Further evidence that *de novo* missense and truncating variants in ZBTB18 cause intellectual disability with variable features

Cohen J.S., Srivastava S., Farwell Hagman K.D., Shinde D.N., Huether R., Darcy D., Wallerstein R., Houge G., Berland S., Monaghan K.G., Poretti A., Wilson A.L., Chung W.K., Fatemi A. Further evidence that *de novo* missense and truncating variants in ZBTB18 cause intellectual disability with variable features. Clin Genet 2017: 91: 697–707. © 2016 The Authors. Clinical Genetics published by John Wiley & Sons A/S. Published by John Wiley & Sons Ltd., 2016

Identification of rare genetic variants in patients with intellectual disability (ID) has been greatly accelerated by advances in next generation sequencing technologies. However, due to small numbers of patients, the complete phenotypic spectrum associated with pathogenic variants in single genes is still emerging. Among these genes is ZBTB18 (ZNF238), which is deleted in patients with 1q43q44 microdeletions who typically present with ID, microcephaly, corpus callosum (CC) abnormalities, and seizures. Here we provide additional evidence for haploinsufficiency or dysfunction of the ZBTB18 gene as the cause of ID in five unrelated patients with variable syndromic features who underwent whole exome sequencing revealing separate *de novo* pathogenic or likely pathogenic variants in ZBTB18 (two missense alterations and three truncating alterations). The neuroimaging findings in our cohort (CC hypoplasia seen in 4/4 of our patients who underwent MRI) lend further support for ZBTB18 as a critical gene for CC abnormalities. A similar phenotype of microcephaly, CC agenesis, and cerebellar vermis hypoplasia has been reported in mice with central nervous system-specific knockout of Zbtb18. Our five patients, in addition to the previously described cases of *de novo* ZBTB18 variants, add to knowledge about the phenotypic spectrum associated with ZBTB18 haploinsufficiency/dysfunction.

**Conflict of interest**

Nothing to declare.

Key words: cerebellar vermis hypoplasia – corpus callosum abnormalities – intellectual disability – microcephaly – ZBTB18 – ZNF238
ZBTB18 (zinc finger and BTB domain-containing protein 18; formerly known as ZNF238) encodes a transcriptional repressor (1) that plays an important role in cortical and cerebellar development. In mice, Zbtb18 deficiency results in defective growth, differentiation, and maturation of cortical and cerebellar neurons (2–4), leading to a phenotype of microcephaly, cerebellar vermis hypoplasia, and agenesis of the corpus callosum (CC) (5).

Emerging evidence suggests that ZBTB18 haploinsufficiency in humans results in a recognizable neurodevelopmental phenotype. ZBTB18 is located on 1q44, and individuals with chromosome 1q43-q44 microdeletions (MIM # 612337) variably have intellectual disability (ID), microcephaly, CC agenesis/hypogenesis, seizures, facial dysmorphisms (round face, prominent forehead, flat nasal bridge, hypertelorism, epicanthal folds, and malformed low-set ears), growth problems, and other systemic abnormalities (6). Within this deletion, haploinsufficiency of ZBTB18 may be critical to the development of CC anomalies (6–9).

To date, only eight patients with ZBTB18 sequence alterations have been described in the published literature (Table 1). The first patient was part of an exome sequencing study of ID (10). She was a female with severe non-syndromic ID and normal neuroimaging who was found to have a de novo missense alteration, c.1483C>G (p.R495G), in transcript NM_205768.2 [reported as c.1456C>G (p.R486G) based on the non-preferred transcript NM_006352.3]. The authors proposed ZNF238 (ZBTB18) as a candidate gene for ID. The second patient was a female with features overlapping 1q43-q44 microdeletion syndrome except for normal appearance of the CC. Whole exome sequencing (WES) revealed a de novo nonsense variant in ZBTB18, c.397G>T (p.E133*) (11). The third was part of a cohort with clinical features overlapping Rett syndrome. She presented with severe ID, microcephaly, developmental regression, hand stereotypies, bruxism, screaming spells, abnormal breathing, unremarkable brain MRI, and no growth problems. She was found to have a de novo truncating variant in ZBTB18, c. 583C>T (p.R195*) in transcript NM_205768.2 [reported as c. 556C>T (p.R186*) based on transcript NM_006352.3] (12). Most recently, the Deciphering Developmental Disorders (DDD) project released WES findings from 4293 ID patients, which included five patients with unique de novo ZBTB18 alterations: two nonsense variants [c.622G>T (p.G208*) and c.811C>T (p.Q271*)], two frameshift variants [c.1046dup (p.E350Rfs*151) and c.635del (p.P212Hfs*10)], and one missense variant [c.1391G>A (p.R464H)]; however the published data included only limited clinical information without individual patient details (13).

In this report, we present detailed clinical characteristics of five patients with de novo ZBTB18 sequence alterations (two missense alterations and three truncating alterations), underlying their phenotype of global developmental delay/ID with variable additional features.

**Clinical report**

Clinical characteristics of the five patients are summarized in Table 1.

