SHORT REPORT

A *de novo* non-sense mutation in *ZBTB18* in a patient with features of the 1q43q44 microdeletion syndrome

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The phenotype of patients with a chromosome 1q43q44 microdeletion (OMIM; 612337) is characterized by intellectual disability with no or very limited speech, microcephaly, growth retardation, a recognizable facial phenotype, seizures, and agenesis of the corpus callosum. Comparison of patients with different microdeletions has previously identified *ZBTB18* (*ZNF238*) as a candidate gene for the 1q43q44 microdeletion syndrome. Mutations in this gene have not yet been described. We performed exome sequencing in a patient with features of the 1q43q44 microdeletion syndrome that included short stature, microcephaly, global developmental delay, pronounced speech delay, and dysmorphic facial features. A single *de novo* non-sense mutation was detected, which was located in *ZBTB18*. This finding is consistent with an important role for haploinsufficiency of *ZBTB18* in the phenotype of chromosome 1q43q44 microdeletions. The corpus callosum is abnormal in mice with a brain-specific knock-out of *ZBTB18*. Similarly, most (but not all) patients with the 1q43q44 microdeletion syndrome have agenesis or hypoplasia of the corpus callosum. In contrast, the patient with a *ZBTB18* point mutation reported here had a structurally normal corpus callosum on brain MRI. Incomplete penetrance or haploinsufficiency of other genes from the critical region may explain the absence of corpus callosum agenesis in this patient with a *ZBTB18* point mutation. The findings in this patient with a mutation in *ZBTB18* will contribute to our understanding of the 1q43q44 microdeletion syndrome.

INTRODUCTION

The phenotype of patients with a submicroscopic deletion of chromosome 1q43q44 (OMIM: 612337) is characterized by intellectual disability, microcephaly, growth restriction, dysmorphic features, agenesis of the corpus callosum (ACC) and cardiac, gastroesophageal and urogenital anomalies.[1–8] Even for patients with an overlapping deletion, the phenotype varies considerably. So far, no direct association has been identified between the phenotype and the deleted genes. On the basis of overlapping microdeletions, a number of candidate genes have previously been suggested for microcephaly and corpus callosum agenesis. These include *AKT3*, *ADSS*, *CEP170*, *C1orf100*, *C1orf101*, *C1orf121*, *C1orf199*, *EFCAB2*, *FAM36A*, *HNRNPU*, *HNRNPU-AS1*, *PLD5*, *PNAS-4*, and *SDCCA8*.[1,3,8–14] Specifically, *ZBTB18* has repeatedly been identified as a strong candidate gene for microcephaly and/or ACC.[7,9,11,14,15] *ZBTB18* is particularly compelling since a brain-specific knock-out of this gene in mice causes microcephaly and callosal anomalies.[16] However, other studies suggest critical regions that do not include *ZBTB18*.[8,10,12]

To our knowledge, no point mutations in *ZBTB18* have been described so far. Here, we describe a patient with a *de novo* non-sense mutation in *ZBTB18* and phenotypic features of the 1q43q44 microdeletion syndrome.

MATERIALS AND METHODS

Clinical features

This female was the first child of healthy, non-consanguineous parents. The pregnancy was established through *in vitro* fertilization, because of subfertility due to oligospermia. Amniocentesis, performed because of raised maternal serum markers, revealed a normal female karyotype. On ultrasound at 20 weeks of gestation bilateral pyelectasia and choroid plexus cysts were noted. Labor was induced at 36 weeks of gestation because of intra-uterine growth restriction.

Birth length was 45.5 cm (−2.3 SD), with a weight of 2.15 kg (−2.3 SD), and a head circumference of 30 cm (−2 SD). Neonatal examination was unremarkable. Ultrasound investigations of the brain and the abdomen showed no abnormalities.

The girl developed feeding problems after the introduction of formula milk and weight gain was poor. After elimination of cow's milk from the diet, her weight gain remained delayed. Gastroenterological examinations (including biochemistry, endoscopy, small bowel biopsy, and abdominal ultrasound and upper GI tract X-rays) revealed gastroesophageal reflux, but no other explanation for her growth restriction. Because of developmental delay, and suspected absence seizures associated with vomiting episodes, neurological assessment was performed which was unremarkable. Additional investigations included a cerebral MRI, EEG, metabolic screen, and hearing tests. All results were normal (Figure 1c).

