BRAIN
LETTER TO THE EDITOR

Biallelic *ZBTB11* variants associated with complex neuropsychiatric phenotype featuring Tourette syndrome

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We read with great interest the recent article by Sumathipala *et al.*[1] delineating the role of biallelic variants in *ZBTB11* (OMIM *618181) in a rare neurodevelopmental condition known as autosomal recessive intellectual developmental disorder 69 (MRT69, OMIM #618383).

ZBTB11 encodes the zinc finger and BTB (broad-complex, tramtrack, bric-à-brac) domain containing 11, a member of the ZBTB zinc fingers (also known as BTB-ZF or POK, Pox virus and Krüppel-like zinc fingers) superfamily of ~50 proteins consisting of a BTB domain and a variable number of zinc fingers.[2] Zinc fingers mediate the DNA binding, while the BTB domain can homo- or heterodimerize with several cell-specific interactors, including transcriptional repressors such as histone deacetylases (HDACs), thus allowing the ZBTB zinc fingers to act as either transcriptional repressors (in most cases) or activators.[2]

In humans, the link between ZBTB11 and brain development is supported by the identification of biallelic *ZBTB11* variants in three consanguineous families showing moderate intellectual disability (ID), microcephaly, ataxia, spasticity, drooling and brain MRI abnormalities.[3,4] More recently, five new cases from three independent families have been described.[1] These subjects presented with mild-to-profound ID, variably associated with microcephaly, ataxia, spasticity or hypotonia, drooling and heterogeneous neuroimaging findings, such as corpus callosum and cerebellar atrophy.[1] Combined malonic and methyl malonic aciduria (CMMA) was also detected in three of five subjects.[1]

We report a patient harbouring novel biallelic variants in *ZBTB11* and presenting with a complex neuropsychiatric phenotype, expanding the molecular and phenotypic spectrum of MRT69. Written informed consent was obtained from the parents and the study was approved by the Gaslini Children's Hospital ethics committee. The boy was born at term after an uneventful pregnancy and neonatal course. Developmental milestones were regularly met, but speech was impaired due to persistent dyslalia. At the age of 2 years the patient was diagnosed with attention deficit hyperactivity disorder (ADHD), and 4 years later, complex motor tics started, consisting of hand rubbing and repetitive anterior trunk flexion. Tics became recurrent and expanded to facial grimacing, tongue protrusion and clicking, hand robbing, and shoulder abduction. The patient developed coprolalia and experienced recurrent episodes of psychomotor agitation with auto- and heteroaggressive outbursts, occurring in response to even mild frustration and often associated with suicidal ideation.

At the age of 6 years, the occipitofrontal circumference was 50 cm [−1.5 (standard deviation score, SDS)], weight 27.5 kg (1.73 SDS) and height (2.33 SDS). A neuropsychological evaluation revealed normal total IQ (88) and confirmed the diagnosis of Tourette syndrome. His sleep EEG showed bilateral biphasic spikes and spike-and-wave complexes in the centro-temporo-occipital regions (Supplementary Fig. 1). At 13 years, assessment revealed oppositional behaviour, restlessness and easy distractibility (Supplementary Video 1 available at FigShare doi:10.6084/m9.figshare.20935894). Speech was limited to single words with persistent dyslalia. Neurological examination showed paratonia and clumsiness. Additional features were selective eating and occasional enuresis and encopresis. A combined therapy of aripirazole

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(5 mg, b.i.d), valproate (375 mg, b.i.d.), promazine hydrochloride (8 mg, b.i.d.) and lithium sulphate (83 mg/day) failed to control the psychomotor agitation episodes. Metabolic testing excluded malonic acid in the urine, and revealed normal levels of plasma methylmalonic acid (MMA) (0.14 μmol/l, normal <1) and urine MMA (0.64 μmol/mmol, normal <5), with urine MMA/creatinine ratio of 0.05 μmol/mmol (normal <2). Array comparative genomic hybridization (aCGH) detected a *de novo* duplication in 22q11.21 [arr22q11.21(20,719,112–21,464,119)×3] (Supplementary material).

For exome sequencing, genomic DNA was extracted from peripheral blood lymphocytes of the proband and the parents, and trio-exome sequencing was performed (Supplementary material). After filtering for population genetics (allele frequency <0.001), conservation of involved residues and *in silico* predictions (Supplementary Table 1), biallelic missense variants in *ZBTB11* emerged as best candidates: the paternal (NM_014415.4): c.2008A>G (p.Met670Val) and the maternal c.101G>A (p.Arg34Gln) variants (Fig. 1). Both variants are extremely rare (allele frequency of 0.00000398 and 0, respectively), absent in the homozygous state in gnomAD and predicted to be pathogenic by *in silico* tools (Supplementary Table 1). No other putative pathogenic variants were detected in other genes, including major Tourette syndrome-related genes.

