Citation for final published version:

Publishers page: http://dx.doi.org/10.1002/ajmg.a.62492

Heterozygous variants in *ZBTB7A* cause a neurodevelopmental disorder associated with symptomatic overgrowth of pharyngeal lymphoid tissue, macrocephaly and elevated fetal hemoglobin

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Abstract

By clinical whole exome sequencing, we identified 12 individuals ages three to 37 years old,

including three individuals from the same family, with a consistent phenotype (intellectual

disability (ID), macrocephaly and overgrowth of adenoid tissue). All 12 individuals harbored

a rare heterozygous variant in *ZBTB7A* which encodes the transcription factor Zinc finger and

BTB-domain containing protein 7A, known to play a role in lympho- and hematopoiesis. ID

was generally mild. Fetal hemoglobin (HbF) fraction was elevated 2.2% - 11.2% (ref. value

<2% in individuals > six months) in four of the five individuals for whom results were

available. Ten of 12 individuals had undergone surgery at least once for follicular lymphoid

hypertrophy limited to the pharynx. In the most severely affected individual (individual 1),

airway obstruction resulted in 17 surgical procedures before age 13 years. Sleep apnea was

present in eight of 10 individuals. In the nine unrelated individuals, all *ZBTB7A* variants were

novel and *de novo*. The six frameshift/nonsense and four missense variants were spread

throughout the gene. This is the first report of a cohort of individuals with this novel

syndromic neurodevelopmental disorder.

Key words: developmental delay, intellectual disability, *ZBTB7A*, pharyngeal lymphoid hypertrophy, macrocephaly, whole exome sequencing

Introduction

Whole exome sequencing (WES), a recommended first-tier test for individuals with global developmental delay / cognitive impairment and congenital malformations yields a molecular diagnosis in ~50% of individuals in well-selected cohorts (Srivastava et al., 2019). Resources such as DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources (DECIPHER) (Firth et al., 2009) and GeneMatcher (Sobreira et al., 2015) facilitate contact between clinicians and researchers with an interest in the same gene. Benefits of a known etiological diagnosis include an improved basis for reproductive decision-making, the option of contact with other individuals and families, and relief from uncertainty about causation.

The zinc finger and BTB domain-containing 7A transcription factor, located on chromosome 19p13.3, also known as Pokemon and lymphoma/leukemia-related factor (LRF), is a widely expressed in humans (Davies et al., 1999). It is involved in several cellular activities (Constantinou et al., 2019). Notch signaling in progenitor cells drives T-cell development at the expense of B-cell development. *ZBTB7A* is required to block Notch signaling in bone marrow progenitor cells, allowing their development into B-cells, thus *ZBTB7A* is a master up-regulator of T-cell proliferation (Maeda et al., 2007). The thymus expresses an abundance of ligands that activate the Notch pathway, which plays an important role in neuronal development (Salzar et al., 2020). Mouse knockouts of *ZBTB7A* exhibit aberrant activation of

the Notch pathway (Maeda et al., 2007) and deletion of *ZBTB7A* may contribute to the developmental delay in individuals with 19p13.3 deletions (de Smith et al., 2011). Changes on chromosome 19 are uncommon. A few cases of 19p13.3 deletions are reported with variable size of the deletions and variable phenotypes (Risheg et al., 2013; de Smith et al., 2011). Common features in individuals with similar 19p13.3 deletions include intellectual disability and developmental delay, cardiac defects, and minor facial anomalies (Risheg et al., 2013). *ZBTB7A* has been proposed as a candidate gene for several phenotypic traits (macrocephaly, obesity, sleep apnea, umbilical hernia, and ID) in individuals with 19p13.3 microdeletions (Risheg et al., 2013; de Smith et al., 2011). A single case report describes a boy with macrocephaly, ID and sleep apnea, with a *de novo* variant in *ZBTB7A* (Ohishi et al., 2020).

We report 12 individuals with heterozygous variants in *ZBTB7A* [MIM: 65878] encoding a transcription factor that represses expression of a wide range of genes involved in cell proliferation and differentiation. In our cohort, individuals had macrocephaly (11/12), some degree of ID (12/12), autistic features (7/12) and hypertrophy of pharyngeal lymphoid tissue (12/12).

We aim herein to begin to delineate the molecular and phenotypic spectrum in individuals with a neurodevelopmental syndrome caused by pathogenic variants in *ZBTB7A*.

Materials and Methods

The study was performed according to the declaration of Helsinki and approved by the Norwegian Regional Committee for Medical Research Ethics in South-East Norway (no. 185548) and the Institutional Data Protection Authority (PVO) at Telemark Hospital Trust.

Individual 1 was identified by trio whole exome sequencing (trio WES) in 2018, registered in GeneMatcher the same year and in DECIPHER in 2020. Individuals 2-12 were identified via contact through DECIPHER (Firth et al., 2009) and GeneMatcher (Sobreira et al., 2015).

In individual 1 copy number variant (CNV) analysis, and investigations for *PTEN* variants (MLPA/sequencing) and Beckwith-Wiedemann syndrome (methylation-sensitive MLPA for chromosome 11p15.5), and sequencing of a panel of ~2000 genes were performed because of overgrowth of pharyngeal lymphoid tissue, macrocephaly and delayed motor and language development. Trio WES was subsequently performed on DNA from blood and the lymphatic tissue.

Informed written consent was obtained from each family for publication of genetic and clinical information. The parents of individual 1 consented to the publication of radiological and histological images.

Results

This cohort consists of four females and eight males aged three to 37 years with rare heterozygous variants in *ZBTB7A*. Clinical features and genotypes are summarized in Table 1. The most frequent features are summarized in Table 2. Supplemental case reports are available online (Supplement 1).

