmorphism such as right palpebral ptosis. Other features included scapular winging and pectus excavatum, hands with long and narrow palms, and long fingers. His weight was 26.6 kg (<3rd centile), height 137 cm (<25th centile), and his head circumference 50.5 cm (−2 SD). At this time, he showed moderate to severe ID (WISC: IQ 35) and behavioral problems with impulsivity and aggressiveness. His motor development was within the normal range but he showed language delay.

MATERIALS AND METHODS

DNA was isolated from peripheral blood lymphocytes of the patient and the parents by using BioRobot EZ1 (Qiagen, Solna, Sweden). DNA concentration and purity were determinate with a ND-1000 spectrophotometer (NanoDrop Technologies, Berlin, Germany) while whole-genome copy number variation (CNV) analysis was carried out using the Genome-Wide Human SNP 6.0 Array (Affymetrix, Santa Clara, CA) as previously described [Palumbo et al., 2012a]. Data analysis was performed with the Genotyping Console software version 4.1 (Affymetrix) using annotation file version NA32 (hg19) and an in-house reference file consisting of 90 samples (45 males and 45 females) with the setting for marker count at 25, size at 50 kb and confidence at 85.

A higher genomic microarrays analysis of the patient was performed using the CytoScan HD array platform (Affymetrix). This array contains more than 2.6 million markers for copy number analysis and approximately 750,000 SNPs that fully genotype with greater than 99% accuracy. The CytoScan HD assay was performed according to the manufacturer's protocol, starting with 250 ng DNA. Briefly, total genomic DNA was digested with a restriction enzyme (*Nsp*I), ligated to an appropriate adapter for the enzyme, and subjected to PCR amplification using a single primer. After digestion with DNase I, the PCR products were labeled with a biotinylated nucleotide analogue, using terminal deoxynucleotidyl transferase and hybridized to the microarray. Hybridization was carried out in the Hybridization Oven 645 while subsequent washing and staining were performed using the Fluidics Station 450. The array was then scanned with the Scanner 3000 7G and both quality control step and copy number analysis were performed using the Chromosome Analysis Suite Software version 2.0: (1) the raw data file (.CEL) was normalized using the default options; (2) an unpaired analysis was performed using as baseline 270 HapMap samples in order to obtain Copy numbers value from .CEL files while the amplified and/or deleted regions were detected using a standard Hidden Markov Model (HMM) method. Karyotype was designated according to ISCN 2009 [Shaffer et al., 2009] and base pair position were derived from the University of California Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway), build GRCh37 (hg19).

RESULTS

SNP array analysis performed by using the Genome-Wide Human SNP 6.0 Array showed an interstitial microdeletion of ~0.100 Mb in band 2q24.2. The deleted region was covered by 43 SNP array probes (from CN_830726 to CN_832815) and included only one gene: *TBR1*. No additional clinically significant copy number changes were identified. The subsequent microarray analysis of the patient's parents using the same platform revealed normal chromosome 2 in both, proving the de novo occurrence of the deletion (Supplementary Fig. S1 in supporting information online). For higher resolution analysis of breakpoints, DNA from the patient was run on the Affymetrix CytoScan HD Array. A loss of 0.122 Mb was identified at 2q24.2, which appeared slightly larger than previously detected due to the increased density of the array. The deleted region was covered by 80 SNP array probes and again encompassed only the *TBR1* (Fig. 1). The proximal breakpoint (centromeric) was located between the last present probe C-6XDCN (162,269,784 bp) and the first deleted probe C-4GTRG (162,269,888 bp), while the distal breakpoint (telomeric) was located between the last deleted probe C-7MWPW (162,391,666 bp) and the first present probe C-7MLNM (162,398,343 bp). The molecular karyotype of the patient was arr2q24.2 (162,269,784 × 2, 162,269,888–162,391,666 × 1, 162,398,343 × 2) dn.

