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De novo missense variants in *ZBTB47* are associated with developmental delays, hypotonia, seizures, gait abnormalities, and variable movement abnormalities

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DECLARATION OF INTERESTS

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing completed at Baylor Genetics Laboratory. MJGS is an employee of GeneDx, LLC.

Editorial Policies and Ethical Considerations.

This study was approved by ethics committees at the respective institutions involved. All patients or their parents provided written signed consent under a research protocol that was approved by an Institutional Review Board (IRB) at the National Institutes of Health for Patient 1 (P1), Baylor College of Medicine (BCM, P2, P3), UT Health San Antonio (P5), or the Department of Clinical Genetics at Erasmus MC in Rotterdam (in agreement with Dutch research legislation, P4).

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Abstract

The collection of known genetic etiologies of neurodevelopmental disorders continues to increase, including several syndromes associated with defects in zinc finger protein transcription factors (ZNFs) that vary in clinical severity from mild learning disabilities and developmental delay to refractory seizures and severe autism spectrum disorder. Here we describe a new neurodevelopmental disorder associated with variants in *ZBTB47* (also known as *ZNF651*), which encodes zinc finger and BTB domain-containing protein 47. Exome sequencing (ES) was performed for five unrelated patients with neurodevelopmental disorders. All five patients are heterozygous for a *de novo* missense variant in *ZBTB47*, with p.(Glu680Gly) (c.2039A>G) detected in one patient and p.(Glu477Lys) (c.1429G>A) identified in the other four patients. Both variants impact conserved amino acid residues. Bioinformatic analysis of each variant is consistent with pathogenicity. We present five unrelated patients with novel, *de novo* missense variants in *ZBTB47* and a phenotype characterized by developmental delay with intellectual disability, seizures, hypotonia, gait abnormalities, and variable movement abnormalities. We propose these variants in *ZBTB47* are the basis of a new neurodevelopmental disorder.

Keywords

ZBTB47; ZNF651; zinc finger; developmental delay; seizure; neurodevelopmental; movement disorder

INTRODUCTION

Neurodevelopmental disorders are characterized by childhood onset and impairment in personal, social, academic, and occupational functioning. Their presentation includes a variable phenotype of intellectual disability, developmental delay, language and speech disorder, autism spectrum disorder, attention deficit/hyperactivity disorder, and motor and movement disorder (Neurodevelopmental Disorders, 2013). The identification and understanding of the genetic basis of neurodevelopmental disorders and neurologic disease are rapidly expanding. The broadening accessibility and growing clinical use of next-generation sequencing (NGS) has resulted in the identification of numerous new disease genes associated with neurodevelopmental phenotypes, including genes encoding transcription factors. There is mounting evidence of the critical role played by transcription factors in the growth and development of the brain, particularly in the differentiation of neural stem cells and the downstream delineation of central nervous system structures (Al-Naama et al., 2020; Lein et al., 2017; Santiago et al., 2014; Silbereis et al., 2015).

Zinc finger protein transcription factors (ZNFs) make up the largest family of transcription factors in eukaryotes (Al-Naama et al., 2020; Fedotova et al., 2017), including humans (Ladomery and Dellaire, 2002), and serve to regulate cell development and differentiation. They are characterized structurally by finger-like protrusions that bind directly to their related DNA (or other macromolecular) sequence with a zinc ion that creates structural stability through an ionic bond with cysteine or histidine residues of the finger (Al-Naama et al., 2020; Cassandri et al., 2017). Cys2His2 (C2H2) is the most prevalent and widely characterized zinc finger domain. On binding to the targeted DNA sequence, ZNFs containing a C2H2 domain (C2H2-ZNFs) recruit cofactors and other transcription factors to regulate downstream transcription of the targeted gene (Al-Naama et al., 2020). There is evidence that ZNFs are involved in multiple categories of human disease, including immune regulation (Fu and Blackshear, 2017; Maeda and Akira, 2017; Scott and Omilusik, 2019), cancer (Cassandri et al., 2017; Jen and Wang, 2016), skeletal development (Funato et al., 2020), and cardiac disease (Cassandri et al., 2017; Reamon-Buettner and Borlak, 2005). There is also increasing evidence that defects in ZNFs may be associated with neurodevelopmental disorders, neuropsychiatric disorders, and neurological disease. At least 68 ZNF genes have been reported in association with brain development, neurodevelopmental disabilities, and/or other neuropsychiatric disorders (Al-Naama, et al., 2020).

Here we present five unrelated patients with a neurodevelopmental phenotype that includes global developmental delay, intellectual disability, hypotonia, seizures, gait abnormalities, and, in some, abnormal movements unrelated to seizure. Exome sequencing (ES) in all five patients revealed a heterozygous *de novo* missense variant in the gene *ZBTB47* (also known as *ZNF651*), which encodes a C2H2-ZNF in which variants have not yet been associated with a human phenotype.

MATERIALS AND METHODS

Editorial Policies and Ethical Considerations.

This study was approved by ethics committees at the respective institutions involved. All patients or their parents provided written signed consent under a research protocol that was approved by an Institutional Review Board (IRB) at the National Institutes of Health for Patient 1 (P1), Baylor College of Medicine (BCM, P2, P3), UT Health San Antonio (P5), or the Department of Clinical Genetics at Erasmus MC in Rotterdam (in agreement with Dutch research legislation, P4).

Ascertainment.—Following the identification of the *de novo ZBTB47* candidate variant in P1, additional patients with *ZBTB47* variants were identified through case matching efforts, including responses to P1's Participant Page on the Undiagnosed Diseases Network (UDN) website (http://undiagnosed.hms.harvard.edu/), connection through GeneMatcher (Sobreira et al., 2015), and collaboration with clinical genetic testing laboratories (GeneDx).