**Patient 1**

Patient 1 is a 7-year-old boy who was the first child born to a non-consanguineous couple of European ancestry with no notable family history. He was born at term gestation weighing 3799 g (69th centile) with an uncomplicated prenatal and neonatal course. He demonstrated poor head control at 3 months of age, and thereafter he was globally delayed in development. From a motor standpoint, he first rolled after one year, sat with support and combat-crawled after two years, crawled properly at three years of age, walked with support by five years, and could ambulate with a walker at six years. His cognitive abilities are in the severe-to-profound ID range. He has no spoken words but is learning to use an assistive communication device that provides picture choices. On general examination at 7 years, he had relative macrocephaly (head circumference 50.5 cm, 10th centile) and tall stature (height 136.5 cm, 98th centile). He has multiple minor anomalies including bifid uvula, bilateral single palmar creases, wide-spaced nipples, and cryptorchidism but no significant craniofacial dysmorphism compared to his parents. On neurological examination, he was happy and attentive, maintaining good eye contact. He exhibited stereotyped movements and vocalizations without aggressive behaviors. He did not follow commands. His cranial nerves were intact. He had truncal hypotonia and lower extremity spasticity. With support, he walked with a spastic diplegic gait pattern juxtaposed with ataxia. Electroencephalogram at 8 months of age showed slowing and multifocal spike activity, but repeat study was normal; he has never had...
Table 1. Clinical features of patients with ZBTB18 variants

| Genotype                  | Inheritance | Gender | Age at last exam | Head Circumference | Height | Weight | Development Motor delay | Speech delay | Cognitive delay | Neurological and other findings | Behavioral features |
|---------------------------|-------------|--------|------------------|--------------------|--------|--------|------------------------|-------------|-----------------|-------------------------------|----------------------|
| c.1382A>C                 | de novo     | Male   | 7 years          | 50.5 cm (10th)     | 136.5 cm (98th)    | 23.9 kg (44th)       | +                      | +                        | +                            | None                 |
| c.943_944delAG            | de novo     | Male   | 3 years          | 47.5 cm (10th)     | 90.5 cm (10th)     | 12.7 kg (10-25th)    | +                      | +                        | +                            | Screaming spells       |
| c.1183C>T                | de novo     | Male   | 4 years          | 47 cm (c-1st, 50th for 16 months) | 178 cm (50th)  | 13.15 kg (2nd)        | +                      | +                        | +                            | None                 |
| c.1390C>T                | de novo     | Male   | 6 years          | 56 cm (90th)       | 122 cm (65th)      | 20.6 kg (42nd)       | -                      | +                        | +                            | Screaming spells       |
| c.133C>T                | de novo     | Female | 34 months        | 50 cm (10th)       | 169 cm (50th)      | 7.9 kg (1<st)         | +                      | +                        | +                            | None                 |
| c.1483C>G                | de novo     | Male   | 5 years          | 57 cm (90th)       | 79 cm (c-1st)      | Individual values not reported | Individual values not reported | Individual values not reported | Mild ID, Low average cognitive ability, Severe ID | None                 |
| c.3970>T                | de novo     | Female | 18 years         | 45 cm (c-1st, 50th for 7 months) | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | +/− with cognitive impairment | None                 |
| c.583C>T                | de novo     | Female | 34 months        | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Mild generalized hypotonia, Limited speech | None                 |
| c.622G>T                | de novo     | Male   | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Hypotonia: 4/8 | None                 |
| c.635del                 | de novo     | Female | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Not reported | None                 |
| c.811C>T                | de novo     | Male   | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Not reported | None                 |
| c.1046dup               | de novo     | Female | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Not reported | None                 |
| c.1319G>A               | de novo     | Male   | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Not reported | None                 |
| c.1118del               | de novo     | Female | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Not reported | None                 |
| c.160T>C                | de novo     | Male   | Not reported     | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Individual values not reported | Not reported | None                 |

| (Reference) | (14) | (10) | (11) | (12) | (13) | (SCV0002267599.1) | (SCV000265568.1) |
|-------------|------|------|------|------|------|----------------|----------------|
| c.1118del   |      |      |      |      |      | Microcephaly   | Normal (70th)   |
| c.160T>C    |      |      |      |      |      | Absence of microcephaly | 6/15 (60%)       |
| (p.S373Ter) |      |      |      |      |      | 6/15 (60%)     | Normal (70th)   |
| (p.C54R)    |      |      |      |      |      | 6/15 (60%)     | Normal (70th)   |
| c.1319G>A   |      |      |      |      |      | Short stature: | Normal (65th)   |
| c.1118del   |      |      |      |      |      | Failure to thrive: 2/13 (15%) | Normal (65th)   |
| c.160T>C    |      |      |      |      |      | Failure to thrive: 2/13 (15%) | Normal (65th)   |
| (p.R464H)   |      |      |      |      |      | Motor delay: 12/13 (92%) | Normal (65th)   |
| (p.E133*)   |      |      |      |      |      | Speech delay: 15/15 (100%) | Normal (65th)   |
| c.1319G>A   |      |      |      |      |      | Cognitive delay: 15/15 (100%) | Normal (65th)   |
| c.1118del   |      |      |      |      |      | Hypotonia: 4/8 | Normal (65th)   |
| c.160T>C    |      |      |      |      |      | Hypotonia: 4/8 | Normal (65th)   |
| Patient # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference | (14) | | | | | (10) | (11) | (12) | (13) | | | | | |
| Neuroimaging findings | Global CC hypoplasia, inferior cerebellar vermis hypoplasia | Hypoplasia of posterior CC | Hypoplasia of posterior CC, mild enlargement of the posterior horn of the left lateral ventricle | Not performed | Mild global CC hypoplasia with possible dysplasia | Normal brain MRI | Normal brain MRI | Normal brain MRI | Not reported | Not reported | Not reported | CC abnormalities: 4/7 (57%) | | |
| Craniofacial dysmorphism | None | None | Prominent maxilla and thick lips | Small low-set ears, narrow palate with crowded teeth | None | Arched eyebrows, telecanthus, short palpebral fissures, long nose with prominent tip, thin upper lip, flattened philtrum | None | | 2/5 with abnormality of nasal alae, no other findings reported | None | Unspecified abnormal face shape | | |
| Minor anomalies | Bilateral single palmar creases, wide-spaced nipples, bilateral antebrachial folds, bifid uvula | Bilateral 2-3 toe synacthely | None | None | None | Slightly broad first fingers and toes | Small cold hands/feet | None | Not reported | Variable minor anomalies in at least 8/14 (57%) | | |
| Other symptoms | Myopia | Strabismus | Strabismus, single fertile seizure as an infant | Drooling | Epilepsy | None reported | Gastrointestinal problems | Regression at 8 months, bruising, breathing disturbances | Not reported | Not reported | | | | |