In individual 1, results were normal in all instances as well as in sequencing of a panel with ~2000 genes, the majority of which are associated with pediatric neurodevelopmental disorders. The only finding of interest in trio WES was a heterozygous *de novo* variant in *ZBTB7A,* detected in DNA from both blood and lymphatic tissue. *ZBTB7A* is a candidate gene for a syndromic neurodevelopmental disorder (Ohishi et al., 2020). The variant was registered in GeneMatcher and DECIPHER which allowed us to recruit an additional eleven individuals. Genotypes and clinical phenotypes were provided by collaborating clinicians.

Molecular findings

Figure 1 shows the locations on *ZBTB7A* (NM_015898.4) of both the 12 new cases and the previously reported case. Eight individuals had confirmed *de novo* variants, two sibs had inherited variants from an affected parent and two had unknown inheritance. One individual had two *de* novo variants in *cis*. All individuals carried variants that either affect highly conserved amino acids (missense- and indel variants), lead to truncation of ZBTB7A or are predicted to undergo nonsense mediated decay (NMD) (LoF-variants). All variants are absent from gnomAD (gnomad.broadinstitute.org).More information on the variants is shown in Table 1.

No other variants of interest were detected in any of the patients.

Pregnancy and delivery

Pregnancy and birth history were uneventful with a few exceptions. Individual 2 is a dizygotic twin conceived after *in vitro* fertilization and born by planned Cesarean section at 34 weeks gestation. Maternal age was 40 years at delivery and paternal age was 39 years. Birthweight (BW) was 1899 grams and birth length (BL) was 43 cm. She was slow to feed, received phototherapy for hyperbilirubinemia, and was discharged home after four weeks. Individuals 7 and 8 are half-sibs and inherited the identical *ZBTB7A* variant from their father (individual 9). Individual 7 was born to a 27-year-old mother by Cesarean section at 39 weeks gestation. Pregnancy was complicated by fetal macrosomia. BW was 4394 grams and BL 54,6 cm. Transient respiratory distress was treated with supplemental oxygen; he was weaned quickly to room air and discharged home at four days old. He was readmitted the following day due to increased respiratory effort, poor feeding, and jaundice requiring phototherapy. An echocardiogram identified two small ventricular septal defects (VSDs) and an atrial septal

defect (ASD) that will require surgical intervention. He was discharged home at 10 days of age. His half-sister (individual 8) was born to a 22-year-old mother by spontaneous vaginal delivery at 39 weeks gestation. Pregnancy was complicated by gestational diabetes. BW was 3827 grams and BL was 53.3 cm. She also had a persistent ductus arteriosus (PDA) and moderate sized atrial septal defect (ASD), which required surgical closure. She was discharged home at five days of age, then readmitted shortly after for phototherapy for jaundice and discharged home at two weeks of age.

Lymphoid hypertrophy and adenoid overgrowth

Symptomatic lymphoid hypertrophy or adenoid overgrowth was present in all individuals. Upper airway obstruction, in many resulting in documented or suspected sleep apnea, was present in all individuals, except in individuals 4 and 5. In individual 12 sleep apnea has been suspected but polysomnography has not been performed. Ten individuals had undergone surgical excision of adenoid tissue. The most severely affected individual (individual 1) underwent 17 surgical procedures before 13 years of age. Individual 4 was reported to snore but had a normal sleep study. Frequent upper airway infections were a consistent feature (10/12). Three individuals (individual 5, 7 and 9) were reported to have had recurrent pneumonias. CT thorax, performed only in individual 1 at ten years of age, showed an enlarged thymus. MRI of the neck, at the age of 13 years old, showed excess lymphatic tissue in the pharynx (Figure 2) which on histological examination was classified as adenoid tissue with lymphoid follicular hyperplasia (Figure 3).

Craniofacial features

Another striking finding was macrocephaly; 11 of 12 individuals had an occipito-frontal head circumference (OFC) above or equal to the 90th percentile, including seven with an OFC

above the 97.5th percentile. Except for macrocephaly, there were no common characteristic facial features. Dental crowding was present in five (individuals 2, 3, 5, 8 and 10). Individual 11 had numerous retained deciduous teeth. Individuals 3 and 10 had facial asymmetry and hemifacial hypertrophy, respectively.

Neurodevelopment

Nine individuals had delayed achievement of motor milestones, usually mild delay, with walking generally achieved before age 24 months. One individual (individual 5) with severe ID who underwent surgery for congenital bilateral pes cavus walked at age six years. Cognitive impairment was generally mild. Speech was delayed in nine of 12 and autistic features were common (7/12).

Seizures

Two individuals (individuals 2 and 5) had seizures and were treated with anticonvulsants. Individual 7 had a single seizure when he was two years old, after which he did not receive an anticonvulsant. Individual 11 had seizures presumably as a sequela of brain abscesses.

Gastrointestinal

Three individuals had an umbilical (individuals 1, 4, and 7) and one a hiatal hernia (individual 9). Laryngomalacia was reported in two and gastro-esophageal reflux in four individuals. Only one individual who had mild developmental delay (individual 10) had severe feeding difficulties. At age nine years she remains gastrostomy dependent. Weight was above the 97th percentile in seven of ten individuals.

Visual issues

No serious visual issues were noted. Three individuals had astigmatism, one had pronounced hypermetropia, one mild myopia, and one mild hypermetropia.

Cardiovascular issues

In an adult male (individual 9) with cardiac hypertrophy there was suspicion of congenital heart defect that resolved spontaneously. Both his children (individuals 7 and 8) inherited his *ZBTB7A* variant. One child (individual 7) had two ventricular septal defects (VSD) and an ASD and his half-sister had a PDA and ASD. Individual 11 had a small patent foramen ovale.

Hematological issues

Two individuals (individuals 10 and 11) had consistently low monocyt counts. One individual (individual 5) had a consistently moderately elevated monocyte count, and hypogammaglobulinemia compatible with a diagnosis of common variable immunodeficiency (CVID) requiring immunoglobulin replacement therapy (IgRT). Normal monocyte counts were recorded in the four individuals (individuals 1, 2, 7 and 8) for whom information was available. Neutropenia was reported in one individual.