In summary, we report a complex neuropsychiatric phenotype associated with *ZBTB11* variants not strictly overlapping the previously described classic phenotype.[1,3,4] However, milder phenotypes have been reported in subjects boring the homozygous c.154C>T (p.Arg52Trp) variant.[1] These patients showed a mild cognitive impairment, subtle brain malformations, and no evident metabolic abnormalities, thus partially resembling our patient.[1] The (p.Arg52Trp) change affects a conserved residue outside ZBTB11 functional domains and lies very close to the Arg34 residue, which is affected in our patient. Although further confirmation is needed, this would lead to suggest that variants located early in the ZBTB11 protein, outside the main functional domains, may be associated with a likely milder and more heterogeneous clinical phenotype.

Behavioural abnormalities reported in *ZBTB11* patients include autism, stereotypies and ritualistic behaviour (Table 1).[1] The neuropsychiatric manifestations in our patient are instead suggestive of Tourette syndrome, a complex condition characterized by tics involving any part of the body (especially the face), usually starting in childhood and often improving in adult life.[5] Although Tourette syndrome is a highly heritable disorder, it has complex genetics involving both common mildly deleterious and rare highly damaging variants in several crucial genes for neuronal function.[5] Recently, a potentially relevant pathophysiological role has been suggested for genes encoding proteins involved in mitochondrial transport (*NDE1*, *DISC1*, *OPA1*), fusion (*OPA1*) and fission (*ADCY*).[6] Of note, *ZBTB11* variants were found to affect ZBTB11 binding to several target genes, including those encoding proteins involved in mitochondrial functions.[1] This is also supported by the mitochondrial dysfunction observed in ZBTB11 patient fibroblasts, mainly resulting from the downregulation of genes implicated in the mitochondrial respiratory complex biogenesis and translation.[1] Overall, these findings suggest a pathophysiological link between Tourette syndrome and the *ZBTB11* variants detected in our patient. The lack of similar neuropsychiatric manifestations in other ZBTB11 patients might depend on several factors, including the localization of the variants, and further studies should confirm this association. However, our case raises the possibility that ZBTB11, as an essential transcriptional regulator, plays a role as a risk gene in Tourette syndrome.

The c.2008A>G (p.Met670Val) variant detected in our patient affects the third cysteine 2/histidine 2 (C2H2) zinc finger domain of ZBTB11, which is implicated in the DNA binding.[2] This change likely affects the binding to the DNA sites, which is crucial for ZBTB11 regulatory function.[2] Variants localized to one of the 12 C2H2 domains of ZBTB11 have already been reported in affected individuals.[1,3] These variants decrease ZBTB11 binding to several target genes, suggesting an underlying loss-of-function mechanism in MRT69.[1] The c.101G>A (p.Arg34Gln) variant affects instead a region preceding the BTB domain. This variant does not affect a functional domain but lies close to the p.Arg52Trp detected in homozygous state in two affected sisters from a consanguineous family, suggesting that variants in this region are deleterious, possibly through a negative impact on the adjacent BTB domain (Fig. 1).[1]