<sup>a</sup>Variant reported as c.1456C>G (p.R486G) based on transcript NM_006352.3.
<sup>b</sup>Variant reported as c.556C>T (p.R186*) based on transcript NM_006352.3.
<sup>c</sup>Ambry Genetics Laboratory, manuscript in preparation.
Clinical seizures. Brain MRI at 44 months showed global hypoplasia of CC and hypoplasia of inferior cerebellar vermis (Fig. 1). These findings were overall stable compared to a prior MRI at 9 months, which was notable for delayed myelination. Workup prior to WES was overall unremarkable and included karyotype, Fragile X trinucleotide repeat analysis, X-linked ID sequencing panel (81 genes, Ambry Genetics), and biochemical testing for a wide range of inborn errors of metabolism. Chromosomal microarray showed a 507 kb duplication on chromosome 12p11.23 that was subsequently determined to be inherited from his phenotypically normal father and thus interpreted to be likely benign. This patient was included with limited clinical information in the series published by Srivastava et al. (14).

Patient 2
Patient 2 is a 3-year-old boy who was the second child born to parents of European ancestry. He was delivered at 39 weeks of gestation without prenatal or neonatal complications. His birth weight was 3685 g (76th centile). His main issue has been developmental delay. He sat at 9 months, crawled at 11 months, and walked at 24 months. At 3 years of age, he can go up and down stairs. He has some word approximations, but they are inconsistent. He is able to follow one step commands. He gets frustrated with his inability to communicate at times. Medically, his only concern has been strabismus that was surgically corrected. At 3 years of age, his height was 12.7 kg (10th–25th centile), height was 90.5 cm (10th centile), and head circumference was 47.5 cm (10th centile). On examination, he was happy, interactive, attentive, and maintained good eye contact. His cranial nerves were intact. He had truncal hypotonia. Brain MRI at 19 months was remarkable for hypoplasia of the posterior part of the CC (Fig. 1). Workup prior to WES was unremarkable and included a normal karyotype, chromosome microarray, Prader–Willi methylation analysis, and Fragile X trinucleotide repeat analysis.

Patient 3
Patient 3 is a 4-year-old boy of Mexican ancestry who was born at 38 weeks after a pregnancy that was uncomplicated aside from pre-eclampsia near delivery. His birth parameters included a weight of 2600 g (10th centile) and length 45.7 cm (6th centile). He had one febrile seizure as an infant. He has a history of truncal hypotonia and strabismus. He communicates using signs and can put 2–3 signs together in a sentence. He follows simple commands, smiles, and makes eye contact. He occasionally screams for no reason, lasting 30 minutes about twice a day but is otherwise pleasant and active. He walked after 2 years but has ataxia and tends to trip often. At 4 years, his height was 98 cm (12th centile), weight was 13.15 kg (2nd centile), and head circumference was 47 cm (<1st centile, 50th centile for a 16-month-old). Dysmorphic features include downslanting palpebral fissures, flaring of the medial eyebrows, bulbous nasal tip, thin upper lip, flattened philtrum, and bilateral 2–3 toe syndactyly. Brain MRI at age 47 months revealed hypoplasia of posterior part of CC and mild enlargement of the posterior horn of the left lateral ventricle (Fig. 1). Fragile X trinucleotide repeat analysis, chromosomal microarray, and thyroid studies prior to WES were all normal.
Despite severe cognitive impairment, he is able to live and function semi-independently with accommodations. He lives by himself in apartment with close follow up by caretakers who assist with activities of daily living such as preparing meals and selecting clothing, though he can feed and dress himself. He has a custom working place, can stay alone for hours at home doing his activities, and can make telephone calls with a simple modified phone and web camera. On clinical examination, he made good eye contact, smiled, and liked to shake hands, something he did repeatedly. He has a coarse face with a prominent maxilla and thick lips, but no overt dysmorphism. Height and head circumference have been within normal range. EEG has been normal, and no cerebral imaging has been performed. Genetic analysis prior to WES was normal, including a chromosomal microarray, Angelman syndrome methylation and copy number analysis, and TCF4 sequencing.