Fetal hemoglobin (HbF) was only available for five individuals (individuals 1, 4, 5, 11, and 12). Four (individuals 1, 4, 11, and 12) had HbF levels above the upper reference limit (2%), respectively 4.4% (age 12 years and 10 months), 2.2% (age five years), 11.1% / 2.8% (at ages 22 months / 10 years, respectively), and 5.3% (age 17 years). Individual 5, who had beta thalassemia minor, had an HbF level of 0.5% at age 26 years.

Discussion

We identified 12 individuals within a phenotypic spectrum with pathogenic heterozygous variants in the *ZBTB7A*. All 12 had clinically significant pharyngeal lymphoid hypertrophy, in some instances quite striking and recurrent, with adenoid overgrowth. Frequent upper airway infections were present in ten. Delayed gross motor development was present almost without exception. Some degree of cognitive impairment, from specific learning difficulties to severe ID was a consistent finding. Other salient features included macrocephaly, without structural brain abnormalities, sleep apnea, and increased weight for height, autistic features and attention deficit hyperactivity disorder (ADHD). No common dysmorphic feature, other than macrocephaly was noted. An increased HbF fraction may be characteristic for individuals with pathogenic *ZBTB7A* variants.

The gene constraint metrics for *ZBTB7A* suggest intolerance to haploinsufficiency (gnomAD pLI = 0.96, o/e = 0.33) and missense variation (gnomAD missense Z-score = 4.04) in humans (Karczewski et al., 2020). In our cohort, six loss-of-function (LoF) variants are predicted to lead to nonsense mediated decay (NMD). Two variants are predicted to produce a truncated protein: Val417Glyfs*123 will disrupt three of the C2H2 zinc finger domains and the nuclear localization signal, and Arg530Glyfs*27 will remove 29 amino acids from the well conserved C-terminal portion of the protein. All missense variants except Ala287Leu affect well conserved amino acids that are located in the BTB-domain or one of the C2H2 zinc finger domains. The individual carrying the Ala287Leu variant also carries Ala111Pro which affects a highly conserved amino acid in the BTB-domain. All variants are absent from gnomAD.

All 12 individuals in our cohort had some degree of learning difficulties or ID. There was no difference in phenotype between patients with truncating and missense variants.

Nine of the 12 individuals were reported to have sleep apnea. All 12 had lymphoid hypertrophy/adenoid overgrowth. Ohishi et al. (2020) did not report lymphoid/adenoid overgrowth in their patient who, however, had sleep apnea and recurrent upper airway infections. Ten individuals in our cohort experienced recurrent upper airway infections. Two were noted to have low monocyte counts and one hypogammaglobulinemia. In the others, no obvious immunological factor was detected that would likely contribute to increased susceptibility to upper airway infection. In individual 1 hypertrophy of lymphoid tissue was located in Waldeyer's lymphatic ring in the absence of enlarged lymph nodes (Figure 2). Inhaled antigens meet host immune cells initially in the lymphoepithelial tonsils and adenoids (Nave et al., 2001). The pathogenesis of chronic non-malignant tonsillar disease is largely unknown: however, a few mechanisms have been proposed. Respiratory viruses are prevalent in the adenoids in individuals with chronic tonsillar disease and may cause persistent inflammation resulting in adeno-tonsillar hypertrophy (Proenca-Modena et al., 2012). Gastro-esophageal reflux may also contribute to tonsillar hypertrophy (Kim et al., 2017). We think that the detected *ZBTB7A* variants are causally linked to an overgrowth of lymphoid tissue, however, chronic virus infections and reflux may aggravate hypertrophy. Enlarged tonsils and adenoids are the most common cause of sleep apnea in children without craniofacial anomalies or neuromuscular disorders (Garg et al., 2017). None of the previously published cases of 19p13.3 deletions (Risheg et al., 2013; de Smith et al., 2011) reported hypertrophy of adenoids or tonsils: however, whether they were specifically evaluated for this is not clear from the publications. We suggest that all children with pathogenic variants in *ZBTB7A* or with 19p13.3 deletions be investigated with polysomnography and evaluated for hypertrophy of the adenoids and tonsils, noting that adenotonsillectomy/tonsillectomy is recommended in children with obstructive sleep apnea (Mitchell et al., 2019).

Individual 5, an 18-year-old male with microcephaly, hypogammaglobulinemia, beta thalassemia minor and severe ID, diverges phenotypically from the rest of our cohort. Genetic testing prior to trio WES detected a maternally inherited duplication (chrX:7,932,124-8,565,153 (hg19)). This CNV includes *VCX2*, *VCX3B* and a portion of *KAL1*. Duplication or gain of function of these genes, has not been reported as a cause of X-linked ID. It seems unlikely therefore that the duplication explains or contributes to the severity of the phenotype, and an additional, undetected causal mechanism may be present. It could be discussed whether the *ZBTB7A* missense variant in this patient has an opposite effect compared to the other variants.

ZBTB7A upregulation represses expression of fetal hemoglobin and downregulation of ZBTB7A results in increased HbF expression (Masuda et al., 2016, Chumchuen et al., 2019). The developmental switch from fetal to adult hemoglobin occurs just before birth. Normally, fetal hemoglobin (HbF) decreases day by day after birth and constitutes less than 2% of the total hemoglobin after six months (Huisman, 1993), and is on average closer to 1% than 2% (Zertal-Zidani et al., 2002). Hereditary persistence of HbF (Swiss HPFH) and sickle cell anemia may cause elevated HbF (Huisman, 1993). Ohishi et al. (2020) reported an unusually high HbF fraction of 100% at one month of age in their case and suggested that the detected *ZBTB7A* variant led to a failure of repression of HbF expression (Ohishi et al., 2020). HbF fraction was available for five of the 12 individuals in our cohort. Four had elevated HbF fractions of 4.4%, 2.2%, 2.8% and 5.3% at ages 12 years and 10 months, five years, 10 years, and 17 years, respectively. In individual 11 the elevated HbF decreased with age, and in individual 5, who had beta-thalassemia minor, HbF was in the normal range (0.5%) at the age of 26 years. This may indicate that the decrease in HbF fraction is slowed but may normalize.