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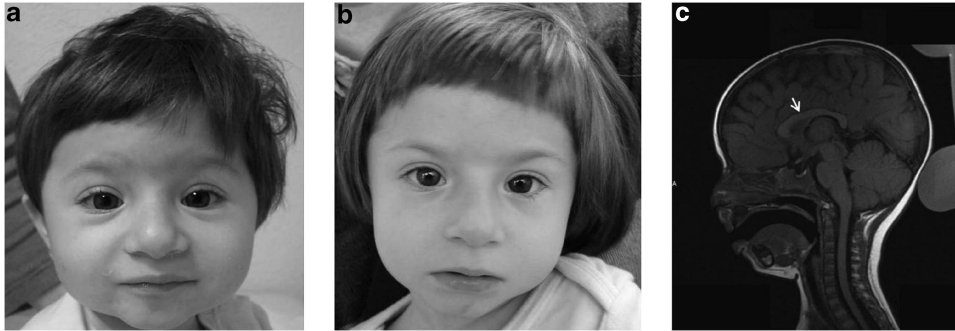
Figure 1 Female with a *de novo* non-sense mutation in *ZBTB18*: c.397G>T (p.(Glu133*)). (**a**) Patient at age 16 months. Dysmorphic features include arched eyebrows with telecanthus, short palpebral fissures, a long nose with a prominent tip, short philtrum, and micrognathia; (**b**) Patient at age 34 months. Dysmorphic features are similar to those at age 16 months; however, the nasal tip is less prominent, the philtrum is longer, and the upper lip is thinner. (**c**) Lateral view of the brain at age 15 months. Note the presence of a normal corpus callosum (white arrow).

At the age of 16 months, she was referred to the genetics clinic for further assessment. At this time, all growth parameters were below −2.5 SD. Her height was 70.5 cm, weight 7 kg, and head circumference 41.5 cm. On clinical examination, she had proportionate short stature, mild facial dysmorphic features (Figure 1a and b), and slightly broad first fingers and toes. At later reviews, her delay became more evident and more marked in the speech domain. She walked unsupported at 26 months. At 34 months, her cognitive development was at the level of 2 years old with prominent speech delay. Her speech was hyper nasal. Her height was 79 cm (−4.5 SD), with a weight of 7.9 kg (−4.6 SD), and a head circumference of 43 cm (−4.1 SD).

At this time, a diagnosis of possible Floating-Harbor syndrome was considered.

Subsequent mutation analysis of the *SRCAP* gene revealed no abnormalities. MLPA assays for telomeric and recurrent genomic rearrangements (MRC-Holland kits) were also normal, as was a genomic microarray.

Molecular analysis

Exome sequencing was performed in a female patient from a cohort of patients with short stature, developmental delay, predominantly speech delay, with or without facial dysmorphic features. Exome sequencing was done on DNA from the female and both parents as described previously.[17,18] In brief, genomic DNA was isolated from blood with the use of a QIAgen DNA mini kit (QIAgen, Venlo, The Netherlands). Exomes were enriched using a SOLiD-optimized SureSelect human exome kit (Agilent v2, 50 Mb, Agilent Technologies Inc., Santa Clara, CA, USA), followed by sequencing performed using the 5500xl platform (Life Technologies, Carlsbad, CA, USA). After sequencing the trio, candidate *de novo* events were selected by excluding variants seen in either parent, whereas recessive models were evaluated using bi-allelic variants inherited from both parents. Candidate mutations were validated by conventional Sanger sequencing methods in DNAs of the patient and her parents (Supplementary Tables 1–3) and annotated according to HGVS recommendations. After publication, the variant will be submitted to the Human Gene Mutation Database (HGMD; URL: http://www.hgmd.cf.ac.uk/ac/index.php).

RESULTS

Exome sequencing detected three *de novo* variants. Two *de novo* missense variants were not confirmed with Sanger sequencing (Supplementary Table 3). Sanger sequencing confirmed the presence of a single *de novo* non-sense mutation located in *ZBTB18*: c.397 G>T (p.(Glu133*)) (NM_205768; UCSC, hg19). This mutation is predicted to cause a premature stop codon and may lead to non-sense mediated decay. No additional mutations in *ZBTB18* were detected through Sanger sequencing in nine patients with an overlapping phenotype. Furthermore, neither stop mutation nor any other truncating event (stop-loss, frameshift mutation) in *ZBTB18* was

identified in 6500 individuals from the exome variant server (Exome Variant Server, NLHBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) (accessed July 2013)).

DISCUSSION

We report a *de novo* non-sense mutation in *ZBTB18* in a patient with global developmental delay, prominent speech delay, microcephaly, short stature, and discrete facial dysmorphisms. These clinical features are consistent with the phenotype of patients with microdeletions of chromosome 1q43q44 (Table 1). Overlapping clinical features include microcephaly (97%), developmental delay (100%), intra-uterine growth retardation (62%), and postnatal growth retardation (45%). We suggest that haploinsufficiency of *ZBTB18* contributes to these features of the chromosome 1q43q44 microdeletion syndrome.