**Patient 5**

Patient 5 is a 6-year-old boy of African-American ancestry who was born to a non-consanguineous couple with no significant family history. He was born at term gestation with an uncomplicated prenatal course. His birth weight was 3969 g (80th centile), and there were no neonatal complications. His motor milestones were not delayed; he sat at 6 months, crawled at 10 months, and began walking independently at 14 months. However, he had significant language delay in that he did not speak his first words until 2 years and did not put together 2–3 word sentences until 3 years. He was diagnosed with epilepsy close to 3 years of age, when he developed both focal motor seizures without impairment of consciousness as well as focal seizures with impairment of consciousness. His EEGs have shown multifocal epileptiform activity (spikes and sharp waves). Since initiation of levetiracetam, his seizures have been fairly well controlled. Neuropsychological testing at 5 years revealed variable performance on the Differential Ability Scales-II, with average range verbal reasoning skills (standard score 99) and non-verbal reasoning skills (standard score 94), but extremely low range spatial reasoning skills (standard score 60), placing his overall cognitive ability in the low average range (standard score 80). At 6 years, his height was 122 cm (85th centile), weight was 20.6 kg (42nd centile), and head circumference was 50 cm (10th centile). On neurologic exam, he was alert, playful, interactive, and able to speak in multi-word sentences, though his speech was slightly dysarthric and the content seemed immature for age. His cranial nerves were intact. He had generalized hypotonia. His reflexes and sensation were normal. He was able to run well and could hop on each foot, but he was clumsier doing so on the right. Dysmorphic features include small, low-set ears and narrow palate with crowded teeth. Brain MRI at 37 months showed mild global hypoplasia of the CC (Fig. 1). Workup prior to WES was normal including chromosomal microarray and Fragile X trinucleotide repeat analysis.

**Methods**

For all patients, WES was performed with trios including the proband and both biological parents on genomic DNA isolated from blood, and diagnostically relevant variants were confirmed by dideoxy sequencing. Maternity and paternity were confirmed as part of quality control. For patients 1 and 3, WES was performed by Ambry Genetics Laboratory, Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) and the xGen Exome Research Panel (Integrated DNA Technologies, Coralville, IA), respectively, and sequenced on the Illumina HiSeq or NextSeq (Illumina, San Diego, CA). Data annotation and interpretation were performed as previously reported (15). For patients 2 and 5, WES was performed at GeneDx Laboratory with methods as previously reported (16). For patient 4, DNA samples were prepared with Nextera Rapid Capture Exome Kit and sequenced on Illumina NextSeq 500 (Illumina). The variants were annotated with Cartagenia Bench Lab, NGS module. The analysis was performed diagnostically by Centre for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Norway. Informed consent was obtained from all patients and family members undergoing sequencing. Diagnostically relevant variants were deposited into publicly available databases, namely ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) for patients 1–3 and 5, and DECIPHER (https://decipher.sanger.ac.uk/) for patient 4.

Available brain MRI studies were retrospectively reviewed for morphological abnormalities of the corpus callosum, which has been classified according to Hanna et al. (17).

The patient cohort was assembled with the assistance of GeneMatcher, which is an online tool that facilitates connections among clinicians and investigators with shared interest in a gene (18).

This study was approved by the Johns Hopkins Medicine Institutional Review Board (protocol number IRB00103185).

**Results**

The genotypes and analysis of the variants are presented in Fig. 2. All sequence variants are described in reference to RefSeq transcript NM_205768.2.

In patients 1 and 4, WES independently identified heterozygous de novo missense variants in ZBTB18, c.1382A>G (p.N461S) and c.1390C>T (p.R464C), respectively. Both of these alterations affect evolutionarily conserved amino acids in the third zinc finger domain and are predicted to be deleterious by multiple in silico algorithms (Fig. 2). Both missense variants are absent from ExAC (http://exac.broadinstitute.org) and are classified as likely pathogenic based on ACMG guidelines (19).

In the three other patients, WES independently identified heterozygous de novo truncating ZBTB18 variants: a frameshift alteration c.943_944delAG (p.R315Gfs*4) in patient 2, a nonsense alteration c.1183C>T (p.Q395*) in patient 3, and a missense alteration c.1502G>T (p.D501V) in patient 5.

Cohen et al.
**Fig. 2. Analysis of ZBTB18 variants.** (a) Protein domain architecture and locations of ZBTB18 variants in reference to RefSeq transcript NM_205768.2. The variants depicted above the diagram are reported in this study, whereas those below the image were previously published. Red triangles indicate missense variants, while yellow triangles represent truncating variants. (b) Multiple sequence alignment of ZBTB18 protein homologues centered on the locations of the ZBTB18 missense variants, denoted by arrowheads. An asterisk indicates a position of complete conservation. (c) Results of in silico missense pathogenicity prediction tools: PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html), PROVEAN (http://provean.jcvi.org/index.php), Mutation Taster (http://www.mutationtaster.org), and CADD (http://cadd.gs.washington.edu). (d) Alignment between the C2H2 Zn3 and Zn4 domains in ZBTB18. Positions that make specific base contacts with the DNA are indicated with a number above the alignment. The zinc-coordinating amino acids in the C2H2 motif are bolded and underlined. The observed missense alterations in our patient cohort are highlighted in red font.