DISCUSSION

Only nine patients with deletion in 2q24.2 region have been reported and the associated phenotype includes ID, behavioral problems, and mild or absent dysmorphic features. Genotype–phenotype correlation, by using a reverse dysmorphology approach, has been performed in these cases so that the critical region corresponding to the reported features could be defined. In a recent paper we described a patient carrying a 7.5-Mb deletion in 2q24.1q24.2 with ID and generalized hypotonia. Further comparison of clinical and molecular data of 2q24.2 deleted patients allowed us to narrow the candidate region shared by all to a 2.5-Mb interval containing 15 genes [Palumbo et al., 2012b]. More recently, Burrage et al. [2013] described a patient showing mild ID and short

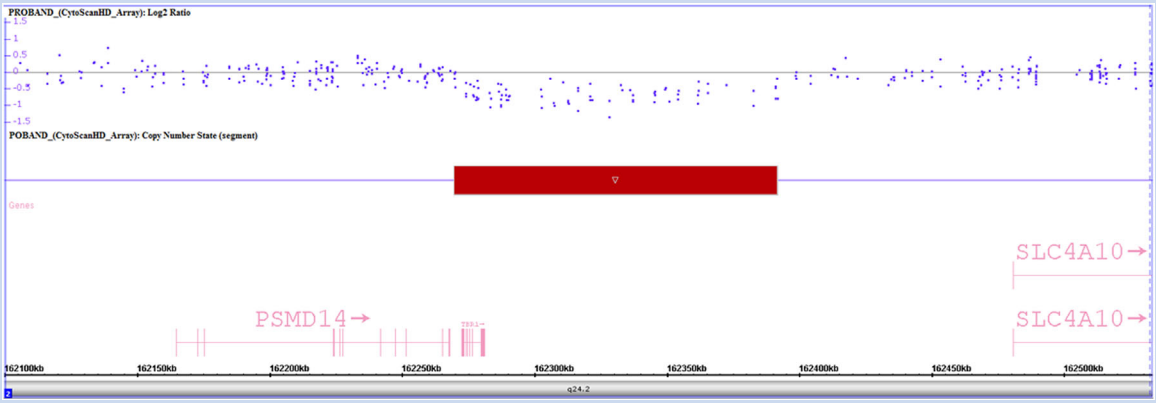

FIG. 1. CytoScan HD Array analysis results for patient. Intensity data (log 2 ratio value) of each probe is drawn along chromosome 2 from 162.10 to 162.53 Mb (USCS Genome Browser build February 2009, hg19). The upper panel shows patient versus reference signal (80 probes with decreased signal) while the red bar in the lower panel represents the 2q24.2 deletion.

stature carrying a mosaic deletion 2q24.2 identified by comparative genomic hybridization. This work allowed to further refine the minimal region of overlap at 0.422 Mb including only three genes: *TBR1*, *TANK*, and *PSMD14*.

Here, we describe a patient with ID and growth retardation harboring a 0.122-Mb microdeletion in 2q24.2, encompassing only *TBR1*. Our patient also showed slight dysmorphisms (right palpebral ptosis), skeletal alterations (scapular winging and pectus excavatum) and aggressive–impulsive behavior. The phenotypic features and molecular findings in our present patient and in other previously reported individuals with the 2q24.2 deletion are summarized in Table 1, while the schematic representation of the deletions is given in Figure 2. Deletion mapping and genotype–phenotype correlation in these individuals further support that haploinsufficiency of *TBR1* is the main cause of the ID, speech delay, and behavioral problems. In fact, the deletion affecting only *TBR1* in our patient is associated with a milder phenotype (no significant brain malformations/physical abnormalities) than those observed in patients carrying larger deletions, suggesting that haploinsufficiency in other genes contributes to the clinical presentation associated with the 2q24.2 deletion.

An association between *TBR1* abnormalities and ID has been proposed by many studies [Palumbo et al., 2012b; Burrage et al., 2013] and supported by its functional role. In fact, *TBR1* is a member of a conserved family of genes encoding transcription factors expressed in post-mitotic projection neurons with an important role in corticogenesis. This gene is known to regulate frontal cortex identity [Bedogni et al., 2010a], to be part of the *PAX6–TBR2–NEUROD–TBR1* cascade that controls the glutamatergic neuronal cell fate [Méndez-Gómez et al., 2011], and to have a role in the *CASK–TBR1–RELN* pathway important for neuronal migration [Hevner et al., 2006]. Mutations of these genes in humans have been associated with alterations in embryological brain development [Mitchell et al., 2003] and neuronal migration disorders [Hong et al., 2000]. Also, one of *TBR1*'s targets is the *AUTS2*, whose altered expression has been documented in patients with autism and/or intellectual disabilities [Bedogni et al., 2010b; Nagamani et al., 2013].