There are a number of reports on patients with deletions of 1q43q44 not including *ZBTB18*.[1,8–12] Out of 27 patients with deletions distal or proximal (including the *AKT3* gene) to *ZBTB18*, only 5 (19%) showed corpus callosum abnormalities and 10 (37%) had microcephaly, compared with 82 and 97%, respectively, of patients with deletions including *ZBTB18*.[1,8–12] Furthermore, IUGR was seen in 3 out of 14 of these patients (21%) and postnatal growth retardation was seen in 7 out of 23 patients (30%). Collectively, these results support a crucial role for *ZBTB18*, but with contributions to the phenotype of other genes in patients with larger deletions.

ZBTB18, also known as ZNF238 or RP58, acts as a transcriptional repressor of key proneurogenic genes such as Neurogenin2 and NeuroD1.[16] ZBTB18 is activated during neuronal differentiation in pin-like cells of the ventricular zone, and in migrating multipolar cells.[19] ZBTB18 participates in neuron and astrocyte differentiation by mediating cell-cycle control of neural stem cells.[20] Mice with loss of ZBTB18/RP58 die at birth with neocortical defects. CNS-specific loss of ZBTB18 is associated with microcephaly, ACC, and cerebellar hypoplasia.[16] ZBTB18-mutant brains maintain precursor pools, but have reduced neuronal and increased glial differentiation. This is consistent with a role for ZBTB18 in favoring neuronal differentiation and brain growth by repressing multiple proneurogenic genes in a timely manner. On the basis of the phenotypes observed in mutant mice, Xiang *et al*[16] proposed ZBTB18 as a strong candidate gene for the 1qter deletion syndrome. Our finding of intellectual disability and microcephaly in a female with a *de novo* non-sense mutation in ZBTB18 strongly supports this hypothesis.

Dysmorphic features in our female were mild, whereas variable facial features have been previously reported with 1q43q44

Table 1 Comparison of the clinical features of a patient with a *de novo* non-sense mutation in *ZBTB18*, c.397G>T (p.(Glu133*)), and the phenotype of patients with a chromosome 1q43q44 microdeletion including *ZBTB18* described in literature

Features	Patient with ZBTB18 mutation	Patients with 1q43q44 microdeletion described in literature (N=29)[1,8–12]
Deletion size (range in Mb)	NA	1.26−4.9
Mutation	397G>T (p.(Glu133*))	NA
Sex	F	16F/12M (57/43%)
Growth parameters		
Intra-uterine growth retardation	+	6/15 (40%)
Postnatal growth delay	+	11/26 (42%)
Microcephaly (OFC< −2SD)	+	28/29 (97%)
Neurology		
Developmental delay	+	18/18 (100%)
Speech delay	+	3/3 (100%)
Corpus callosum anomalies	−	23/28 (82%)
Hypotonia	−	14/18 (78%)
Seizures	−	19/29 (66%)
Facial dysmorphic features		
Hypertelorism	−	5/19 (26%)
Strabismus	−	2/18 (11%)
Prominent nasal tip/Bulbous nose	+	10/19 (53%)
Abnormal philtrum	+	6/19 (32%)
Abnormal lips	+	8/19 (42%)
Micro-/retrognathia	+	1/19 (5%)
Abnormal ears	−	4/19 (21%)
Abnormal fingers/toes	+	8/19 (42%)
Cardiac anomalies	−	7/19 (37%)
Urogenital anomalies	−	6/19 (32%)
Gastrointestinal problems	+	8/17 (47%)

NA, not applicable.

microdeletions of different sizes and gene content. Clearly, other genes from the 1q43q44 region may still contribute to the phenotypic outcome in patient cases with the 1q43q44 microdeletion syndrome. Most (24 out of 29) previously reported patients with a 1q43q44 microdeletion including *ZBTB18* had corpus callosum abnormalities on brain imaging. The majority (66%) developed seizures.[1,8–12,15] Incomplete penetrance is a possible explanation for the absence of ACC in this girl, given that the corpus callosum was apparently normal in at least five patients with 1q43q44 microdeletions that included *ZBTB18* as described previously.[1,9,12] We note that brain-specific loss of *ZBTB18* in mice leads to a small brain phenotype with ACC and cerebellar hypoplasia.[16]

In conclusion, we describe the first patient with a *de novo* non-sense mutation in *ZBTB18*. The phenotype of the patient reported here suggests that *ZBTB18* haploinsufficiency contributes to the microcephaly and global developmental delay that are core features of the 1q43q44 microdeletion syndrome.

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