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Investigation of *TBR1* Hemizygosity: Four Individuals with 2q24 Microdeletions

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Key Words
2q24 · aCGH · Cortical development · Microdeletion · *TBR1*

Abstract
TBR1 encodes a transcription factor with critical roles in corticogenesis, including cortical neuron migration and axon pathfinding, establishment of regional and laminar identity of cortical neurons, and control of glutamatergic neuronal cell fate. Based upon *TBR1*'s role in cortical development, we sought to investigate *TBR1* hemizygosity in individuals referred for genetic evaluation of intellectual disability and developmental delay. We describe 4 patients with microdeletions identified by molecular cytogenetic techniques, encompassing *TBR1* and spanning 2q24.1q31.1, ranging in size from 2.17 to 12.34 Mb. Only the patient with the largest deletion had a possible cortical malformation. Mild ventriculomegaly is the only common brain anomaly, present in all patients; a Chiari I malformation is seen in 2 patients, and mega cisterna magna is seen in a third. Our findings are consistent with *Tbr1* mouse models showing that hemizygosity of the gene requires additional genetic factors for the manifestation of severe structural brain malformations. Other syndromic features are present in these patients, including autism spectrum disorders, ocular colobomas, and craniosynostosis, features that are likely affected by the deletion of genes other than *TBR1*.

TBR1 encodes a T-box family transcription factor expressed in postmitotic projection neurons and functionally significant in embryological corticogenesis [Bulfone et al., 1995; Hevner et al., 2001]. In mice, Tbr1 regulates regional and laminar identity, and the cortex of null mutants displays disturbed laminar organization from defective preplate splitting and *Reln* downregulation [Hevner et al., 2001; Bedogni et al., 2010a]. Tbr1 also acts as a direct transcriptional repressor to restrict the formation of the corticospinal tract to layer 5 of the cortical plate [Han et al., 2011] while specifying corticothalamic neuron identity in layer 6 [McKenna et al., 2011], and it functions in cortical and thalamic axonal pathfinding [Hevner et al., 2002].

TBR1 is part of the PAX6-TBR2-NEUROD-TBR1 transcription factor cascade that is critical for controlling glutamatergic neuronal cell fate in the cortex, cerebellum, and hippocampus [Englund et al., 2005; Hevner et al.,

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2006; Mendez-Gomez et al., 2011], and TBR1 also functions in the CASK-TBR1-RELN pathway necessary for proper neuronal migration during corticogenesis [Hevner et al., 2006]. Mutations of these genes in humans are associated with defects in embryological brain development; for example, individuals with heterozygous *PAX6* mutations have been reported to have eye malformations and absence or hypoplasia of the anterior commissure, reduced olfaction, absence of the pineal gland, and polymicrogyria [Sisodiya et al., 2001; Mitchell et al., 2003]. Likewise, heterozygous mutation or deletion of *CASK* results in microcephaly, simplified gyral pattern, thin brainstem with flattening of the pons, and severe cerebellar hypoplasia [Najm et al., 2008]. Homozygous mutation of *RELN* has been associated with lissencephaly, a neuronal migration disorder [Hong et al., 2000]. Disruption of these pathways in animals demonstrate their crucial function in embryological brain development [Englund et al., 2005; Atasoy et al., 2007; Arnold et al., 2008; Tuoc et al., 2009; Bedogni et al., 2010a], though homozygous *Tbr1* deletion is required in mice before brain abnormalities manifest. Heterozygous mice do not display an obvious phenotype [Bulfone et al., 1998]. Given its roles in neurodevelopment, we targeted *TBR1* for study to investigate whether hemizygosity would result in cortical malformations and neurological impairment.

Materials and Methods

Patient Ascertainment

Patients 1, 3, and 4 were ascertained by Signature Genomic Laboratories (Spokane, Wash., USA) following referral for clinical microarray-based comparative genomic hybridization (aCGH) testing. Patient 2 was ascertained by Nemours Children's Clinic. Written consent was obtained to publish images using an Institutional Review Board Spokane-approved consent form.

Oligonucleotide-Based aCGH

Oligonucleotide-based aCGH analysis was performed at Signature Genomics on DNA from patients 1, 3, and 4 using a 105K-feature, whole-genome microarray (SignatureChipOS® version 1, custom-designed by Signature Genomics; manufactured by Agilent Technologies, Santa Clara, Calif., USA) as previously described [Ballif et al., 2008]. Results were analyzed and visualized using aCGH analysis and web-based data visualization software (Genoglyphix®; Signature Genomics).