The hypothesis that *TBR1* is the candidate for the observed ID phenotype was further supported by previously reported *TBR1* knockout mice, which showed a cortical migration disorder [Hevner et al., 2001]. Together, this functional, clinical, and molecular evidence supports the role of *TBR1* in human neurodevelopment and it is reasonable to assume that the haploinsufficiency of *TBR1* is sufficient to result in a neurological phenotype characterized by ID. Finally, our patient also showed growth defects. Burrage et al. [2013] proposed as a candidate for this feature *PSMD14*, located upstream of *TBR1*. Although the deletion reported in our patient does not affect *PSMD14*, we cannot exclude a positional effect impairing the expression of the flanking genes, as previously reported and proposed for other rearrangements such as 22q11.2 microdeletions [Garavelli et al., 2011]. In summary, ours is the first description of a patient with ID in whom SNP-array analysis led to identification of a 2q24.2 microdeletion encompassing only *TBR1*.

This finding provides compelling evidence that alteration of this gene is responsible for the ID observed in patients with a 2q24.2 microdeletion. We suggest that researchers investigating nonsyndromic, sporadic ID may consider looking for *TBR1* mutations. Further research investigating how various *TBR1* deletions impact the function of the *TBR1* protein isoforms and the report of additional individuals with *TBR1* abnormalities will be helpful in understanding how molecular defects of this gene contribute to neurodevelopmental disease.

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TABLE 1. Clinical and Molecular Features of Patients With Deletions Encompassing *TBR1*

		Krepischi et al. [2010]				Traylor et al. [2012]				
	Present case	**Patient_1**	**Patient_3**	**Takatsuki et al. [2010]**	**Magri et al. [2011]**	**Patient_1**	**Patient_2**	**Patient_3**	**Palumbo et al. [2012b]**	**Burrage et al. [2013]**
Age	12 years	14 years	5 years	5 months	3.5 years	11.5 years	8 years	33 months	3 years	18 years
Gender	Male	Female	Female	Female	Male	Male	Male	Male	Female	Male
Deletion coordinates (hg19)	162,269,888–162,391,666	156,761,199–163,169,595	161,229,701–167,823,275	160,624,368–168,116,454	159,910,206–165,173,808	159,684,612–162,827,915	162,105,737–164,272,433	162,211,060–166,303,506	155,818,224–163,350,648	161,967,633–162,285,346
Inheritance	De novo	De novo	De novo	De novo	De novo	Unknown	De novo	De novo	De novo	De novo
Genes	*TBR1*	31; includes *TBR1*	27; includes *TBR1*	33; includes *TBR1*	22; includes *TBR1*	15; includes *TBR1*	9; includes *TBR1*	16; includes *TBR1*	33; includes *TBR1*	3; includes *TBR1*
Growth parameters										
Birth weight	<3rd centile	<3rd centile	50th centile	<3rd centile	3rd centile	10th–25th centile	25th–50th centile	50th–75th centile	50th centile	NR
Last examination: weight, height, and OFC	<3rd centile, <25th centile, −2 SD	50th–75th centile, <3rd centile, 5th centile	NR, NR, <3rd centile	<3rd centile, 3rd–10th centile, NR	NR, 10th centile, NR	3rd centile, 5th–10th centile, 2nd centile	95th centile, 71st centile, 51st centile	<3rd centile, 3rd–10th centile, 10th–25th centile	25th centile, 50th centile, 10th centile	<3rd centile, <1st centile, <3rd centile
Neurological profile										
Developmental delay/ intellectual disability	Delays in speech, and cognitive development, ID	+, no speech	Severe, no speech	+	Severe	Moderate, apraxia	+, no speech	+, speech delays	+, speech delays	Mild
Behavioral problems	Impulsivity aggressiveness	Autism	−	NA	−	Attention deficit hyperactivity disorders	Pervasive developmental disorder	Autistic features	−	−
EEG	Some abnormalities during sleep	Abnormal	Abnormal	NR	NA	Normal	Abnormal	NA	NA	NR
Brain malformations	Normal MRI	Normal MRI	Normal MRI	NR	Normal ultrasound	Mega cisterna magna, ventriculomegaly	Thick corpus callosum, venticulomegaly	Chiari I, ventriculomegaly, possible cortical dysplasia	Normal MRI	Normal MRI
Hypotonia	−	−	Moderate	+	Severe	+	+	+	Generalized	−
Others										
Facial/physical features	Right palpebral ptosis,scapular winging, pectus excavatum, long, narrow palms and long fingers	Small feet and hands, tapered fingers, valgus feet, abduction of upper limb	Coloboma, brachycephaly, thin nose, broad nasal bridge, anteverted nares, short philtrum, tapered fingers, displaced anus	Low set ears, thick and arched eyebrows, upslanting palpebral fissures, long eyelashes, short nose, flat nasal bridge, long philtrum, small mouth, micrognathia	Flat occipit, prominent forehead, sparse eyebrows, large mouth, short philtrum, micrognathia, camptodactyly of the hallux, joint laxity, hypotrophic muscles	Hyperextensible elbows	Facial asymmetry, downslanting palpebral fissures, wide alae nasi,turned-in foot	Small and low-set ears, epicanthal folds, short palpebral fissures, tapered fingers, joint laxity	Bruxism, hip dislocation	High palate, 5th finger clinodactyly