Exome Analysis Methods.

P1—Trio ES was performed on a clinical basis by a commercial clinical genetics laboratory using previously published methods (Yang et al., 2014). The clinical exome data were transferred to the Undiagnosed Diseases Network (BCM site) for research reanalysis, which was performed using Codified Genomics variant review software. *De novo* and biallelic variants with frequency less than 1% in gnomAD (Karczewski et al., 2020) and in BCM's local exome database were prioritized for review. Copy number analysis was performed using single nucleotide polymorphism (SNP)-based chromosomal microarray (CMA) at a commercial clinical genetics laboratory.

P2, P3, P5—ES (trio in P2, P5 and proband in P3) was performed on a clinical basis by a commercial clinical laboratory with protocols previously described (Sacoto et al., 2020), and variants reported were confirmed by an orthogonal method, as appropriate. No further exome data analysis beyond the clinical report was performed. Copy number variant analysis via CMA was performed at a commercial clinical genetics laboratory.

P4—Trio ES was performed on a clinical basis by Erasmus MC University Medical Center Department of Clinical Genetics. DNA was enriched using Agilent SureSelect DNA + SureSelect OneSeq 300kb CNV backbone + Human All Exon V7 capture and paired-end sequenced on the Illumina platform. The data were demultiplexed with bcl2fastq Conversion Software from Illumina. Reads were mapped to the genome using the Burrows-Wheeler Alignment Tool (BWA-MEM algorithm). Sequence variant detection was performed by the Genome Analysis Toolkit HaplotypeCaller. The detected sequence variants were filtered and annotated with Alissa Interpret software and classified with Alamut Visual. Copy number variant analysis via CMA (IL-C12_NEXUS) was performed by Erasmus MC University Medical Center Department of Clinical Genetics.

RESULTS

Case Presentations

P1—P1 was a 2-year, 5-month-old female at the time of evaluation by the UDN and 5 years old at her most recent clinical evaluation. Pregnancy was complicated by possible placenta previa per maternal report but without known infection or teratogen exposure. Delivery was via Cesarean section at full term and uncomplicated, and the patient was discharged home in the first few days of life. Family history is unremarkable. Developmental delay was first noticed at 6 months of age, when the patient was unable to sit unsupported and did not respond to sound, reach for objects, or make eye contact. At 8 months she began to demonstrate myoclonic spasms, characterized by flexion of the trunk and upper extremities with head falling forward. Shortly afterward, she began to demonstrate constant movement of hands and feet. At most recent evaluation, she was unable to sit unassisted or bear weight, but she was able to roll over. She vocalized and babbled but did not have any words. She demonstrated some eye tracking but did not fix and follow, make eye contact, or respond to sounds or commands. She ate by mouth, but gastric tube placement was being considered. At 5 years of age, no significant dysmorphic features were present on physical examination. Musculoskeletal examination and imaging were consistent with mild neuromuscular scoliosis and neuromuscular hip dysplasia. Growth parameters remained in the normal range (between 20-50th percentile for weight and between 30-80th percentile for height) until around 4 years of age, at which time she began to demonstrate weight loss and poor growth. Most recent growth parameters were as follows: less than the 0.01st percentile for weight (Z-score −4.09), 0.58th percentile for height (Z-score −2.53), and 11th percentile for occipital frontal circumference (OFC, Z-score −1.23). She was evaluated by gastroenterology (GI), diagnosed with severe malnutrition, and started on caloric supplementation. She was referred to neurology due to concern for significant developmental delay, abnormal movements, and seizure activity. On neurological examination, she demonstrated significant generalized hypotonia and near continuous choreiform movements of hands, feet, trunk, and face. She was started on anti-epileptic drugs (AEDs) with poor control of her seizures and continued to have approximately 45 seizures per day. Most recent epilepsy management included clobazam and cannabidiol, with consideration of starting a modified Atkins diet.

Electroencephalogram (EEG) showed multifocal spikes and generalized tonic seizures. Multiple magnetic resonance imaging (MRI) studies of the brain were normal, as was MR spectroscopy. Audiology examinations via otoacoustic emissions and auditory brainstem response were normal. Cerebrospinal fluid (CSF) neurotransmitters, CSF lactate, serum lactate, ammonia, and pyruvic acid were all normal. Metabolic studies were within normal limits, including plasma amino acids, acylcarnitine analysis, and urine organic acids. NMDA, thyroglobulin, and GAD antibodies were negative. Metabolomic studies of the CSF and plasma were performed and were remarkable for elevated plasma pipecolic acid, but other metabolites associated with peroxisomal disorders were unremarkable. CMA was normal. Angelman syndrome methylation analysis and myotonic dystrophy type 1 repeat expansion analysis were normal/negative. Sterol studies for investigation of cholesterol synthesis disorders were normal. Clinical trio exome sequencing (ES) was performed and

identified a heterozygous variant of uncertain significance (VUS) in *CHRNB2* inherited from the unaffected mother. Additional details on the *CHRNB2* variant as well as other variants reported are provided in supplementary table 1. *CHRNB2* is associated with nocturnal frontal lobe epilepsy 3. ES was otherwise considered non-diagnostic clinically with no pathogenic, likely pathogenic variants, or *de novo* VUS in known disease genes. The patient was referred to the UDN for further evaluation, and research reanalysis of the ES data highlighted a heterozygous *de novo* variant in *ZBTB47* [NM_145166.3:c.2039A>G, p.(Glu680Gly)] as a candidate of interest.