Single Nucleotide Polymorphism Array

A single nucleotide polymorphism (SNP) array (Genome-Wide Human SNP Array 6.0; Affymetrix, Santa Clara, Calif., USA), which consists of 906,600 SNPs and 946,000 probes for analyzing copy number variation, was performed at LabCorp (Burlington, N.C., USA) on patient 2. The mean intermarker distance for the SNP array is 700 bp. In brief, DNA was extracted from whole blood, and 250 ng of patient DNA was digested with restriction enzymes. Next, the fragments were ligated to adaptors that are recognized by a particular primer necessary for polymerase chain reaction amplification within a certain size range. Finally, the amplified DNA was fragmented and labeled prior to array hybridization, and visualization was performed following washing and staining of the array. GeneChip Genotyping Analysis Software (Affymetrix, Santa Clara, Calif., USA) was used to statistically analyze data and obtain breakpoints that were reported via graphic visualization.

FISH

Copy number abnormalities detected by microarray in patients 1–4 were visualized by metaphase FISH using one or more BAC clones located within the abnormal regions as previously described [Traylor et al., 2009]. When available, parental samples were also analyzed using FISH.

Results

Microarray analysis in each of the patients identified a deletion of 2q24, including *TBR1* (fig. 1). No additional clinically significant copy number changes were identified in any of the 4 patients. Parental FISH testing for patients 2 and 3 showed the deletions to be apparently de novo in origin. All other parental samples were unavailable for testing (table 1).

Clinical Summaries

Patient 1 is an 11.5-year-old male with global developmental delay (DD), moderate intellectual disability, attention deficit hyperactivity disorder, speech apraxia, dysphagia, and small size. He was born at 42 weeks gestation to a G2P1 mother following a pregnancy complicated by bleeding at 3 months and a choroid plexus cyst identified by ultrasound. His birth weight was at the 10th–25th percentile, and length was at the 75th–90th percentile. His postnatal course was complicated by a sebaceous cyst that was removed at 6 months of age. DD and constitutional small size were first noted in the early newborn period. Developmentally, he sat at 7 months, stood at 9 months, walked at 1.5 years, and had his first words at 2 years. A formal psychological evaluation performed at 5 years 11 months showed a Pictorial Intelligence Quotient in the range of 52–58 (normal 85–115) and a General Adaptive Composite in the range of 50–63 (normal 85–115). Staring spells prompted an MRI and electroencephalogram (EEG) at 5 years of age, but both were reportedly normal. MRI at 6 years 5 months showed mild ventriculomegaly and mild mega cisterna magna with normal versus borderline small size of the cerebellar ver-

Table 1. Features of individuals with deletions including *TBR1*

Features	Patient 1	Patient 2	Patient 3	Patient 4	Langer et al. [2006]	Krepischi et al. [2010] Patient 1	Krepischi et al. [2010] Patient 3	Takatsuki et al. [2010]	Magri et al. [2011]	Palumbo et al. [2010]
Sex	male	male	male	male	female	female	female	female	male	female
Age, years	11.5	8	33 months	16 months	2 years 8 months	14	5	5 months	3.5	3
Minimum deletion coordinates (hg18)	159,392,858–162,536,161	161,813,983–163,980,679	161,919,306–166,011,752	159,307,955–171,648,560	159,108,825–171,723,298	156,469,445–162,877,841	160,937,947–167,531,521	160,332,614–167,824,700	159,618,452–164,882,054	155,526,470–163,058,894
Deletion size, Mb	3.14	2.17	4.09	12.34	12.61	6.41	6.59	7.49	5.26	7.53
Number of genes	15	9	16; includes *SCN2A*	64; includes *SCN1A*	65; includes *SCN1A*	31	27; includes *SCN1A*	33; includes *SCN1A*	22	33
Inheritance	unknown	de novo	de novo	unknown	de novo	de novo	de novo	de novo	de novo	de novo
Growth features										
Birth weight percentile	10th–25th	25th–50th	50th–75th	3rd	15th	<3rd	50th	<3rd (–2.4 SD)	3rd	50th
Weight percentile	<3rd (–4.2 SD)	95th	<3rd (–2.1 SD)	<3rd (–5 SD)	80th	50th–75th	NS	<3rd (–2.3 SD)	NS	25th
Height percentile	5th–10th	71st	3rd–10th	<3rd (–4 SD)	50th	<3rd (–2.3 SD)	NS	3rd–10th	10th	50th
OFC percentile	2nd	51st	10th–25th	<3rd (–7.2 SD)	3rd	5th	<3rd (–4.3 SD)	NS	NS	10th
Neurological features										
DD/ID	moderate	+	+	severe	severe	+	profound	+	severe	+
Speech	apraxia; nasal voice	no speech; uses signs	single word	no speech	no speech	no speech	no speech	NA	NS	first words at 3 years
Behavior problems	ADHD; some perseverations	PDD; tantrums; hyperactivity	autistic-like	NA	–	autistic	–	NA	–	–
Hypotonia	+	+	+	–	+	–	moderate	+	severe	generalized
Seizures	staring spells at 5 years	–	–	onset 11 week, infantile spasms and complex partial seizures, intractable	onset 3.5 months, initially 40 daily	onset 10 years, tonic-generalized	onset 2 months, intractable epilepsy, varied seizure types	at 2 months, continuous myoclonic jerks, intractable; at 3 months, tonic-clonic	–	–
EEG	normal	abnormal	NA	abnormal	NS	abnormal	abnormal	NS	NA	NA
Brain malformations	mega cisterna magna, mild ventriculomegaly	thick CC, mild ventriculomegaly	Chiari I, thick CC, mild ventriculomegaly	Chiari I, possible cortical dysplasia, mild ventriculomegaly	ultrasound: lack of homogeneity around thalamus	normal MRI	normal MRI	NS	normal ultrasound	normal MRI
Other						ataxic gait				bruxism
Ophthalmologic features	–	strabismus, surgically repaired	intermittent left esotropia	bilateral colobomas	microphthalmia, coloboma, blepharophimosis	–	bilateral coloboma	–	–	–