Whether HbF fraction is a helpful diagnostic marker for pathogenicity of a *ZBTB7A* variant in children remains to be determined.

Dysregulated cellular processes, such as proliferation, growth, and differentiation are mechanisms in both developmental disorders and cancer. Among individuals with developmental delay, germline damaging *de novo* variants are more enriched in cancer driver genes than non-drivers (Qi et al., 2016). There are well-known genes and pathways implicated in both cancer and developmental disorders, with recurrent somatic mutations in cancer and germline variants in developmental disorders. One example is *PTPN11* (MIM#176876), a phosphatase in RAS/MAPK signaling pathway, implicated in both Noonan syndrome and leukemia (Tartaglia et al., 2003). *ZBTB7A* has been reported to be a tumor suppressor in several cancers (Shen et al., 2017, Wang et al., 2013). One individual (individual 5) was reported to have acute myeloblastic leukemia at age of one month. Beyond this, we have no indication of increased risk of malignancy in our limited cohort (mean age 14.5 years) with constitutional heterozygous pathogenic variants in *ZBTB7A*.

Conclusion

Pathogenic variants in *ZBTB7A* cause a syndromic neurodevelopmental disorder characterized by overgrowth of pharyngeal lymphoid tissue often with clinically significant upper airway obstruction, macrocephaly and ID. Increased HbF fraction in children may be a useful marker for the disorder.

Limitations

Subjects were recruited from different primary centers in different countries, and clinical information is based on information provided by each contributor. In addition, not all

individuals had undergone all the same investigations, for example HbF measurements or extended immunological investigations.

Acknowledgements

Contributors

CvdL, KT, TP, ØLH, ØLB, WT, FHF, AF, DL contributed to the conception and design of the study.

CvdL, KT, TP, ØLH, ØLB, WT,SC, RU, JH, FP, FHF, AF, DL, KBB, JB, SJ, JE, DO, CM, IB, FF, ASAC, KE, DZ, SMA, MJ, FB-B, JL, SD, ND contributed to data acquisition and interpretation of data for the work.

CvdL, KT, TP, ØLH, ØLB, WT, SC, RU, JH, FP, MGH, FHF, AF, DL, KBB, JB, SJ, JE, DO, CM, IB, FF, ASAC, KE, DZ, SMA, MJ, FB-B, JL, SD, ND contributed by providing genetic and clinical data and editing of the manuscript.

CvdL, KT, TP, ØLH, ØLB, WT contributed to preparing the figures and original draft preparation.

CvdL, KT, TP, ØLH, ØLB, WT, JH, FP, MGH, FHF, AF, DL KBB, JB, SJ, JE, DO, CM, IB, FF, ASAC, KE, DZ, SMA, MJ, FB-B, JL, SD, ND contributed to drafting the report and participated in final draft revisions.

Competing interests

None declared.

Ethics approval This study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the Norwegian regional ethic committee for south east Norway (REK Ref. 185548), data protection officer at Telemark Hospital Trust, and site-specific institutional review boards. Written informed consent was obtained from all individuals or their legal guardians for publication of genetic, clinical, and radiological data.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1. Genetic and clinical findings in 12 individuals with heterozygous variants in *ZBTB7A*

Findings and features	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6
ZBTB7A variant NM_015898.4	c.832G>T p.(Glu278*)	c. 167_168delGCinsTT p.S56I	c.1108C>T; p.(Gln370*)	c.1588del p.(Arg530GlyfsTer27)	c.1354 G>A p.(Asp452Asn)	c.[331G>C; c.859_860delinsCT] p.[(Ala111Pro); (Ala287Leu)]
Variant type	Nonsense	Missense (indel)	Nonsense	Frameshift	Missense	Missense (indel) + missense
Inheritance	*De novo*	Unknown	*De novo*	*De novo*	*De novo*	Two *de novo* variants in *cis*
Gender, age at last assessment	Female, 13y	Female, 10y	Male, 18y	Male, 10y 11m	Male, 18y	Male, 19y
Prenatal / delivery	Uneventful / Elective CS, 38 wk	Twin, IVF / Elective CS, 34 wk	Uneventful / Uneventful, 42 wk	Uneventful / Elective CS, 38 wk	IUGR / uneventful, 36 wk	Uneventful / Uneventful, 40 wk
Motor milestones	Mild delay Walked 18m	Mild delay Walked 21m	Mild delay Walked 18m	Mild delay Walked 20m	Severe delay Walked 6y	Normal
Speech	Mild delay	Mild delay	Mild delay	Delay	Severe delay	Mild delay
Cognitive impairment/ID	Mild delay	Mild delay	Moderate ID	Moderate ID	Severe ID	ID
Autistic features	Yes	Yes	No	Yes	Yes	Yes
Seizures	No	Yes	No	No	Yes	No

Sleep apnea	Yes	Yes	Yes	Snores, normal sleep study	No	Yes
Recurrent upper airway infections	Yes	No	Yes	Yes	Yes Recurrent pneumonias	Yes
Lymphoid hyperplasia/adenoid overgrowth	Yes	Yes	Yes	Yes	Yes	Yes
Weight, percentile	>97,5p	18p	98p	99p	<<3p	>97p
OFC, percentile (mother's/father's OFC)	97,5p (25p / 75p)	>97,5p (50-75p / NA)	91-98p (25p / 25p)	94p (NA)	<<3p (50p/25-50p)	>99p (75p / 75p)
Visual issues	Astigmatism	Normal	Astigmatism Red green color blindness	Normal	Pronounced hypermetropia	Normal
Hearing issues	No	No	Hyperacusis	No	No	No
Cardiovascular issues	No	No	No	No	No	No
Gastrointestinal issues	Reflux	No	No	No	Severe reflux	No
Dysmorphic features	Short neck	Congenitally absent upper right lateral incisor Dental crowding	Facial asymmetry Downslanting palpebral fissures Dental crowding	Uplifted earlobes Finger pads Inverted nipples High arched palate	Low hairline High arched palate Dental crowding Long philtrum	NA