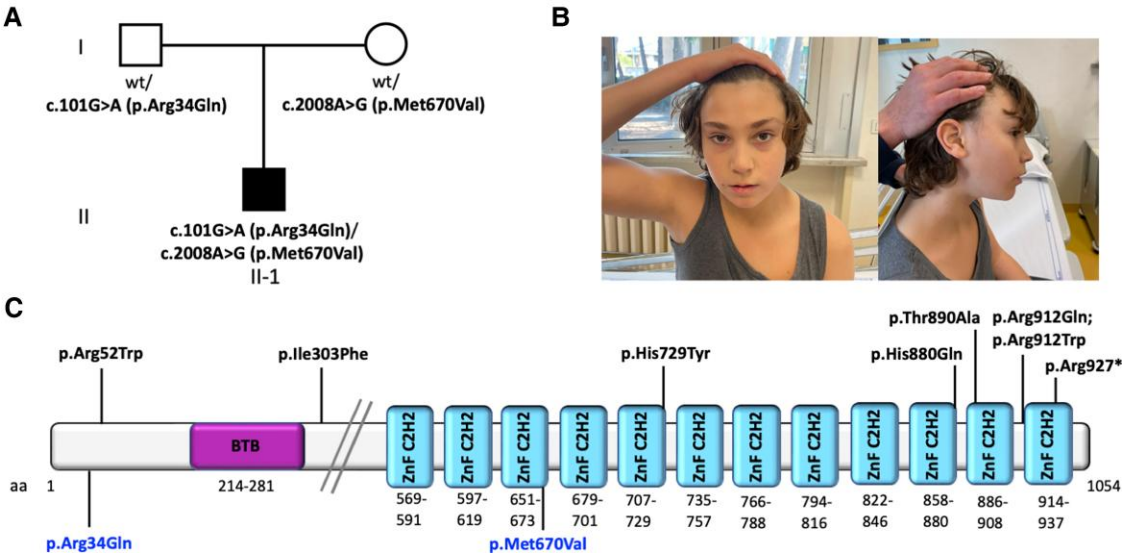

Figure 1 Clinical and genetic features. (**A**) Pedigree of the reported family and segregation of *ZBTB11* variants. (**B**) The patient shows some mild dysmorphic features, including thick eyebrows, upslanting and almond-shaped palpebral fissures, and uplifted ear lobes. (**C**) Schematic diagram of ZBTB11 (NM_014415.4, NP_055230.2, UniProtKB: O95625, ZBT11_HUMAN) with functional domains and localization of pathogenic variants. Pathogenic amino acid changes reported in previous papers and identified in this study are reported above and below, respectively.

Table 1 Behavioural and neuropsychiatric manifestations in *ZBTB11* patients

	Our patient	Family A[3]	Family B[3]	FB.II-1[1]	FA.II-3[1]
ADHD	+	–	–	–	–
ASD	–	–	–	+	–
Tourette syndrome	+	–	–	–	–
Stereotypies and ritualistic behaviour	–	–	–	–	+

ASD = autism spectrum disorder.

The duplicated region identified in our patient lies distally to the region of 22q11.21 duplication syndrome, characterized by dysmorphic features, cognitive impairment, abnormal behaviour and congenital malformations.[7] A similar maternally-inherited duplication was reported in a male patient with dysmorphic features and psychomotor delay.[8] However, the phenotype displayed by our patient is not suggestive of 22q11.21 duplication syndrome and his neuropsychiatric manifestations are more severe as compared to this case.[8] Furthermore, an overlapping duplication is reported in several controls in the Decipher database and the Database of Genomic Variants, suggesting that this rearrangement has uncertain significance.

Behavioural features of patients with the 22q11.21 duplication syndrome include autism, ADHD, schizophrenia and obsessive-compulsive disorder (OCD).[9] One patient harbouring a *de novo* 22q11 duplication (breakpoints not available) presented with Tourette syndrome, stereotypy, OCD and Klippel–Feil anomaly.[10] However, this subject also carried a *de novo* ~3 Mb hemizygous 22q11.2 microduplication within the region of classical 22q11 microdeletion syndrome and a 675A allele of COMT haplotype, causing lower COMT expression in the brain.[10] Both the 22q11 microdeletion syndrome and the 675A allele of COMT haplotype confer risk for neuropsychiatric manifestations, leading to the conclusion that the association of the *de novo* 22q11 duplication with Tourette syndrome was incidental.[10] Although a possible contribution of the 22q11.21 duplication to the ADHD features of our patient cannot be completely excluded, his neuropsychiatric manifestations appear to be distinct and more complex as compared to those reported in subjects with 22q11.21 duplication.[9] It is possible that this rearrangement confers risk for neuropsychiatric disorders and that the *ZBTB11* variants act as genetic modifiers, leading to the full phenotypic expression observed in our patient.

Hence, our case raises the possibility that the phenotypical spectrum of MRT69 may be wider than expected based on previous reports, suggesting that patients harbouring biallelic variants in *ZBTB11* may show a more heterogeneous neurodevelopmental and neuropsychiatric involvement. In particular, Tourette syndrome in our patient suggests a possible pathophysiological link between this condition and the mitochondrial dysfunction resulting from loss of function *ZBTB11* variants. Further studies will play a pivotal role in the refinement of *ZBTB11*-related neuropsychiatric features and the investigation of the potential role of *ZBTB11* as a risk gene for Tourette syndrome.

Data availability

This work includes no new software and/or algorithms. Data are available upon reasonable request.

Web resources

The following URLs were used for data presented herein:

ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/

Combined Annotation Dependent Depletion: http://cadd.gs.washington.edu/

Decipher: https://decipher.sanger.ac.uk

Database of Genomic Variants: http://dgv.tcag.ca/dgv/app/home

Ensembl: https://www.ensembl.org/index.html

Gene Cards: http://www.genecards.org/

Gene Matcher: http://www.genematcher.org

GnomAD: http://gnomad.broadinstitute.org/

Human Splice Finder: http://www.umd.be/HSF/

Iranome: http://www.iranome.ir

Mutalyzer: https://mutalyzer.nl

Mutation Taster: http://www.mutationtaster.org

OMIM: http://www.ncbi.nlm.nih.gov/Omim

PubMed: http://www.ncbi.nlm.nih.gov/pubmed/

RefSeq: https://www.ncbi.nlm.nih.gov/refseq/

SIFT: https://sift.bii.a-star.edu.sg

The 1000 Genomes Browser: http://browser.1000genomes.org/index.html

The Greater Middle East (GME) Variome Project: http://igm.ucsd.edu/gme/index.php

UCSC Human Genome Database: http://www.genome.ucsc.edu

Varsome: https://varsome.com

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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