Table 1 (continued)

Features	Patient 1	Patient 2	Patient 3	Patient 4	Langer et al. [2006]	Krepischi et al. [2010] Patient 1	Krepischi et al. [2010] Patient 3	Takatsuki et al. [2010]	Magri et al. [2011]	Palumbo et al. [2010]
Dysmorphic features										
Head	–	facial asymmetry	–	craniosynostosis	bulging metopic suture	–	brachycephaly	–	flat occiput, fine hair, prominent forehead	–
Auricular region	–	–	small and low-set ears	low-set and rotated ears	low-set and rotated ears	–	–	low-set ears	–	–
Periocular region	–	downslanting PF	epicanthal folds, short PF	small and downslanting PF	short and downslanting PF, hypertelorism	–	–	thick and arched eyebrows, upslanting PF, long eyelashes	sparse eyebrows	–
Midface	–	wide alae nasi	–	–	broad nasal root	–	thin nose, depressed and broad nasal bridge, anteverted nares	flat nasal bridge, short nose	–	–
Perioral region	–	–	–	retrognathia	–	–	short philtrum	long philtrum, small mouth, micrognathia	large mouth, short philtrum, micrognathia	–
Limb features	hyperextensible elbows	turned-in foot	tapered fingers; joint laxity	brachydactyly; STPC	–	small feet and hands, tapered fingers, valgus feet, abduction of upper limbs	tapered fingers	–	camptodactyly of the hallux; joint laxity	–
Respiratory features	frequent infections	normal	–	respiratory failure	central apnea	–	recurrent infections due to swallowing difficulties	emphysema caused by viral infection	–	–
Gastrointestinal features	dysphagia	GERD	GERD, resolved	G-tube feedings	–	–	–	–	–	–
Endocrine features	–	–	–	hypothyroidism	–	–	hypothyroidism	–	–	–
Other features			t(7;10)(q22;q26)	wide-spaced nipples, chordee			displaced anus		hypotrophic muscles	hip dislocation

+ = Feature present; – = feature absent; ADHD = attention deficit hyperactivity disorder; CC = corpus callosum; GERD = gastroesophageal reflux disease; ID = intellectual disability; NA = not applicable; NS = not specified; PDD = pervasive developmental disorder; PF = palpebral fissures; SD = standard deviations; STPC = single transverse palmar crease.

mis (fig. 2). He has a tendency for frequent respiratory infections. He takes methylphenidate daily for attention deficit hyperactivity disorder. At the age of 11.5 years his weight is <3rd percentile, his height is at the 5th–10th percentile, and his occipitofrontal circumference (OFC) is at the 2nd percentile. He has verbal apraxia and hypernasal speech. He speaks in sentences and phrases but is not easily understood. He has both fine motor and gross motor delays. He can write his first name only. He has tight heel cords, decreased muscle tone, foot slap, and does some toe walking. Oligonucleotide aCGH, performed at 11.5 years, revealed a 3.14-Mb, apparently de novo 2q24.1q24.2 deletion (fig. 1).