P2—P2 was an 11-month-old female at initial presentation and 6 years old at most recent clinical evaluation. Pregnancy history was complicated by preeclampsia. The patient was born at 37 weeks gestational age via vaginal delivery and was transferred to the neonatal intensive care unit (NICU) briefly due to respiratory difficulties. She was discharged at 2 days of life. Breathing and feeding difficulties were present since birth. The patient was diagnosed with laryngomalacia, laryngeal cleft, reflux, and dysphagia. She underwent supraglottoplasty with improvement in respiratory disease. The patient also underwent G-tube placement due to dysphagia and growth concerns. The patient's history was remarkable for hypotonia and global developmental delays. She sat at 13 months and walked at 18 months. Her first words were reported at 2.5 years of age, and two-word phrases were noted at 4 years. The patient also demonstrated fine motor delay. At most recent evaluation, she continued to undergo speech, occupational, and physical therapy twice per week. Physical examination demonstrated dysmorphic features including tented mouth, deep-set eyes, high-arched palate, small nose, relative microcephaly, wrinkly skin, and sagging cheeks. Most recent growth parameters were as follows: 69th percentile for height (Z-score 0.50), 41st percentile for weight (Z-score −0.23), and 4th percentile (Z-score −1.75) for head circumference. The patient was diagnosed with generalized seizures at 2 years of age. Seizures presented during sleep as startling/shuddering attacks. The patient was most recently on valproic acid but continued to have 2-4 seizures per month. She also continued to startle easily. Neurological examination was remarkable for Gowers sign, ataxic gait, diffuse weakness, hypotonia, myoclonus, and hand tremor. Due to developmental and behavioral concerns (tantrums and poor sociability), she received neuropsychological evaluation and was found to have moderate intellectual disability and autism spectrum disorder. Other health concerns of note included muscular ventricular septal defect (VSD), frequent infections, sleep apnea, gastroesophageal reflux disease (GERD), constipation, pressure equalization (PE) tubes, and the requirement of glasses.

Brain MRI was normal. EEG showed primary generalized epilepsy with occasional runs of bifrontal predominant generalized spike and wave discharges with shifting bilateral predominance, occipital intermittent rhythmic delta activity (OIRDA), and occasional irregular and rhythmic delta activity in left greater than right hemispheres. Genetic evaluation included SNP-CMA, which revealed small regions of homozygosity (<10Mb) but was otherwise unremarkable, Prader-Willi methylation studies that were normal, and *DMPK* sequencing that was negative. Trio ES with mitochondrial sequencing identified a heterozygous VUS (ACMG criteria: PM2) in *SLC12A5* [NM_020708.4:c.1955C>T, p.(Ala652Val)] inherited from the unaffected mother. Additional details on this variant may

be found in supplementary table 2. This gene is associated with autosomal dominant susceptibility to idiopathic generalized epilepsy and autosomal recessive developmental and epileptic encephalopathy. No other pathogenic variants, likely pathogenic variants, or VUS related to the clinical phenotype were reported. However, ES reanalysis two years later identified a heterozygous *de novo* variant in *ZBTB47* [NM_145166.3:c.1429G>A, p.(Glu477Lys)].

P3—P3 was a 5-year-old male on initial evaluation and 8 years old at most recent clinical evaluation. Pregnancy was complicated by maternal hypothyroidism treated with levothyroxine. There was no known infection or teratogen exposure during pregnancy. Delivery was vaginal, full-term, and uncomplicated. Family history was unremarkable. Weight at birth was 3.572 kg (51st percentile for age, Z-score 0.03); length at birth was 48.3 cm (24th percentile for age, Z-score −0.71). The neonatal period was uncomplicated. The patient walked at 18 months. His first words occurred at 2 years after receiving speech therapy, and he started using two-word phrases at 5 years. Early language was dominated by echolalia. At most recent evaluation he remained unable to read or write and did not know letters or numbers. His behavior was remarkable for frequent tantrums, screaming with frustration, poor eye contact, and poor sociability. His neuropsychological evaluation was notable for behavioral dysregulation, language and cognitive impairment, fine motor difficulties, and moderate intellectual disability. He was diagnosed with autism spectrum disorder. At most recent evaluation he was feeding by mouth and demonstrated poor growth with the following parameters: weight at the 1st percentile for age (Z-score −2.47), height at the 5th percentile for age (Z-score −1.66), and OFC at the 74th percentile for age (Z-score 0.64). Physical examination demonstrated dysmorphisms, including prominent forehead, deep orbits, borderline hypertelorism, and anteverted nose. Neurological examination was remarkable for hand tremor, wide-based gait, and poor coordination that worsened with time. He was also affected by partial seizures with onset at 17 months old that remained refractory to AEDs (2-4 seizures per month), and he continued off AEDs. Of note, the patient's history was also remarkable for frequent infections, including recurrent otitis media, Salmonella gastroenteritis, and sepsis that required hospitalization. Other medical problems included mild hearing loss, sleep apnea, constipation, intermittent tachycardia, joint laxity, and aortic valve abnormality. Surgical history included tonsillectomy, adenoidectomy, and bilateral PE tube placement.