**ZBTB18 intellectual disability**

(c) | c.160T>C (p.C54R) (Patient 15) | c.1382C>T (p.N461S) (Patient 1) | c.1390C>T (p.R464C) (Patient 4) | c.1391G>A (p.R464H) (Patient 13) | c.1483C>G (p.R495G) (Patient 6) |
---|---|---|---|---|---|
**Protein domain** | BTB/POZ | Zn3 | Zn3 | Zn3 | Zn4 |
**PolyPhen2** | 1.00 (Probably Damaging) | 0.97 (Probably Damaging) | 0.99 (Probably Damaging) | 0.99 (Probably Damaging) | 0.99 (Probably Damaging) |
**SIFT** | 0.00 (Deleterious) | 0.03 (Deleterious) | 0.01 (Deleterious) | 0.03 (Deleterious) | 0.01 (Deleterious) |
**Provean** | -9.09 (Damaging) | -2.93 (Damaging) | -5.49 (Damaging) | -3.44 (Damaging) | -6.03 (Damaging) |
**MutationTaster** | Disease Causing | Disease Causing | Disease Causing | Disease Causing | Disease Causing |
**CADD** | 18.30 | 17.49 | 19.65 | 21.40 | 15.92 |
in patient 3, and another nonsense alteration c.133C>T (p.R45*) in patient 5. They are absent from ExAC and are classified as pathogenic based on ACMG guidelines.

CC abnormalities (CCAs) were evident in all four patients who underwent neuroimaging (Fig. 1). Patient 1 had global CC hypoplasia. Patients 2 and 3 had hypoplasia of the posterior part of the CC (apple core CCA). In addition to mild global CC hypoplasia in patient 5, there was a small protuberance within the superior part of the middle-posterior CC, which could represent CC dysplasia.

Discussion

We report on five patients with global developmental delay/ID with de novo variants in ZBTB18 identified through WES. The frequency of ZBTB18 alterations in ID is not known. Two of the patients in our cohort (patients 1 and 5) were ascertained from the same clinic at Kennedy Krieger Institute, where approximately 300 patients with neurodevelopmental disorders have undergone clinical WES over a 4-year-period. The five DDD patients were ascertained from a cohort of 4293 patients with ID. An analysis of 188 patients with ID undergoing multiplex targeted sequencing that included ZBTB18 identified a patient with a truncating alteration c.1118del (p.S373Tfs*26), though parental samples were not available to determine if the variant was de novo or inherited (Ambry Genetics Laboratory, manuscript in preparation; ClinVar submission accession number SCV000267599.1). This patient’s clinical features included severe ID, normal head circumference and growth parameters, and absence of dysmorphic features and congenital anomalies (Table 1). Additionally, ClinVar contains one entry of a patient with a truncating ZBTB18 missense variant c.160T>C (p.C54R), which is located in the BTB/POZ domain; limited clinical details available for this patient include moderate ID, microcephaly, and abnormal face shape (ClinVar submission accession number SCV000265569.1). Including these unpublished cases brings the total to 15 patients with likely pathogenic ZBTB18 sequence alterations in the published literature and/or publicly available databases.

Phenotype of patients with ZBTB18 variants

Global developmental delay/ID is present in all 15 patients. Among the patients in our cohort, patient 5 had notably higher functioning compared to the others, with overall low average cognitive abilities; his variant was a nonsense alteration, so it does not appear that null variants are associated with more severe phenotype compared to missense variants. Head circumference is reduced in over half of the patients, with six (40%) having absolute microcephaly and two (13%) having relative microcephaly. A minority of patients have short stature (1/14, 7%) and/or failure to thrive (2/13, 15%). Craniofacial dysmorphism and/or minor anomalies are present in approximately half of patients, though there is no consistent pattern. Importantly, detailed clinical descriptions are not available for the DDD and unpublished ClinVar patients, so it is possible that additional features are present in some of those patients.

The neuroimaging findings in our patient cohort demonstrate that ZBTB18 haploinsufficiency or dysfunction in humans may result in structural brain abnormalities similar to those seen in animal models. Mice with ubiquitous deletion of Zbtb18 have evidence of disrupted neocortical and hippocampal formation and die at birth (4). Meanwhile, mice with neural-specific knockout of Zbb18 survive to around 3 weeks and demonstrate microcephaly, cerebellar vermis hypoplasia, agenesis of the CC, and cerebral cortical dysplasia (5). All four of the patients in our cohort with neuroimaging had CC hypoplasia of varying degrees, and patient 1 additionally had hypoplasia of the inferior cerebellar vermis. This is in contrast to the three previously published cases with de novo ZBTB18 variants who had normal neuroimaging (patients 6–8), though it is possible that these patients could have microstructural differences not evident on MRI. Patient 4 in our cohort did not undergo neuroimaging, and neuroimaging information is not known for patients 9–15.