HbF	*HbF 4,4% (12y 10m)	HbF NA	HbF NA	*HbF 2,2% (5y)	*HbF 0,5% (26y)	HbF NA
Other findings / issues	Umbilical hernia Laryngomalacia ADHD Monocytes normal (several times)	ADHD Dyslexia Monocytes normal (5y)	High pain threshold Monocytes N/A	Umbilical hernia Drooling Swallowing difficulties Pancytopenia Monocytes normal	AML (1m) GH deficiency Pes cavus Hypogammaglobinemia HbA2 5,5 beta-thalassemia minor Monocytes elevated (several times)	No Monocytes NA

Findings and features	Individual 7	Individual 8	Individual 9	Individual 10	Individual 11	Individual 12	Ohishi *et al.* 2020
***ZBTB7A* variant NM_015898.4**	c.642dupC p.(Asn215GlnfsTer35)	c.642dupC p.(Asn215GlnfsTer35)	c.642dupC p.(Asn215GlnfsTer35)	c.983del p.(Gly328AlafsTer26)	c.1247dupA p.(Val417Glyfs*123)	c.1214C>A p.(Thr405Lys)	c.1152C>G p.Cys384Trp
Variant type	Frameshift	Frameshift	Frameshift	Frameshift	Frameshift	Missense	Missense
Inheritance	Paternal	Paternal	Unknown	*De novo*	*De novo*	*De novo*	*De novo*
Gender, age at last assessment	Male, 3y 2m	Female, 8y 11m	Male, 37y	Female, 9y 8m	Male, 10y	Male, 17y	Male, 6y 5m
Prenatal/ delivery	Fetal macrosomia / CS, 39 wk	Gestational diabetes / Uneventful, 39 wk	NA	Maternal SLE / Uneventful	Uneventful / Uneventful, 39+5 wk	Uneventful / vaginal delivery, 40 wk	Uneventful / Uneventful 40wk

Motor milestones	Mild delay Walked 20m	Normal	NA	Mild delay Walked 24m	Mild delay Walked 19m	Mild delay	Mild delay Walked 17m
Speech	Repetitive and expressive language disorder	Normal	Normal	Mild delay	Mild delay	Mild delay	Mild delay
Cognitive impairment/ID	Global developmental delay	Specific learning disorder in mathematics	Mild learning disability	Mild developmental delay	Mild ID	Learning difficulties/ specific learning disorders/ language develop- mental disorder	Mild ID
Autistic features	Yes	No	No	No	No	Yes	N/A
Seizures	One seizure at 2y of age, no medication	No	No	No	Yes (after brain abscesses)	No	No
Sleep apnea	Yes	Yes	Yes	Yes	Yes	No	Yes
Recurrent upper airway infections	Yes Recurrent pneumonias	Yes	Yes Recurrent pneumonias	Unknown	Yes	Yes	N/A
Lymphoid hyperplasia/aden oid overgrowth	Yes	Yes	Yes	Yes	Yes	Yes	N/A

Weight, percentile	>99p	98p	N/A	79p	98p	95p	>97p
OFC, percentile (mother's/father's OFC)	>97,5p (NA / >97,5p)	90p (NA / >97,5p)	>97,5p (NA / NA)	93p (41st/ 71st)	>99p (NA / NA)	99p (70p/93p)	>97p
Visual issues	Normal	Normal	Normal	Normal	Mild hyper-metropia	Astigmatism Mild myopia	Normal
Hearing issues	Recurrent acute otitis media, ventilation tubes, normal hearing	Recurrent chronic serous otitis media, normal hearing	Ventilation tubes in childhood x several, mild hearing loss	Hyperacusis, Serous otitis media	No	No	Normal
Cardiovascular issues	VSD x 2 spontaneous closure, ASD surgically repaired	ASD (moderate) PDA	Mild cardiomyopathy	No	Small patent foramen ovale	ASD (+ positive family history for same)	PDA
Gastrointestinal issues	Reflux	Reflux	NA	PEG at 2y 10m for feeding difficulties	No	No	No
Dysmorphic features	Prominent forehead Downslanting palpebral fissures Epicanthal folds Large, slightly protruding ears Long philtrum Thin upper lip Fifth finger clinodactyly	Thick eyelashes Broad nasal tip Dental crowding High narrow palate Mildly tapered fingers Mild genu valgum Bilateral palmar erythema	NA	Plagiocephaly Hemifacial hyperplasia Dental crowding	Flat nasal bridge Numerous retained deciduous teeth	Short palpebral fissures Epicanthal folds	Prominent forehead

HbF	HbF NA	HbF NA	HbF NA	HbF NA	*HbF 11,1% (22m) / 2,8% (10y)	*HbF 5,3%	*HbF 100% (at 1 month old)
Other findings / issues	Hypotonia Pectus excavatum Small umbilical hernia Monocytes normal (several times)	Hypotonia Monocytes normal (several times)	Pectus excavatum Hiatal hernia Monocytes NA	Monocytes low (at 1y and 8y)	Laryngo-malacia Bilateral multiple brain abscesses (Strep milleri), drained at 1y 8m von Willebrand disease (inherited) Dyspraxia Nasal speech Monocytes consistently low	Pectus excavatum	Umbilical hernia Hypotonia Monocytes NA

Abbreviations: ASD: atrial septal defect, CS: cesarean section, GH: growth hormone, HbF: Fetal hemoglobin, ID: intellectual disability, IUGR: intrauterine growth restriction, IVF: *in vitro* fertilization, m: month(s), NA: not available, OFC: occipitofrontal head circumference, PDA: persistent ductus arteriosus, PEG: Percutaneous endoscopic gastrostomy, SLE: systemic lupus erythematosus, VD: vaginal delivery, VSD: ventricular septal defect, wk: week(s), y: year(s)