EEG was remarkable for independent left and right parietal epileptiform discharges. MRI of the brain demonstrated cortical dysplasia of the left temporal lobe and Chiari I malformation, as well as haziness of the gray-white matter interface at the temporal poles. Positron emission tomography (PET) of the brain was consistent with decreased glucose metabolism in the temporal lobes and left frontal lobe. Serum lactate was below normal, and 2-hydroxyglutaric acid and acetoacetic acid were elevated, but these findings were considered nonspecific and non-diagnostic. CMA was normal. Proband ES identified a heterozygous paternally inherited VUS (ACMG criteria PM2+PM+PP3) in *PPOX* (NM_003009.5:c.338+2dupT). Additional details on this variant may be found in supplementary table 3. Heterozygous pathogenic variants in *PPOX* are associated with porphyria variegata. The variant observed in the patient has been previously reported as

likely pathogenic in association with this disorder (Rossetti et al., 2008). Pathogenicity is supported by the variant's location at a consensus splice site. However, this variant is paternally inherited from a currently unaffected father suggesting the possibility of incomplete penetrance or benign status. In addition, porphyria variegata was determined to be an incomplete phenotype match for the patient. No other variants were reported in genes related to the clinical phenotype. However, a heterozygous variant in *ZBTB47* was identified [NM_145166.3:c.1429G>A, p.(Glu477Lys)] and determined to be *de novo* after parental testing.

P4—P4 was a 3-year-old female at first evaluation and 13 years old at most recent clinical evaluation. Pregnancy was complicated by gestational diabetes and prolonged rupture of membranes. The patient was born at 38 weeks gestation. Weight at birth was 2.690 kg (8^{th} percentile for age; Z-score −1.4); length at birth was 50 cm (58^{th} percentile for age; Z-score 0.20); OFC at four weeks of age was 35.5 cm (16^{th} percentile for age; Z-score −0.99). The neonatal period was uncomplicated. Family history was remarkable for a paternal half-aunt with epilepsy that presented after a case of meningitis. The patient's development was relatively unremarkable early in life with some indication of gross motor delay. She sat unassisted at 5 months, crawled at 10 months, and walked at 16 months. She spoke her first word at 12 months and was toilet trained by 18 months. Her developmental delay became more apparent with age. By the age of two years, she was noted to have some difficulty learning. At 3 years old she was noted to have stiff movements with poor gross motor skills and was unable to speak in three-word phrases. She was diagnosed with moderate intellectual disability at 7 years of age, and she received a diagnosis of autism spectrum disorder, characterized by poor eye contact, echolalia, difficulty with social interaction, difficulty accommodating change, anger outbursts, and anxious behavior. The patient was also diagnosed with epilepsy. She was first noted to have staring episodes around 2-3 months of age, followed by febrile seizures at 13 months and 3 years and recurrent tonic-clonic seizures with multiple episodes of status epilepticus starting at age 5 years. She continued to have severe and refractory multifocal epilepsy at most recent evaluation with frequency of 12 seizures per month. Her seizures were managed with clobazam, lamotrigine, midazolam, and sulthiame with improvement. Her most recent growth parameters were as follows: weight at the 58^{th} percentile (Z-score 0.21), height at the 87^{th} percentile (Z-score 1.12), and OFC at the 32^{nd} percentile (Z-score −0.47). Physical examination was notable for dysmorphic features including deep orbits, full cheeks, prominent forehead, small bitemporal diameter, and full upper eyelids. Neurological examination was also remarkable for hypotonia, generalized hypermobility, unsteady gait, poor coordination, and hand flapping with excitement. The patient's medical history was otherwise notable for chronic constipation.

EEG demonstrated multifocal epileptic pattern with consistent focus in the left centroparietal region. Brain MRI was notable only for slightly wide occipital horns. Non-diagnostic genetic testing included epilepsy gene panel, intellectual disability gene panel, *SCN1A* sequencing, *PCDH19* sequencing, *CDKL5* sequencing, mitochondrial DNA sequencing, and CMA. Trio ES identified a heterozygous *de novo* variant in *ZBTB47*

[NM_145166.4:c.1429G>A, p.(Glu477Lys)]. Additional variants identified may be found in Supplementary table 4.

P5—P5 was a 6-year-old male at evaluation. Pregnancy was uncomplicated, and he was born at full term via vaginal delivery. Weight at birth was 3.529 kg (64th percentile; Z-score 0.36); length at birth was 53 cm (89th percentile; Z-score 1.20); OFC at birth was 34.5 cm (50th percentile; Z-score 0.0). His neonatal period was complicated by a brief NICU hospitalization for transient tachypnea of the newborn. Family history was remarkable for febrile seizures in the patient's father and autism spectrum disorder in a paternal first cousin. The patient's infancy was complicated by global developmental delay. He sat unassisted at 7 months of age and walked at 15 months of age. He continued to walk with an unsteady gait at most recent evaluation. His first words occurred at 2 years old, and at most recent evaluation he had only 30-40 words with noted echolalia. His receptive language was better than his expressive language, and he was diagnosed with autism spectrum disorder. He began having seizures at 9 months of age and was diagnosed with generalized tonic-clonic epilepsy with focal motor seizures. At most recent evaluation his seizures were controlled on lacosamide and valproic acid with breakthrough seizures noted every 2-3 months. He was also noted to have a tic disorder and hyperactivity. His most recent growth parameters were as follows: weight at the 83rd percentile (Z-score 0.95), height at the 98th percentile (Z-score 2.22), OFC at the 14th percentile (Z-score −1.08). Physical examination was notable for dysmorphic features including cupped ears, short philtrum with thick upper lip and small café au lait macule on the left thigh. The patient's neurological examination was also remarkable for hypotonia. Other medical problems included dysphagia and chronic rhinitis.

EEG was consistent with the patient's clinical epileptic activity. MRI of the brain was normal. Metabolic workup, including plasma amino acids, urine organic acids, acylcarnitine profile, lactic acid, and pyruvate, was normal. Further genetic workup was also non-diagnostic, including CMA, fragile X testing, an autism/intellectual disability gene panel, and an epilepsy gene panel. Trio ES performed during a genetics reevaluation identified a heterozygous *de novo* variant in *ZBTB47* [NM_145166.3:c.1429G>A, p.(Glu477Lys)]. No other variants were reported in genes related to the clinical phenotype.