Patient 2 is an 8-year-old male with significant verbal expressive language delay and possible pervasive developmental disorder. He was born at 39 weeks gestation by Cesarean delivery after his mother developed preeclampsia late in the pregnancy. She suffered from frequent emesis but was otherwise healthy during the pregnancy. Following an abnormal maternal serum screen,

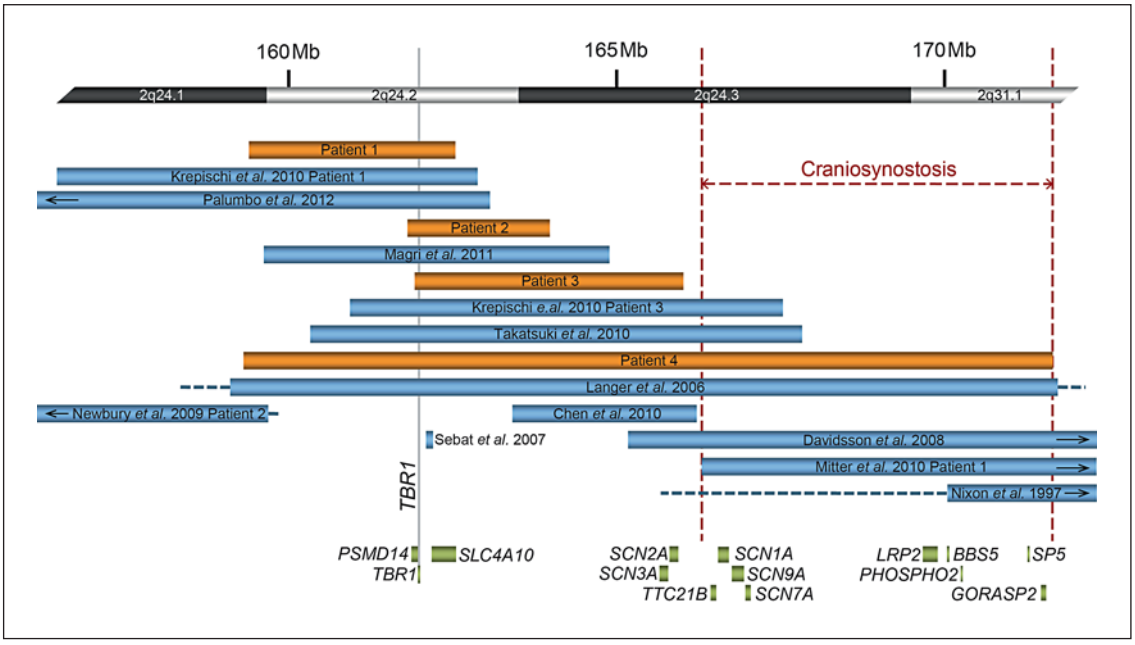
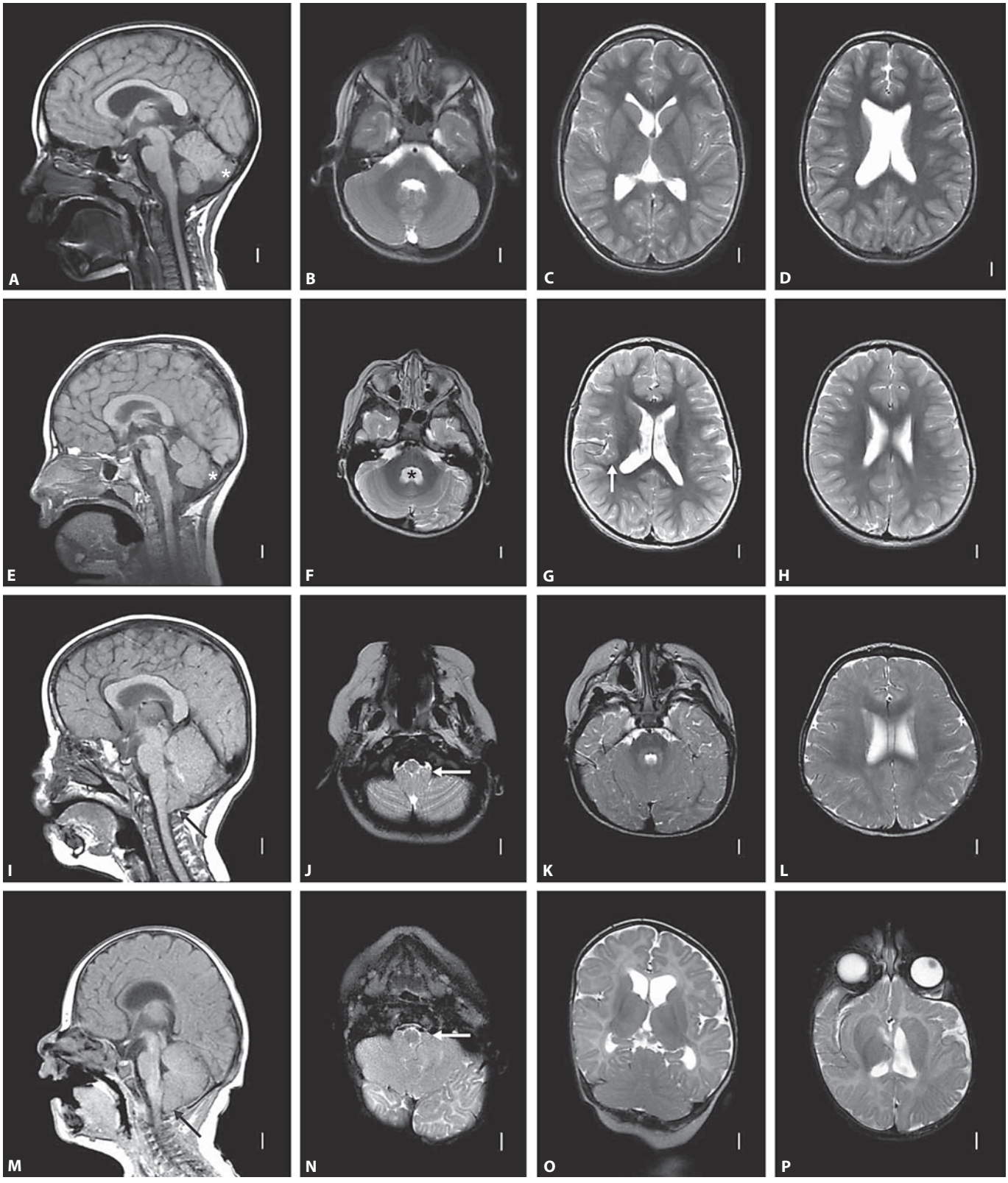