*Reference range: HbF<2% after 6 months postnatal age (Huisman, 1993)

Table 2. Summary of central features

Feature	Number/Total # investigated	Ohishi *et al.* 2020
ZBTB7A-variant Nonsense/Frameshift	8/12	
ZBTB7A-variant Missense	4/12	c.1152C>G p.Cys384Trp
Lymphoid hypertrophy/**adenoid overgrowth**	12/12	N/A
Mild learning difficulties/**Cognitive impairment/ID**	12/12	Yes
Motor milstones delayed	9/11	Yes
Speech delay	9/12	Yes
Macrocephaly (≥ 90p)	11/12	Yes
Weight > 97th percentile	7/11	Yes
Frequent upper airway infections	10/11	N/A
Sleep apnea	9/11	Yes
Autistic features	7/12	N/A
Elevated HbF	4/5	Yes

Supplement 1: Case reports

Individual 1

This is a 12-year-old girl delivered by elective Cesarean section (due to a previous difficult delivery) at 38 weeks' gestation after an uncomplicated pregnancy. Birthweight (BW) was 3560 g (50p), birth length (BL) 50 cm (50p), and occipito-frontal head circumference (OFC) 36 cm (75p). She had an umbilical hernia measuring three centimeters in diameter and laryngomalacia at birth, neither of which required surgery. She had frequent upper airway infections as a young child, persistent serous otitis media from infancy and obstructive sleep apnea. She continues to have upper airway infections, although they are milder now. Her gastroesophageal reflux is treated with Esomeprazole and Famotidine. Strikingly, starting at age 15 months, she has undergone 17 surgical procedures for recurrent symptomatic pharyngeal lymphoid hyperplasia (Figure 1 and Figure 2). Her thymus is large for her age. Generalized lymphadenopathy/ hepatosplenomegaly are absent. She has mild fine and gross motor delay. She started to walk at age 18 months, had language delay, and has attention deficit disorder (ADHD) and autism spectrum disorder (ASD). Measurements are height 25 p, weight 4 kg > 97.5 p, OFC 97.5 p.

Her blood counts at the age of 12 years and 10 months revealed an elevated HbF of 4.4% (normal <2%).

Whole exome sequencing (WES) trio identified a *de novo* nonsense variant in *ZBTB7A*: NM_015898.3:c.832G>T p.(Glu278*) which is predicted to result in NMD. The variant is not present in gnomAD. The gene is LoF-intolerant (pLI=0.96; O/e=0:07). No other SNVs of interest were detected, and a 180k array CGH analysis was unremarkable. Investigations of *PTEN* (MLPA and sequencing) and for Beckwith-Wiedemann syndrome (MLPA and methylation) were unremarkable.

Individual 2

This 10-year-old girl is Twin B of dizygotic girls conceived by in vitro fertilization and born by planned Cesarean section at 34 weeks gestation. Maternal age was 40 years and paternal age 39 years at delivery. BW was 1899 g and BL was 43 cm. She was discharged from hospital after four weeks; neonatal complications included being slow to feed and hyperbilirubinemia treated with phototherapy. Hypotonia was noted at six months and physical therapy was initiated. Development was delayed; she walked at 21 months, had delayed fine motor milestones and spoke her first words at 12 months. Social milestones were also delayed, and she was diagnosed with ASD and ADHD at four years and nine months. Her most recent neuropsychological evaluation at eight years and eight months showed ASD with associated language disorder, ADHD predominantly inattentive type, developmental coordination disorder, and dyslexia. She attends a general education classroom with an individualized education plan, and receives special education instructions in reading and mathematics, occupational therapy, dyslexia therapy, speech therapy and applied Behavior Analysis Therapy. She has a writing device and the services of a paraprofessional at school. ADHD is managed with atomoxetine.

Evaluation of nocturnal arousals from sleep at age five years showed seizures associated with multifocal epileptiform discharges. Seizures were controlled with oxcarbazepine monotherapy until after her adenotonsillectomy for obstructive sleep apnea at six years eight months, when she had breakthrough seizures that recurred on maximal oxcarbazepine therapy. Repeat EEG showed persistence of multifocal epileptiform discharges and new bifrontal predominant generalized waves. Her anticonvulsant medication was migrated to clobazam monotherapy, and seizures have been controlled since. MRI brain at five years three months was normal.

Physical examination is remarkable for a hyperpigmented sebaceous nevus 0.5 cm above the nasal bridge, a capillary hemangioma at nape of neck, absent upper right lateral incisor, mild generalized hypotonia and macrocephaly.

Individual 3

This 18-year-old male is the 2nd child of unrelated Caucasian parents. Uneventful pregnancy. Born at 42 weeks gestation by normal vaginal delivery. BW was 4.765 kg (99th centile, +2.4SD). Admitted to hospital at two weeks of age for bronchiolitis and low oxygen saturations requiring nasal cannula oxygen. In hospital for seven days before discharge. Concerns with developmental delay affecting motor and speech developed. He was late crawling and achieved walking at age 19 months of age. He received speech and language therapy during childhood and his speech has improved. There were initial concerns with ASD due to speech delay and play skills but following formal assessment, he did not have enough features to achieve a diagnosis of autism.

He developed recurrent ear and throat infections and sleep apnea confirmed on sleep study. Tonsillectomy and adenoidectomy were performed at age 3 years. ENT review at age seven years and four months due to persistence of mouth breathing and nasal speech showed regrowth of adenoids but in absence of sleep apnea no further surgical intervention was required.

He was found to have microcytic anemia with normal ferritin levels at age six years and required treatment for iron deficiency anemia despite a good balanced diet. HbF levels were not investigated.