Variant Interpretation

P1 is heterozygous for a *de novo* p.(Glu680Gly) (c.2039A>G) missense variant in *ZBTB47*. This variant localizes to the last exon (exon 6) of *ZBTB47* and the final zinc finger domain of the ZBTB47 protein (The UniProt Consortium, 2021; Kopanos et al., 2019) (Figure 1). Analysis of the exome data from P2, P3, P4, and P5 revealed the same *de novo* heterozygous p.(Glu477Lys) (c.1429G>A) missense variant in *ZBTB47*. This variant localizes to exon 2 of *ZBTB47* and the second zinc finger domain, which is noted to be degenerate (The UniProt Consortium, 2021; Kopanos et al., 2019) (Figure 1). Of note, the UniProt database annotates 9 total zinc finger domains in ZBTB47 (The UniProt Consortium, 2021) (Figure 1), while the original paper characterizing ZBTB47 only noted seven zinc finger domains (Kumar, Cheney, Neilsen, et al., 2010), excluding the two where the patients' variants reside, suggesting that these regions less stringently correlate with a canonical C2H2 zinc finger sequence. Despite residing in less typical zinc finger domains, the bioinformatics of

both variants support pathogenicity (Table 1). Neither variant was present in the gnomAD (Karczewski et al., 2020) or ClinVar databases. The GERP scores, a measure of conservation at nucleotide position, are 4.5799 and 4.28 respectively (Cooper et al., 2005), which demonstrate a high level of conservation at these positions. Amino acid conservation is also high across species at both loci (The UniProt Consortium, 2021; Figure 1C). The combined annotation dependent depletion (CADD) scores, a measure of the deleteriousness of variants, are high at 29.9 and 25.3 respectively, and pathogenicity prediction models SIFT and PolyPhen report that both variants as “deleterious” and “probably damaging” respectively.

DISCUSSION

Over 68 ZNF genes have been reported in association with brain development, neurodevelopmental disabilities, and/or other neuropsychiatric disorders (Al-Naama et al., 2020). ZNFs reported in association with developmental delay, autism spectrum disorder, and/or seizures include *ZEB2* (Baxter et al., 2017; Buraniqi and Moodley, 2015; Ghoumid et al., 2013; Yuan et al., 2015), *BCL11A* (Dias et al., 2016; Shimbo et al., 2017; Yoshida et al., 2018), *PLZF* (Fischer et al., 2008), *ZBTB11* (Fattahi et al., 2018), *ZNF292* (Mirzaa et al., 2020), *ZBTB18* (Depienne et al., 2017; van der Schoot et al., 2018), *ZNF462* (Cosemans et al., 2018; Kruszka et al. 2019; Weiss et al., 2017), *ZNF778* (Willemsen et al., 2010), *ZNF41* (Shoichet et al., 2003), *ZNF711* (van der Werf et al., 2017), *ZBTB20* (Cordeddu et al., 2014; Jones et al., 2018; Mattioli et al., 2016), *ZNF407* (Kambouris et al., 2014), *ZNF148* (Stevens et al., 2016), *ZNF142* (Khan et al., 2019), *TSHZ3* (Caubit et al., 2016), *GLI3* (Biesecker, 2004), *ZDHHC8* (Yang et al., 2018), *ZNF81* (Kleefstra et al., 2004), *ZNF804A* (Anitha et al., 2014), and *ZNF674* (Lugtenberg et al., 2006). Other reports document copy number variants which encompass multiple ZNFs and a neurodevelopmental phenotype including developmental delay and seizures (Andrieux et al., 2007; Spreiz et al., 2014). To date, *ZBTB47* has not been associated with human disease. In this report, we present five patients with one of two novel *de novo* heterozygous missense variants in *ZBTB47* with bioinformatics supportive of pathogenicity (Table 1) and a phenotype that includes developmental delay, intellectual disability, seizures, hypotonia, and variable abnormal movements (Table 2).

ZBTB47 (also known as *ZNF651*) is a *ZNF652* paralogue that was first described as a classical C2H2-ZNF located on chromosome 3 (Kumar, Cheney, Neilsen, et al., 2010). The currently recognized canonical transcript (NM_145166.3) encodes a 747 amino acid protein which includes a longer N-terminal region with a BTB domain not recognized in this previous publication. ZBTB47 and ZNF652 demonstrate high conservation in their zinc finger domains (95%) and short carboxy-terminal proline-rich sequence (85%) (Kumar, Cheney, Neilsen, et al., 2010). ZNF652 has been previously demonstrated to interact at its proline-rich carboxy-terminus with the CBFA2T3 protein of the ETO family of transcriptional regulatory proteins to repress transcription (Kumar, Cheney, McKirdy, et al., 2008; Kumar, Manning, et al., 2006). ZBTB47 demonstrates binding of the same DNA sites and analogous interaction with CBFA2T3 at its proline-rich region of the carboxy-terminus, suggesting it also represses transcription (Kumar, Cheney, Neilsen, et al., 2010). However, tissue expression of the two paralogues is different, suggesting their

developmental function may differ. *ZBTB47* is widely expressed, with highest expression noted in cardiovascular tissues, skeletal muscle, and central nervous system tissues per GTEx (The GTEx Consortium, 2020), a tool for comparison of protein expression amongst different tissue types. The probability of being loss-of-function intolerant (pLI) for *ZBTB47* is 1.0, the missense Z-score for the gene is 2.45, and the observed over expected (o/e) missense score is 0.66 (Karczewski et al., 2020), all of which support pathogenicity of our reported missense variants.