Fig. 1. Overview of molecularly defined deletions within 2q24.1q31.1. Schematic of cases in the literature (shown in light blue) with deletions characterized by molecular cytogenetic techniques and those in patients 1–4 (shown in orange). At the top of the figure there is a partial idiogram showing chromosome bands 2q24.1q31.1, with genomic coordinates corresponding to the hg18 build of the human genome. Blue and orange bars represent minimum deletion sizes, and horizontal dashed lines extend to show maximum deletion sizes. Genes of note within the region are represented by green bars. The vertical gray solid line indicates the location of *TBR1*. The largest possible region of overlap among individuals with craniosynostosis is bordered by red dashed lines.

Fig. 2. MRI images of patients 1–4. Brain MRI in patients 1 (**A–D**), 2 (**E–H**), 3 (**I–L**) and 4 (**M–P**) with deletion 2q24 include T1-weighted midline sagittal (left column) images and T2-weighted axial images at multiple levels (all others except 1 coronal image in **O**). The midline sagittal images in patients 1 (**A**) and 2 (**E**) show normal skull shape and mildly prominent extra-axial fluid below and behind the cerebellum, indicating subtle mega cisterna magna (asterisks in **A** and **E** overlie the hemispheres, not vermis). The fourth ventricle is mildly enlarged in patient 2 (asterisk in **F**), and the lateral ventricles are mildly enlarged in both patients (**C**, **D**, **G**, **H**). Midline sagittal images in patients 3 (**I**) and 4 (**M**) show brachycephaly, small posterior fossa with reduced extra-axial spaces surrounding the cerebellum, small 'pinched' fourth ventricle, and marked cerebellar tonsillar ectopia consistent with Chiari malformation type 1 (long arrows in **I** and **M**). The low lying cerebellar tonsils are also seen on low axial images (arrows in **J** and **N**). The coronal image in patient 4 (**O**) shows mild upward displacement of the anterior (superior) cerebellum, suggesting a small posterior fossa. The lateral ventricles appear normal in patient 3 and mildly enlarged and asymmetric in patient 4. The angle of the axial images in **P** is nonstandard, lending an unusual appearance to the ventricles.