Despite his early receptive and expressive language delay, he has a good vocabulary and speaks in full sentences but has pronunciation/articulation difficulties. He has mild to

moderate learning difficulties. He attended a mainstream primary school initially but required one-to-one assistance in the classroom and then at Year 5, attended a special educational needs school followed by special educational needs secondary school. He is now age 18 years old and currently attends college (mainstream) and requires assistance from a teaching assistant. He has difficulty reading and has slow understanding.

Physical features noted in genetics clinic age nine were facial asymmetry, down-slanting palpebral fissures, narrow arched palate with dental crowding and teeth malalignment and an overbite. He has truncal obesity, gynecomastia and mild pectus excavatum, normal appearance of hands and feet, and no heart murmur on auscultation.

With regards to dental anomalies, he has had four teeth extracted and orthodontic braces fitted to help with the cosmetic appearance. Surgical correction for the overbite requiring major surgery was declined by the patient and family but being kept under review.

His growth parameters continue to follow along the 98th centile for weight, 98th centile for height and 91-98th centile for head circumference. His growth parameters are not in keeping with parents and sibling (OFC for both parents on 25th centile).

Individual 4

This 10 year and 11 months old boy was born at term to unrelated parents; one older brother was well. Elective Caesarean at 39 weeks. BW 4370 g. OFC 39 cm. Born in good condition but after birth develop signs of mild respiratory distress (transient tachypnea of the newborn) and was admitted to NICU for one week. He needed supplemental oxygen (head box) for two days, received intravenous antibiotics for 48 hours and nasogastric tube feeding. He developed physiological jaundice and was treated with phototherapy. He fed well on bottle at discharge.

The boy showed early signs developmental delay: sat at nine months, crawled at 11 months, walked between 19 and 20 months. He could say "mama" and "dada" at one year, but at four years he only had ten words. He was referred to the community pediatrician at the age of three years and five months because of a short attention span, poor language skills and repetitive play.

He had a history of recurrent tonsillitis, otitis media and chest infections. At the age of five years during acute tonsillitis he developed a non-blanching rash and was admitted to hospital; he had pancytopenia and was kept under regular follow up with the hematologist. Pancytopenia resolved but a cause was not found. He had no more episodes of pancytopenia. During investigations he was found to have slightly raised HbF 2.2% and microcytic anemia. The patient had persistent small umbilical hernia aged four and had surgery for it. In the past medical history he also suffered with asthma. The patient had loud snoring but not signs of obstructive sleep problems on sleep study. He has significant drooling.

He was investigated for global development delay at age five years. He had a normal male karyotype, normal Fragile X and microarray.

He was referred to genetics aged eight years because of developmental delay with learning difficulties and because he was large child with some unusual features and a previous history of pancytopenia of unknown cause despite extensive investigations within the hematology service. A diagnosis was not found when seen in genetics aged eight years and four months. He was enrolled in the 100,000 genome project in 2017. He was reviewed in genetics in 2019 aged 10 years and 11 months. The result from 100,000 genome project was not available at that time.

The boy was diagnosed with autism in 2018. His physical examination at the last genetic appointment was: height 147.2 cm (just above the 75th centile), weight 56.3 kg (on the 99th centile) and head size 57.3 cm (+1.6 standard deviations, 94th centile); earlobes uplifted, high

and narrow palate; finger pads with tapered fingers; feet normal; inverted nipples; a birthmark on the lower back.

Individual 5

This 18-year-old male was born at 36 weeks gestational age by spontaneous vaginal delivery. Intrauterine growth restriction was noted from six months gestation, BW 2300 g. His mother recalls the Apgar score as low, however, this has not been possible to confirm. He had neonatal icterus treated with phototherapy. At the age of one month he was diagnosed with acute myeloblastic leukemia. He had severe developmental delay. He was able to sit three years old, walk six years old and speak nine years old. In addition to microcephaly the following dysmorphic features were noted: a low hairline, high arched palate, dental crowding, and a long philtrum.

Epilepsy started when he was three years old with absences. Tonic-clonic seizures began at age seven years and were treated successfully with phenobarbital. At the age of 15 years old he developed self-harming behavior and coprolalia that were treated with risperidone with success. When he was about 17 years old he was diagnosed with common variable hypogammaglobulinemia. He experienced several severe ear infections, frequent upper airway infections and pneumonia, as well as a tuberculosis infection. He receives monthly infusions of immunoglobulins. Growth hormone (GH) deficiency was diagnosed at age nine years and he was treated with GH hormone. He has had several measurements of monocytes showing elevated values. He has severe eczematous acne pruriginosa dermatitis. Pes cavus was surgically repaired because of severe pain. The HbF value was measured when he was 26 years old, and was in the normal range (0.5%). Investigations for standard karyotype, FRAXA, and methylation analysis for Angelman syndrome showed normal results. The SNPs array analysis (trio) detected a chrX:7,932,124-8,565,153 (hg19) microduplication inherited

from the healthy mother. This duplication was not able to explain the complex clinical picture of the patient. Trio WES detected the *de novo* heterozygous *ZBTB7A* variant and no other candidate variants.

Individual 6

This 19-year-old man was diagnosed with ASD, delayed speech and language development, and delayed motor development in the first years of life. He was born following a pregnancy which was uneventful, as was induced vaginal delivery at 40 weeks gestation with Apgar scores 9/10. BW was 2550 g, BL 46 cm and OFC 33.5 cm. Non-immune jaundice required phototherapy.

His weight during the first six months of life was at the 50th percentile and length at the 25th percentile. OFC at six months of age was above the 90th percentile.

He was admitted to hospital at age one month for bronchiolitis and at 11 months for pneumonia.

At age of 23 months, he underwent surgery for adenoid hyperplasia because of episodes of nocturnal apnea, with altered oxygen saturations. Subsequent to the surgery, he engaged more positively with others visually and in general, although cognitive delay was unchanged. Expressive language delay persisted; he had no two syllable words at three years and two months.