The phenotype of our patients is compatible with the general phenotype of neurodevelopmental disorder and developmental delay described in other reported ZNF syndromes but is distinguished by the presence of variable abnormal movements in all patients. P1's movements are choreiform in nature and are seen in the hands, feet, trunk, and face. P2 also has choreiform movements with ambulation, as well as shuddering attacks, twitching during sleep, and easy startling. As a result, choreiform movements are seen in association with both reported variants. P3 does not demonstrate choreiform movements but does present with a significant hand tremor. P4 demonstrates hand-flapping, and P5 presents with a tic disorder. All five patients have poor coordination and abnormal gait. P1 is not ambulatory, P2 demonstrates a Gowers sign in preparation for ambulation, and P3, P4, and P5 walk with a wide-based or unsteady gait. Abnormal movements have been reported in association with ZNF syndromes at least twice previously (Fattahi et al., 2018; Khan et al., 2019), but this finding remains uncommon and may be distinguishing for patients with pathogenic *ZBTB47* variants.

Other less specific neurodevelopmental abnormalities are also present in our cohort of patients, including developmental delay, the primary trigger for evaluation in all five patients. Early motor and speech milestones were mildly delayed in most patients with P1 (who remains non-ambulatory and nonverbal) being an exception. Delays in achieving more advanced skills became more obvious with age. Patients experienced difficulty with motor skills throughout childhood, with poor coordination likely contributing, and their ability to put words together and form sentences were more delayed than age at first words. All continue to demonstrate persistent speech delay. Developmental regression has not been seen in our patients. Moderate intellectual disability has been confirmed in P2, P3, P4, and P5, and intellectual disability is suspected in P1. Four of five patients (P2, P3, P4, and P5), all sharing the p.(Glu680Gly) variant, have been diagnosed with autism spectrum disorder. All reported patients have been diagnosed with seizures and demonstrate hypotonia on exam. The characterization and severity of seizures reported vary (Table 2), but the seizures are refractory in two patients (P1 and P4) and incompletely controlled in two (P2 and P3). The reported seizures do not demonstrate consistent response to specific AEDs. MRI was normal for P1, P2, and P5. P3 was found to have a Chiari I malformation on MRI, as well as cortical dysplasia of the left temporal lobes and poor definition of gray-white matter interface at the temporal lobe. P4 was noted to have slightly wide occipital horns on imaging.

Three of five patients demonstrate growth discrepancy. Both P1 and P3 were appropriate for gestational age at birth but have since developed failure to thrive. Both patients are followed by GI and are being evaluated for failure to thrive and malnutrition. P2 has relative

microcephaly with head circumference around the 4th percentile but with normal height and weight. No consistent pattern of dysmorphic features is seen across our patients, and there is no clear dysmorphic feature profile to suggest for this disorder at this time, although 4 of 5 patients are noted to demonstrate variable dysmorphic facial features (P2, P3, P4, P5).

There are two other features that stand out as possible distinguishing characteristics when examining the phenotypes of our reported patients: cardiac disease and immune dysregulation. Three patients demonstrate a cardiac abnormality of some kind, including structural anomalies (P2 and P3), arrhythmia (P3), and nonspecific EKG findings (P1). When considering the cardiac phenotypes of these patients in the context of *ZBTB47*'s high expression in cardiovascular tissues and the understanding that other ZNFs are associated with cardiac disease (Cassandri et al., 2017; Reamon-Buettner and Borlak, 2005), the presence of a cardiac disease component of this syndrome is possible. Four of our patients have a history of recurrent or frequent infections, including frequent viral infections (P1 and P2), chronic rhinitis (P5), and recurrent bacterial infection and sepsis (P3). None of the reported patients had abnormal immunological testing. However, a history of frequent infection is difficult to interpret as viral infections are common in children, the frequency and severity of which vary significantly in relation to environmental exposure, health practices, and comorbidities. Nevertheless, the history of infection seen in our patients, along with the knowledge that some ZNFs play a role in immune regulation (Fu and Blackshear, 2017; Maeda and Akira, 2017; Scott and Omilusik, 2019), raises the question of possible immune dysregulation not detected by standard testing in association with this syndrome.

This study is limited in its focus on only five patients. The small size of our current cohort is likely related to the rarity of pathogenic variants in *ZBTB47* and the gene's lack of inclusion in most current sequencing platforms. Further limitations include the general paucity of information on the function of ZBTB47, as well as the lack of functional data and absence of animal models to support pathogenicity of these variants on the biochemical or organismal level. Neurodevelopmental phenotypes are generally nonspecific. As such, it is difficult to completely rule out the influence of other disease-causing variants in our patients, although the presence of the same *de novo* variant in four unrelated cases [c.1429G>A, p.(Glu477Lys)] is supportive of pathogenicity (and suggests this amino acid plays an important role in protein function). In addition, the shared phenotype among our patients, including developmental delay, intellectual disability, hypotonia, seizures, and possible movement abnormalities, is consistent and distinguished enough to attribute to the reported novel *de novo* missense variants in *ZBTB47*.

The mechanism by which these novel *ZBTB47* variants result in the described neurodevelopmental phenotype is unclear and will require further research. Development of the central nervous system is complex, and evidence describing the role that transcription factors play in this process continues to grow (Al-Naama et al., 2020; Gower-Winter and Levenson, 2012; Lein et al., 2017; Santiago and Bashaw, 2014; Silbereis et al., 2016). Biochemically, more work is needed to fully elucidate the function of *ZBTB47* and the pathogenesis of the presented variants at the molecular level to allow for a better understanding of the development of the phenotype, as well as consideration of

future management options. The mechanism of pathogenicity for these variants is currently unclear, though the pLI score of *ZBTB47* is 1.0, suggesting strong intolerance of loss-of-function variants and lending support to a possible hypomorphic or haploinsufficiency mechanism. However, gain of function or dominant negative mechanisms are also possible. Further functional studies are needed to establish the mechanism by which these variants cause the phenotype. Future clinical studies could involve the collection of more patients identified with *ZBTB47* variants into a more extensive review of phenotype and natural history. In addition, RNA sequencing could prove useful to characterize the impact that these variants have on the expression of other genes given that ZBTB47 is a transcription factor.