OFC, occipitofrontal head circumference; EEG, electroencephalogram; MRI, brain magnetic resonance imaging; NR, not reported; NA, not applicable; +, feature present; −, feature absent.

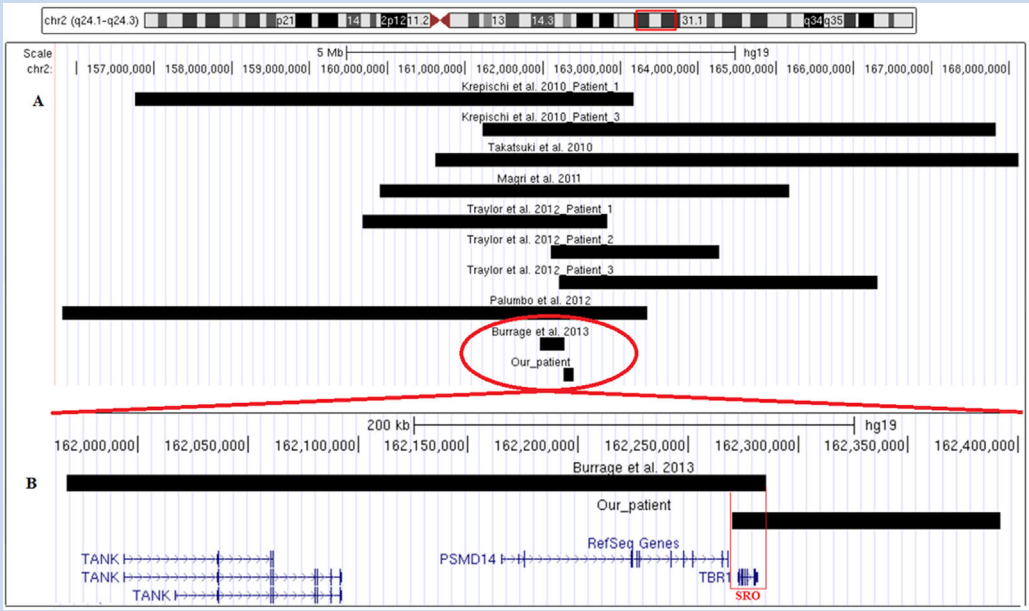

FIG. 2. A: Comparison of the 2q24.2 deletion described in our patient with those of previously reported cases. B: Detailed view of the smallest region of overlap (SRO) between the patients, from 162,269,888 bp (centromeric breakpoint in our patient) to 162,285,346 bp (telomeric breakpoint in the patient described by Burrage at al.). Genomic coordinates are based on assembly GRCh37 (hg19).

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SUPPORTING INFORMATION

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