Insight into 1q43q44 microdeletion syndrome

The range of clinical findings among patients with ZBTB18 variants adds to knowledge about genotype/phenotype correlations among the critical genes in the 1q43q44 microdeletion syndrome. Deletions of 1q43q44 have been reported in more than 100 individuals in the published literature, ranging in size from 80 kb to over 6 Mb. All reported deletions have different breakpoints and many do not overlap. Multiple author groups have published analyses of smallest regions of overlap in attempt to identify genes that may contribute to key clinical features (6, 7, 9, 20–24), although importantly incomplete penetrance and variable expressivity have been shown for most of the features associated with 1q43q44 microdeletion syndrome. There is general agreement that the three most important genes are AKT3, ZBTB18, and HNRNPU, which lie within an interval approximately 1.5 Mb in size mapping from approximately 243.5 Mb to 245 Mb based on human genome assembly GRCh37/hg19. AKT3, ZBTB18, and HNRNPU are known to be important in neurodevelopment, and haploinsufficiency/dysfunction of each of these three genes alone has been documented to cause neurodevelopmental disorders. Among patients with 1q43q44 deletions, AKT3 has been proposed as a critical gene for microcephaly, ZBTB18 as a critical gene for CCA, and HNRNPU as a critical gene for seizures. Ballif et al. (6) found that approximately 90% of cases conform to this model. The neuroimaging findings in our cohort lend further support for ZBTB18 as a critical gene for CCA and affirm that there is reduced penetrance for this feature, since CCAs were not seen in three previously published patients with ZBTB18 variants who had neuroimaging (patients 6–8).
The clinical finding of microcephaly in three of eight patients with *ZBTB18* variants demonstrates that haploinsufficiency/dysfunction of *ZBTB18* alone can cause microcephaly in some patients. Rather than a one-to-one correlation between a single gene and a specific clinical feature, it is evident that multiple genes contribute to the variable phenotype seen in patients with 1q43q44 deletion syndrome.

**Pathogenic mechanisms of *ZBTB18* variants**

*ZBTB18* encodes a C2H2-type zinc finger protein that acts as a transcriptional repressor (1). The preferred transcript NM_205678.2 encodes the longest protein isoform, NP_991331.1, which is 531 amino acids and is characterized by an N-terminal BTB/POZ domain that mediates protein–protein interactions and four zinc fingers (Zn1-4) at the C-terminus that mediate protein binding to regulatory sites within promoters. *ZBTB18* has two exons, although only 13 nucleotides from the first exon are coding.

*ZBTB18* has been found to be highly intolerant to variation. Based on the allele frequencies from the NHLBI-ESP6500 data set, *ZBTB18* has a Residual Variation Intolerance Score (RVIS) of −0.78 and is amongst the 12.77% most intolerant of human genes ([25]; http://genic-intolerance.org/). In fact, using the larger ExAC data set, the RVIS for *ZBTB18* indicates that it is amongst the 6.17% most intolerant of human genes. This is in agreement with the ExAC score for the probability of loss-of-function (LoF) intolerance (pLI=0.97) and the Z-score for missense variants (z = 3.36), which indicate that the *ZBTB18* gene is extremely intolerant to genetic variation (26). In fact, no LoF alterations in *ZBTB18* are seen in ExAC.

The pathogenic mechanism of the *ZBTB18* frameshift and nonsense variants is most likely haploinsufficiency, since these types of variants are presumed to lead to LoF through protein truncation or nonsense-mediated mRNA decay (NMD). All 10 truncating variants described herein are located in the second and final exon and, therefore, likely escape NMD (27). However, they all lead to heterozygous loss of the zinc fingers in the *ZBTB18* protein, so this could be considered haploinsufficient. Furthermore, because the abnormal transcripts likely will escape NMD, and if they lead to the production of truncated proteins that are not removed due to misfolding, these truncated proteins will remain and could potentially have gain-of-function or dominant-negative effects on the wild-type copies (27).

The *ZBTB18* missense variants are clustered in the third and fourth zinc finger domains (Fig. 2a). These regions are nearly devoid of missense variation in ExAC. Alterations in a zinc finger domain could disrupt binding of the ZBTB18 transcription factor to its target DNA sequences. Furthermore, mutating the zinc finger could have a dominant-negative effect by rendering the wild-type protein unable to bind DNA because it dimerizes with a mutant protein. There are a number of zinc finger containing transcription factors in which missense variants are linked to developmental disorders.

A very comparable example is *ZBTB20*, which is located on chromosome 3q13.31 and has a similar structure to *ZBTB18* with an N-terminal BTB domain and several zinc fingers in the C-terminus. Haploinsufficiency of *ZBTB20* contributes to the phenotype of the 3q13.31 microdeletion syndrome (MIM 615433), which is characterized by ID, postnatal overgrowth, hypotonia, and dysmorphic facial features. Heterozygous missense variants in the zinc finger domains of *ZBTB20* cause Primrose syndrome (MIM 259050), which is characterized by a phenotype similar to 3q13.31 microdeletion syndrome but more severe and with additional features of ectopic calcifications, progressive muscle wasting, diabetes, and hearing loss. DNA binding assays demonstrated reduced levels of DNA-bound ZBTB20 and less efficient AFP promoter repression in cells co-expressing the wild-type protein in the presence of each of the missense variants, consistent with the variants having a dominant-negative effect on the wild-type allele (28).

It is noteworthy that patients 1 and 4, who have missense variants located three amino acids apart in Zn3 (p.N461S and p.R464C/H, respectively), are affected with the most severe degree of ID compared to a more variable range of ID among the patients with *ZBTB18* frameshift and nonsense variants in our cohort. The DNA recognition helix of Cys2His2 (C2H2) zinc finger domains contains invariant Cysteine (C) and Histidine (H) residues (underlined in bold font in Fig. 2d) that coordinate a zinc molecule essential for binding to specific DNA sequences. Additionally, amino acids in this recognition helix at four standard positions (−1, 2, 3 and 6, shown in Fig. 2d) around the histidine residues are critical for mediating specific contacts with DNA bases (29, 30). Interestingly, the most impactful zinc finger missense alterations in *ZBTB18* identified in our cohort, p.N461S and p.R464C/H, occur in the 3 and 6 positions respectively, of the DNA specificity residues of Zn3. Engineered variants at these positions have been shown in KRAB-containing zinc fingers to alter the DNA binding amino acid sequence (31).