In summary, this is the first report of *de novo* heterozygous variants in *ZBTB47* in association with a human phenotype. The heterozygous missense variants detected in five unrelated patients via ES are associated with a variable autosomal dominant neurodevelopmental phenotype including developmental delay and intellectual disability, hypotonia, seizures, gait abnormalities, and variable abnormal movements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY

Both reported variants in *ZBTB47* have been deposited in ClinVar: p.(Glu477Lys), accession number: SCV002600228.1); p.(Glu680Gly), accession number: VCV002430193.1.

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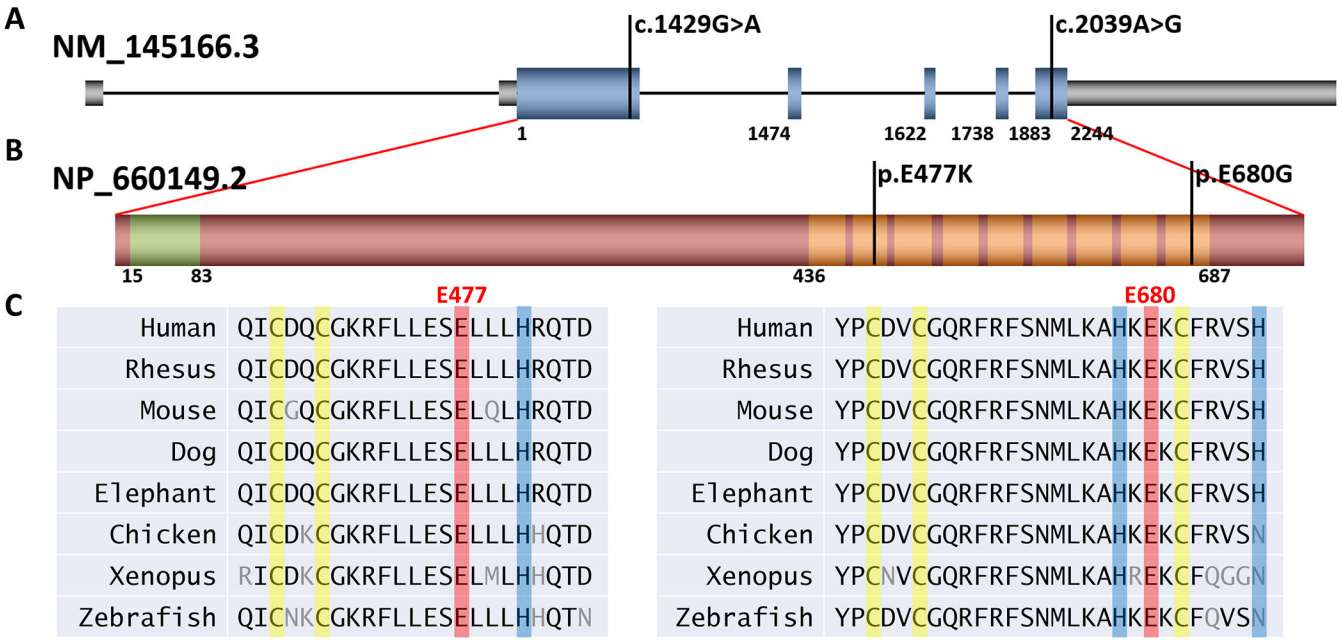

Figure 1.

Visual depiction showing (A) variants' location in exons of *ZBTB47*, (B) variants' location in zinc finger (ZNF) domains of ZBTB47, and (C) high conservation of amino acids for each variant across species, shown within the length of their ZNF domain. Numbers across the bottom of the figures represent coding nucleotides for the starts of the exons (A) or amino acid positions (B). Protein domains shown are the BTB domain (green) and ZNFs (orange). Cysteine residues are noted in yellow, histidine residues are noted in blue, and the affected glutamate residues reported in our patients noted in red (The UniProt Consortium, 2021). This figure shows the current canonical transcript of the protein (NM_145166.3), which differs from the previously reported structure (Kumar, Cheney, Neilsen, et al., 2010) in that it encodes a larger 747 amino acid protein which includes a longer N-terminal region with a BTB domain.

Table 1.

De novo missense variants found in *ZBTB47* in five unrelated patients with neurodevelopmental phenotypes.

	Patient 1 (P1)	**Patient 2 (P2)**	**Patient 3 (P3)**	**Patient 4 (P4)**	**Patient 5 (P5)**
Variant (NM_145166.3)	c.2039A>G, p.(Glu680Gly)	c.1429G>A, p.(Glu477Lys)			
Position (GRCh37/hg19)	chr3:42705885	chr3:42701276			
Exon number (total = 6 exons)	6	2			
Inheritance	*De novo*	*De novo*			
Testing	Trio exome sequencing with Sanger confirmation (Baylor Genetics)	Trio exome sequencing (GeneDx)	Exome Sequencing (GeneDx) with subsequent parental testing	Trio exome sequencing (Erasmus MC University Medical Center Department of Clinical Genetics)	Trio exome sequencing (GeneDx)
Present in ClinVar	Previously unreported	Previously unreported			
Present in gnomAD	No	No			
GERP	4.58	4.28			
CADD	29.9	25.3			
SIFT	Deleterious	Deleterious			
PolyPhenCat	Probably damaging	Probably damaging			
REVEL	Uncertain (0.5)	Uncertain (0.35)			
ACMG criteria	PS2, PM2, PP3	PS2, PM2, PP3			

Table 2.