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fetal karyotyping was performed and showed a normal male result, 46,XY. His birth weight was at the 25th–50th percentile, and his length was at the 50th–75th percentile. At 3 months of age, he was diagnosed with gastroesophageal reflux disease. At 18 months, he was diagnosed with pervasive developmental disorder and placed on a gluten-free, casein-free diet. Examination at 2 years of age indicated DD, hypotonia, and a history of alternating between constipation and diarrhea. While EEG exam was normal, MRI showed a mild dilation of the ventricles and thick corpus callosum (fig. 2). Subsequent EEG revealed abnormal spikes and wave patterns during sleep, but he has never had any overt seizures, and he takes valproic acid, which he tolerates well. He had a tendon transfer due to turning in of his foot and strabismus surgery. He is nonverbal but has about 200 signs and is able to communicate using a tablet computer. He experiences some difficulty interacting with other children, has tantrums, and has some obsessive-compulsive traits. Treatment with guanfacine has helped with focus and decreasing hyperactivity. At 8 years of age, his weight is at the 95th percentile, his height is at the 71st percentile, and his OFC is at the 51st percentile. Minor dysmorphic features include a slight facial asymmetry with the right cheek fuller than the left, mildly downslanting palpebral fissures, and a slight widening of the alae nasi. SNP array analysis revealed a 2.17-Mb, apparently de novo 2q24.2q24.3 deletion (fig. 1).

Patient 3 is a 33-month-old male with DD and hypotonia. He was born at 41 weeks gestation by Cesarean delivery to a 28-year-old G2P1 mother who had a kidney stone during the fourth month of pregnancy. His birth weight was at the 50th–75th percentile, and length was >95th percentile. He was noted to have significant gastroesophageal reflux in the first month of life with recurrent vomiting and poor suck. At 6 months of age, he was noted to have DD as he did not roll over or sit unaided. At 12 months of age, he had tubes placed in his ears for recurrent otitis media. MRI at 12 months showed a moderate to severe Chiari I malformation with cerebellar tonsils herniated ~8 mm below foramen magnum (fig. 2). Chromosome analysis showed an apparently balanced, de novo translocation, 46,XY,t(7;10)(q22;q26). By 14 months, his reflux had resolved, but he occasionally suffered from constipation. He first walked at 27 months, and at 33 months has a single word ('hey'). He is also reported to have autistic-like features. At 33 months, his weight is <3rd percentile, his height is at the 3rd–10th percentile, and his OFC is at the 10th–25th percentile. Dysmorphic features include short palpebral fissures, intermittent left esotropia, slight bilateral epicanthal folds, subtle low-set ears, slight taper in the fingers, and overall small size. Oligonucleotide aCGH, performed at 14 months, revealed a 4.09-Mb, apparently de novo 2q24.2q24.3 deletion (fig. 1).

Patient 4 was a male with severe DD, craniosynostosis, epilepsy, colobomas, and growth retardation, who passed away at 16 months. He was born at 39 weeks via induced vaginal delivery to a 30-year-old G2P1 mother following an uncomplicated pregnancy. He was noted to have dysmorphic features and to be small for gestational age. Dysmorphic features included small downslanting palpebral fissures, posteriorly rotated ears, wide-spaced nipples, chordee of penis, brachydactyly, a single transverse palmar crease on the right hand, and a bridged palmar crease on the left hand. At 1 month, ophthalmology exam showed severe uveal coloboma on the left with likely no potential for central visual development and coloboma of the choroid and retina on the right with likely preserved vision. Cytogenetic studies showed 46,XY,inv(9)(p11q13); the inverted 9 is a known population variant. Head CT at 2 months showed craniosynostosis consisting of fusion of the posterior portion of the sagittal suture and superior portion of the left coronal suture, which was repaired at 6 months. At 11 weeks of age, he began having seizures as frequently as 3 times per day that were characterized by staring with a jerking of his hands and feet and nystagmoid movements of the eye preceded by crying. The seizures were temporarily stopped with the use of phenobarbitol but then recurred. He was placed on levetiracetam and oxcarbazepine, and then phenytoin with clonazepam and diazapam daily, but he continued to have 6–20 seizures daily. EEG at 4 months showed mild, diffuse dysfunction in both hemispheres, at 6 months showed frequent left temporal interictal discharges, and at 9 months showed unusual diffusely slow background pattern and multifocal sharp wave discharges that were potentially epileptogenic. MRI at 4 months showed a Chiari I malformation (fig. 2). While his length was following a curve below the third percentile, he was gaining very little weight. He was diagnosed with hypothyroidism and required G-tube feedings. He died at 16 months of age while asleep, most likely due to respiratory failure in relation to a seizure; bronchoscopy at 14 months had shown pharyngeal collapse, glossoptosis, and laryngomalacia. Oligonucleotide aCGH, performed at 1.5 months, revealed a 12.34-Mb 2q24.1q31.1 deletion (fig. 1).