The p.R495G variant in patient 6 occurs within the di-Histidine motif of Zn4. Although this position is not defined as a specificity residue, the alteration might modulate the DNA binding specificity of the adjacent Zn3 (32). The difference in the severity of the effects of these alterations can be explained by the importance of the position within the zinc finger domain. Altering the positions that interact with DNA will have a direct effect on protein-DNA interaction whereas altering positions that might modulate the DNA interaction would probably have a less severe effect. This is consistent with the severe-to-profound level of ID in patients 1 and 4 compared to the mild level of ID in patient 6; Patient 13 from the DDD cohort has a missense variant p.R464H that alters the same residue as patient 4, however information on this patient’s level of ID in is not known. Functional studies are needed to investigate this hypothesis of a possible dominant-negative effect and determine genotype/phenotype associations for variants in *ZBTB18*. 

**ZBTB18 intellectual disability**
Pathogenesis of ID and structural brain abnormalities in patients with ZBTB18 variants

Though the pathogenesis of ID secondary to ZBTB18 dysfunction is not directly known, it may be related to the multiple functions of ZBTB18 in brain development, as evident from mouse studies. ZBTB18 is essential for proper growth, differentiation, and migration of cortical neurons. It is highly expressed in the cortex (33), where it plays an important role in the survival and maturation of cortical neurons by regulating cortical apoptosis and cell-cycle exit of progenitor cells (4). In addition, ZBTB18 downregulates expression of the neurogenic transcription factors, ngn2 and neuroD1; consequently, its loss interrupts the neurogenic pathway necessary for normal neuronal differentiation (5). Finally, as a requisite to proper neuronal migration, ZBTB18 mediates multipolar-to-bipolar transition of migrating cortical neurons by inhibiting the transcription factors Ngn2 (3) and Rnd2 (34), which together form the Ngn2-Rnd2 pathway closely linked to cortical neuron migration (35).

ZBTB18 is also implicated in cerebellar organization and growth. The presence of ZBTB18 in embryonic granule neuron progenitors is required for normal development of cerebellar architecture, including foliation and lamination, as well as cerebellar growth. Furthermore, ZBTB18 influences proliferation and differentiation of cerebellar glutamatergic and GABAergic neurons by regulating transcription of progenitors to these neuronal populations (2). Though all of these studies occurred in mice, given the close homology between mouse and human ZBTB18 [pair-wise alignment using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi)], these studies suggest several possible contributing factors to ID in humans with ZBTB18 alterations, including, but not limited to, abnormal number, maturity, and migration of cortical and cerebellar neurons.

ZBTB18 is hypothesized to play a role in chromatin assembly and transcriptional repression. Based on molecular biology experiments with bacterial protein expression, ZBTB18 facilitates transcriptional repression through recognition of a specific sequence. Moreover, it associates with condensed chromatin, suggesting it is involved with chromatin organization (1). As discussed above, potential targets of the transcriptional repression of ZBTB18 include many genes involved in neurogenesis. Disruption of the regulatory control of other possible targets of this repressional activity may help explain some of the variability in the phenotype of our patients.

Conclusion

In summary, we present clinical details of five patients with ID who have de novo likely pathogenic ZBTB18 sequence variants, adding to the eight patients previously described in the literature and affirming the causal link between this gene and ID. Moreover, the presence of CCAs in 4/7 of the patients who had neuroimaging provides additional evidence that ZBTB18 is implicated in CC development. It is likely that additional patients will be discovered in the future, adding to knowledge about the clinical spectrum, genotype-phenotype correlations, and frequency of ZBTB18 variants underlying ID and brain development.

Acknowledgements

We would like to thank the families for their involvement. This work is supported in part by a grant to Wendy Chung from the Simons Foundation.

K. D. F. H., D. N. S., and R. H. are employed by and receive a salary from Ambry Genetics. K. G. M. is employed by and receives a salary from GeneDx. Exome sequencing is among the commercially available tests.