Phenotypic comparison of five unrelated patients with *de novo* missense variants in *ZBTB47* and neurodevelopmental phenotype including developmental delay, hypotonia, refractory seizure, and abnormal movements. F, female; GERD, gastroesophageal reflux disease; M, male; OFC, occipital frontal circumference; PWS/AS, Prader-Willi syndrome/Angelman syndrome; y, years.

	Patient 1 (P1)	**Patient 2 (P2)**	**Patient 3 (P3)**	**Patient 4 (P4)**	**Patient 5 (P5)**
Age	5y	6y	8y	13y	6y
Sex	F	F	M	F	M
Pregnancy history	Placenta previa; Full term	Preeclampsia; Born at 37 weeks; Pneumothorax requiring no intervention	Maternal hypothyroidism treated with levothyroxine; Full term	Gestational diabetes, prolonged rupture of membranes; Born at 38 weeks	No complications; Full term
Birth weight (kg; Z-score)	Unknown	Unknown	3.572; 0.03	2.690; −1.4	3.529; 0.36
Birth length (cm; Z-score)	Unknown	Unknown	48.3; −0.71	50; −0.71	53; 1.20
Birth OFC (cm; Z-score)	Unknown	Unknown	Unknown	Unknown	34.5; 0.00
Weight (Z-score)	−4.09	−0.23	−2.47	0.21	0.95
Height (Z-score)	−2.53	0.50	−1.66	1.12	2.22
OFC (Z-score)	−1.23	−1.75	0.64	−0.47	−1.08
Dysmorphic features	Non-dysmorphic	Tented mouth, deep-set eyes, asymmetric facies, sagging cheeks	Deep orbits, prominent forehead, anteverted nose, borderline hypertelorism	Deep orbits, full cheeks, small bitemporal diameter	Short philtrum with thick upper lip, cupped ears
Developmental delay	Yes	Yes	Yes	Yes	Yes
Age at walking	Non-ambulatory	18m	18m	16m	15m
Age at talking	Nonverbal	2.5y	2y	1y	2y
Age at putting words together	Nonverbal	4y	5y	4.5y	Unknown
Intellectual disability	Unknown	Moderate	Moderate	Moderate	Moderate
Hypotonia	Yes	Yes	Yes	Yes	Yes
Coordination	Poor	Poor	Poor	Poor	Poor
Abnormal behaviors	Teeth grinding	Easy startle, strong shudder reflex	Aggressive behavior, frequent tantrums, poor eye contact, poor sociability	Aggressive behavior, anxious behavior, echolalia	Hyperactivity
Autism spectrum diagnosis	No	Yes	Yes	Yes	Yes
Seizures	Yes	Yes	Yes	Yes	Yes
Seizure age of onset	8m	2y	17m	2m	9m
Seizure type	Refractory tonic-clonic seizures, generalized slowing	Generalized epilepsy, multiple seizure types	Partial seizures, Multifocal parietal epileptiform discharges	Refractory tonic-clonic seizures, Multifocal epilepsy with left centroparietal region focus	Generalized tonic-clonic epilepsy with focal motor seizures
Seizure frequency	45 per day	2-4 per month	2-4 per month	12 per month	2-3 per month

	Patient 1 (P1)	Patient 2 (P2)	Patient 3 (P3)	Patient 4 (P4)	Patient 5 (P5)
Anti-epileptic drug therapy	Yes – cannabidiol, clobazam	Yes -- valproic acid	No	Yes – clobazam, lamotrigine, midazolam, sulthiame	Yes – lacosamide, valproic acid
Abnormal movements	Choreiform movements of hands, feet, trunk, and face	Twitching during sleep, shuddering attacks, easy startle, choreiform movements when walking	Hand tremor	Hand-flapping with excitement	Tic disorder
Gait abnormalities	Non-ambulatory	Gowers sign	Wide-based gait	Unsteady	Unsteady
Cardiovascular abnormalities	Nonspecific ST- and T-wave abnormality	Muscular ventricular septal defect	Intermittent tachycardia, aortic valve abnormality	None, pending evaluation	None
Gastrointestinal problems	Severe malnutrition	Constipation, GERD, dysphagia	Poor weight gain, lactose intolerance, constipation	Constipation	Dysphagia
Skeletal abnormalities	Scoliosis, hip dysplasia	None	Joint laxity	Hypermobility	None
Other medical problems	Frequent viral infectious	Frequent viral infections, laryngomalacia with laryngeal cleft, sleep apnea	Frequent infections, recurrent otitis media, Salmonella gastroenteritis, stridor, sleep apnea, hearing loss	None	Chronic rhinitis
MRI results	Normal	Normal	Cortical dysplasia of left temporal lobe, Chiari I malformation, poor definition of gray-white matter interface at temporal poles	Slightly wide occipital horns	Normal
Chromosomal microarray	Normal	Regions of homozygosity (<10Mb)	Normal	Normal	Normal
Other non-diagnostic genetic testing	Myotonic dystrophy panel, PWS/AS methylation	Myotonic dystrophy panel, PWS/AS methylation	None	Epilepsy gene panel, intellectual disability panel, mtDNA sequencing, sequencing of *CDKL5*, *PCDH19*, *SCN1A*	Fragile X, autism/ intellectual disability panel, epilepsy panel