Discussion

Based on its demonstrated role in corticogenesis in mouse models, as well as its involvement in critical developmental pathways, we sought to interrogate *TBR1* hemizygosity for association with cortical malformations and neurological impairment. We gathered detailed clinical records and brain images for a cohort of 4 individuals with 2q24.2 deletions encompassing *TBR1*. Whilst the individuals reported here were referred for genetic evaluation of intellectual disability and DD, other features common to this cohort include hypotonia (3/4), dysphagia (4/4), constitutional small size and/or low weight (3/4), behavior problems (3/3), downslanting palpebral fissures (2/4), and mildly abnormal ears (2/4), all relatively nonspecific features that are common in individuals with chromosomal abnormalities. The absence of a common, distinctive phenotype among the 4 patients leads us to conclude that *TBR1* deletions do not result in a recognizable, novel microdeletion syndrome. In addition, syndromic features present in some of these patients are likely attributable to deletion of multiple genes in the region, in combination with other background genetic/environmental factors, such as the de novo translocation in patient 3.

Within this cohort various brain abnormalities were identified by MRI, and the only common finding was mild ventriculomegaly, another relatively nonspecific finding. Only patient 4, with the largest deletion, had a possible malformation in the right perisylvian region of the cortex (fig. 2), which was our hypothesized *TBR1*-associated malformation, given the role of TBR1 in corticogenesis. Cortical malformations were not seen in the other patients in our study, nor have they been reported in other individuals in the literature with *TBR1* deletions. Patient 2, with the smallest deletion, showed only mild changes on MRI, and patient 1 displayed a mega cisterna magna, a feature opposite from the Chiari I malformation observed in patients 3 and 4. Patients 2 and 3 each had a thick corpus callosum, a feature not seen in patient 1, while patient 4 was too young to ascertain this feature. *TBR1* hemizygosity may possibly contribute to these structural brain changes, but as seen with human mutations of other genes in the PAX6 transcription factor cascade and the RELN pathway, homozygous loss may be required before major brain malformations manifest [Glaser et al., 1994; Baala et al., 2007; Solomon et al., 2009]. Additionally, the more severe malformations, such as the Chiari I malformations in patients 3 and 4, may be attributed to loss of other gene(s) in their ~4-Mb shared deletion region. Alternatively, as Chiari I malformations have been observed in association with craniosynostosis, like in patient 4, and are thought to be due to mechanical forces put on the brain [Raybaud and Di Rocco, 2007], the malformations may have different etiologies in the 2 patients.

Deletions of various sizes spanning the 2q21q31 region have been reported in over 100 cases and are associated with a broad spectrum of phenotypic features [Pereira et al., 2004; Langer et al., 2006; Pescucci et al., 2007; Davidsson et al., 2008; Newbury et al., 2009; Chen et al., 2010; Krepischi et al., 2010; Takatsuki et al., 2010; Magri et al., 2011; Palumbo et al., 2012], including seizure disorder [Grosso et al., 2008]. Substantial evidence indicates that the sodium channel (SCN) α subunit genes of the 2q24.3 region, in particular *SCN1A*, induce the seizure phenotype when mutated or deleted [Davidsson et al., 2008; Escayg and Goldin, 2010], although there is evidence for the contribution of *SLC4A10* at 2q24.2 to a milder seizure phenotype when deleted [Krepischi et al., 2010]. In our cohort, patient 4 had deletion of the entire *SCN* gene cluster and intractable seizures with onset at 11 weeks, while the rest of the patients have deletions that include *SLC4A10,* but spare *SCN1A,* and do not have any confirmed seizure activity. Due to deletion of *SLC4A10* these patients may still be at risk for later-onset seizures, as previously reported [Gurnett et al., 2008; Krepischi et al., 2010], although the oldest patient remains seizure-free at 11.5 years.