References

1. Aoki K, Meng G, Suzuki K et al. RP58 associates with condensed chromatin and mediates a sequence-specific transcriptional repression. J Biol Chem 1998: 273: 26698–26704.
2. Baubet V, Xiang C, Molczan A, Roccograndi L, Melamed S, Dahmene N. Rp58 is essential for the growth and patterning of the cerebellum and for glutamatergic and GABAergic neuron development. Development 2012: 139: 1903–1909.
3. Ohtaka-Maruyama C, Hirai S, Miwa A et al. RP58 regulates the multipolar-bipolar transition of newborn neurons in the developing cerebral cortex. Cell Rep 2013: 3: 458–471.
4. Okado H, Ohtaka-Maruyama C, Sugitani Y et al. The transcriptional repressor RP58 is crucial for cell-division patterning and neuronal survival in the developing cortex. Dev Biol 2009: 331: 140–151.
5. Xiang C, Baubet V, Pal S et al. RP58/ZNF238 directly modulates prioneprogenic gene levels and is required for neuronal differentiation and brain expansion. Cell Death Differ 2012: 19: 692–702.
6. Baliff BC, Rosenfeld JA, Traylor R et al. High-resolution array CGH defines critical regions and candidate genes for microcephaly, abnormalities of the corpus callosum, and seizure phenotypes in patients with microdeletions of 1q43q44. Hum Genet 2012: 131: 145–156.
7. Nagamani SCS, Erez A, Bay C et al. Delineation of a deletion region critical for corpus callosal abnormalities in chromosome 1q43-q44. Eur J Hum Genet 2012: 20: 176–179.
8. Grellana C, Roselló M, Mondonet S et al. Corpus callosum abnormalities and the controversy about the candidate genes located in 1q44. Cytogenet Genome Res 2009: 127: 5–8.
9. Perlman SJ, Kulkarni S, Manwaring L, Shinawi M. Haploinsufficiency of ZNF238 is associated with corpus callosal abnormalities in 1q44 deletions. Am J Med Genet A 2013: 161A: 711–716.
10. Orellana C, Roselló M, Monfort S et al. Identification of novel genetic causes of Rett syndrome-like phenotypes. J Med Genet 2016: 53: 190–199.
11. de Munnik SA, García-Miñaúr S, Hoischen A et al. Adenovirus non-sense mutation in ZBTB18 in a patient with features of the 1q43q44 microdeletion syndrome. Eur J Hum Genet 2014: 22: 844–846.
12. McRae JF, Clayton S, FitzGerald TW et al. Prevalence, phenotype and frequency of ZBTB18 variants as contributors to de novo mutations. Cytogenet Genome Res 2012: 139: 1903–1909.
13. Bhattacharyya B, Khan GD, Nour M et al. De novo mutations in ZBTB18 in patients with RP58/ZNF238 deletion syndrome. Hum Genet 2016: 135: 763–766.
14. Srivastava S, Cohen JS, Vernon H et al. Clinical whole-exome sequencing in child neurology practice. Ann Neurol 2014: 76: 473–483.
15. Farwell KD, Shahmirzadi L, El-Khechen D et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unscreened families with undiagnosed genetic conditions. Genet Med 2015: 17: 578–586.
16. Rutterer K,Juusola J,Cho MT et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med 2016: 18: 696–704.
17. Hanna RM, Marsh SE, Swistun D et al. Distinguishing 3 classes of corpus callosal abnormalities in consanguineous families. Neurology 2011: 76: 373–382.
18. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat 2015: 36: 928–930.
19. Richards S, Aziz N, Bale S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015: 17: 405–424.
20. Boland E, Clayton-Smith J, Woo VG et al. Mapping of deletion and translocation breakpoints in 1q44 implicates the serine/threonine kinase AKT3 in postnatal microcephaly and agenesis of the corpus callosum. Am J Hum Genet 2007: 81: 292–303.
21. van Bon BWM, Koolen DA, Borgatti R et al. Clinical and molecular characteristics of 1qter microdeletion syndrome: delineating a critical region for corpus callosum agenesis/hypogenesis. J Med Genet 2008: 45: 346–354.
22. Caliebe A, Kroes HY, van der Smagt JJ et al. Four patients with speech delay, seizures and variable corpus callosum thickness sharing a 0.44 Mb deletion in region 1q44 containing the HNRPU gene. Eur J Med Genet 2010: 53: 179–185.
23. Gai D, Haan E, Scholar M, Nicholl J, Yu S. Phenotypes of AKT3 deletion: a case report and literature review. Am J Med Genet A 2015: 167A: 174–179.
24. Thierry G, Bénéteau C, Pichon O et al. Molecular characterization of 1q44 microdeletion in 11 patients reveals three candidate genes for intellectual disability and seizures. Am J Med Genet A 2012: 158A: 1633–1640.
25. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. PLoS Genet 2013: 9: e1003709.
26. Lek M, Karczewski K, Minikel E et al. Analysis of protein-coding genetic variation in 60,706 humans. bioRxiv 2015. DOI: 10.1101/030338.
27. Khajavi M, Inoue K, Lupski JR. Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. Eur J Hum Genet 2006: 14: 1074–1081.
28. Cordeddu V, Redeker B, Stellacci E et al. Mutations in ZBTB20 cause Primrose syndrome. Nat Genet 2014: 46: 815–817.
29. Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annu Rev Biochem 2010: 79: 213–231.
30. Persikov AV, Wetzel J, Rowland EF et al. A systematic survey of the Cys2His2 zinc finger DNA-binding landscape. Nucleic Acids Res 2015: 43: 1965–1984.
31. Najafabadi HS, Mnaimneh S, Schmitges FW et al. C2H2 zinc finger proteins greatly expand the human regulatory lexicon. Nat Biotechnol 2015: 33: 555–562.
32. Garton M, Najafabadi HS, Schmitges FW, Radovani E, Hughes TR, Kim PM. A structural approach reveals how neighbouring C2H2 zinc fingers influence DNA binding specificity. Nucleic Acids Res 2015: 43: 9147–9157.
33. Ohtaka-Maruyama C, Miwa A, Kawano H, Kasai M, Okado H. Spatial and temporal expression of RP58, a novel zinc finger transcriptional repressor, in mouse brain. J Comp Neurol 2007: 502: 1098–1108.
34. Heng JT, Qu Z, Ohtaka-Maruyama C et al. The zinc finger transcription factor RP58 negatively regulates Rnd2 for the control of neuronal migration during cerebral cortical development. Cereb Cortex 2015: 25: 806–816.
35. Heng JT, Nguyen L, Castro DS et al. Neurogenin 2 controls cortical neuron migration through regulation of Rnd2. Nature 2008: 455: 114–118.