Common features in several of our, and previously reported, patients may aid in other genotype-phenotype correlations for 2q24q31 deletions. For example, an autism spectrum disorder was present in patient 2 and a previously reported patient [Krepischi et al., 2010], and other behavioral problems were seen in patients 1 and 3. Only 3 genes are commonly deleted in these individuals *(PSMD14, TBR1,* and *SLC4A10),* which are all involved in proper brain development and function [Hevner et al., 2001; Staropoli and Abeliovich, 2005; Jacobs et al., 2008]. Furthermore, *TBR1* interacts with autism susceptibility gene *AUTS2* mRNA [Bedogni et al., 2010b], and there has been a report of a female with autism and a de novo deletion of *SLC4A10* (fig. 1) [Sebat et al., 2007]. We cannot eliminate the possibility of multiple genes in this region contributing to a behavioral phenotype or a positional effect, as 2 other individuals with autism spectrum disorders have been reported with nonoverlapping deletions, one 940 kb distal to *SLC4A10* and the other 2.2 Mb proximal to *PSMD14* (fig. 1) [Newbury et al., 2009; Chen et al., 2010]. Colobomas are present in patient 4 and in 4 previously reported individuals with molecularly defined deletions [Nixon et al., 1997; Langer et al., 2006; Krepischi et al.,

2010; Mitter et al., 2010], 2 of whom have deletions distal to *TBR1* (fig. 1). A possible shared deletion region among these individuals is in the area of the *SCN* gene cluster, and a candidate gene in this region is *TTC21B*; in mice, Ttc21b encodes an axonemal protein required for intraflagellar transport that helps to control Shh signaling [Stottmann et al., 2009]. Heterozygous and homozygous mutations in *TTC21B* are found in some individuals with various ciliopathies, including Joubert syndrome [Davis et al., 2011], of which chorioretinal coloboma can be a feature [Parisi, 2009]. However, as the patient reported by Nixon et al. [1997] did not have any seizures, it is unlikely that the patient's deletion included *SCN1A* and the more proximally located *TTC21B*. Instead, deletion of multiple genes in the region may contribute to the formation of colobomas, which is supported by the report of an additional individual with colobomas and a 2q31.1 deletion [Mitter et al., 2010]. Three additional candidate genes for coloboma are present in patient 3's deleted region, including *TBR1*, which is a candidate due to its role in the *PAX6* transcription factor cascade and *PAX6*'s well-established role in ocular development and mutation in coloboma [Azuma et al., 2003; Nallathambi et al., 2006]. Second, *BBS5* is also involved in ciliary function and implicated in Bardet-Biedl syndrome, although coloboma is rare in Bardet-Biedl syndrome [Li et al., 2004; Hjortshoj et al., 2008]. Third, *LRP2* encodes an endocytic receptor, and autosomal recessive mutations in *LRP2* cause Donnai-Barrow or facio-oculo-acoustico-renal syndrome, of which iris colobomas are a feature [Chassaing et al., 2003; Kantarci et al., 2007]. Craniosynostosis is present in patient 4 and has occasionally been reported in individuals with molecularly characterized 2q deletions [Nixon et al., 1997; Davidsson et al., 2008; Mitter et al., 2010], who share a deletion region distal to *TBR1* (fig. 1). This region has multiple candidate genes including *PHOSPHO2*, which may be involved in the generation of phosphate for bone mineralization [Roberts et al., 2005]. *SP5* encodes a transcription factor that mediates WNT signaling [Weidinger et al., 2005; Fujimura et al., 2007], and inhibition of WNT signaling can lead to craniosynostosis [Behr et al., 2010]. *GORASP2*, also in the region, encodes a Golgi-associated protein that is required for activation of MMP14 and MMP16 [Roghi et al., 2010], metalloproteases required for normal bone formation [Holmbeck et al., 2003, 2005; Shi et al., 2008; Zhou et al., 2009], and copy number gain of another metalloprotease gene, *MMP23*, has been implicated in craniosynostosis [Gajecka et al., 2005].

Our genotype-first approach to establish phenotypic consequences of *TBR1* deletion has shown that *TBR1* hemizygosity alone does not cause significant brain anomalies. This is consistent with animal models, which are essentially phenotypically normal when missing 1 copy of *Tbr1* [Bulfone et al., 1998] but have neocortical and deep cerebellar malformations with homozygous loss [Hevner et al., 2001 2002; Fink et al., 2006]. It is possible that *TBR1* hemizygosity is contributing to the neurodevelopmental phenotypes in these patients, given its role in neurodevelopment and high likelihood of being subject to haploinsufficiency [Huang et al., 2010]. However, our patients show additional clinical features that are likely due to deleted genes other than *TBR1*, and a comparison of our patients to earlier reports has allowed for the identification of some possible critical regions for features including craniosynostosis and colobomas. While this study demonstrates the challenges inherent in the genotype-first approach, when the deletion of the candidate gene may only have mild effects or contribute to a nonspecific phenotype, it also implies potential in mining genomic databases of copy number variations for candidate genes. Through a genotype-first approach, cohorts of patients with loss of developmentally important genes can be evaluated simultaneously to further delineate clinical significance.

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