Genetic tests with normal results included: karyotype (46, XY), CGG expansion analysis of *FMR1*, MLPA of regions related to syndromes associated with intellectual disability including Soros syndrome (*NSD*1) and array CGH. Values in the low normal range or lower were noted for serum cholesterol, HDL cholesterol, triglycerides and apolipoprotein B.

Trio WES identified the heterozygous *de novo* variant c.[331G>C;859_860delinsCT] in the ZBTB7A gene causing the changes of two alanine residues at positions 111 (Ala111Pro) and

287 (Ala287Leu) of the peptide. The cloning and sequencing of a 686 bp amplicon from patient's DNA showed that the two variants were in *cis* (oligonucleotides: F- 5'-ACGCTCACCGTCAGCACAGC-3' and R- 5'-ATCATCTGCTGCAGCAGCGT-3').

Individuals 7, 8 and 9

The proband, a three year and two months old boy (individual 7), presented with a history of macrosomia, macrocephaly, hypotonia, global developmental delay, autism spectrum disorder, gastroesophageal reflux, dysphagia, and multiple aspiration events status post gastro-jejunal tube placement. He also had a history of recurrent infections which improved after taking prophylactic azithromycin, mild adenoid and tonsillar hypertrophy, mild obstructive sleep apnea requiring supplemental oxygen, a type 1 laryngeal cleft, pectus excavatum and congenital heart defects including an atrial septal defect that will require surgical intervention, and two muscular ventricular septal defects that spontaneously closed. Initial chromosome microarray, fragile X syndrome testing, Prader-Willi syndrome methylation analysis, and whole exome sequencing were non-diagnostic. The patient's 8 years and 11 months old sister (individual 8) was subsequently evaluated for similar albeit milder concerns. She had a history of hypotonia, macrocephaly, mild learning delays and ADHD, mild feeding challenges, gastroesophageal reflux, frequent recurrent infections, moderate obstructive sleep apnea and adenoid hypertrophy requiring multiple adenoidectomies. She also had a PDA and moderate sized atrial septal defect, which required surgical closure. Reanalysis of the proband's exome data with the affected sister's sample identified a paternally inherited c.642dupC (p.Asn215GlnfsTer35) variant in *ZBTB7A*, which was present in both siblings. The 37 years old father (individual 9) had a history of macrocephaly, mild

learning delays, ADHD, cardiomegaly, pectus excavatum, and obstructive sleep apnea that resolved with tonsillectomy and adenoidectomy.

Individual 10

The nine year and eleven months old girl is the first child of unrelated parents. Pregnancy was complication by maternal SLE (systemic lupus erythematosus), but lower limb x-rays of the child were normal. She has mild-moderate global developmental delay and is in a special school. She had laryngomalacia and had aryepiglottopexy aged five months and marked feeding difficulties requiring nasogastric feeding then a gastrostomy at two years and one month. She had recurrent tonsillitis and glue ear, and snoring and sleep apnea, and had adenotonsillectomy aged 10 months, then total adenoidectomy aged four years and nine months, repeated at eight years 10 months. She has a craniofacial phenotype with plagiocephaly, hemifacial hypertrophy, a high arched palate and dental crowding. Growth centiles: weight >99.6th, height 70th, OFC >99.6th. Investigations include a normal karyotype, CF (cystic fibrosis)-8 and -33 tests, and fragile X expansion testing. The DDD (Deciphering Developmental Disorders) study showed a normal array. Diagnostic WES (whole exome sequencing) showed a *de novo* heterozygous frameshift variant: *ZBTB7A* ENST00000322357.9 - NM_015898.4:c.983del p.(Gly328AlafsTer26), also identified in the DDD study as a research variant.

Individual 11

This 10-year-old male (DECIPHER 273736) is the first child of unrelated white parents. He has a younger sister who is well. He has a maternal family history of von Willebrand disease. His father was a late walker and bottom-shuffled until he started walking at 18 months.

The proband was born at 39+5 weeks gestation by normal vaginal delivery following an uncomplicated pregnancy. BW was 3.86 kg (73rd centile) and birth OFC 37.5 cm (96th centile). He was admitted to special care for 4 days due to feeding difficulties and required phototherapy for jaundice. He failed his newborn hearing screening due to mild bilateral hearing loss. Hypotonia was noted at the eight-week check. His family reported that he had been floppy since birth. On examination at six months of age he had marked truncal hypotonia. Laryngomalacia were also noted. He was treated for gastroesophageal reflux until one year of age. The boy sat independently at seven months and walked at 19 months. His first word was at 18 months of age.

Hematological investigation in infancy found the patient had inherited von Willebrand disease. In addition, he had a low neutrophil count. Neutrophil levels fluctuated from mild neutropenia (~1.0 x10^9/L, normal 1.5-10) into the low-normal range. Red cell microcytosis (MCV <70fl) was also noted. Iron and B12 stores were normal. Repeat full blood count at 10 months of age showed a microcytic hyper-chromic film with some red cell anisocytosis. Immunoglobulins and lymphocyte subsets were normal. Hemoglobin electrophoresis showed a normal HbA2 of 2% (normal 1.5-3.5%) but an elevated HbF of 19.4% [normal <2%]. Hemoglobin gene analysis found three copies of the alpha-globin gene. Serum creatine kinase, urine organic acids, amino acids, thyroid function assays, biotinidase, ammonia, lactate, urea and electrolytes were all normal.

At 10 months of age the boy was noted to have nasal congestion. Following an admission with bronchiolitis at one year of age a sleep study showed marked obstructive hypoventilation (CO$_2$ of 8 kPa during quiet sleep oxygen but increasing to 10 kPa during active sleep with lowest oxygen saturations in the 60's). Echocardiography showed a structurally normal heart

with a small patent foramen ovale. ECG found right axis deviation suggestive of right ventricular hypertrophy. He was started on non-invasive ventilation by mask.

At 16 months of age the patient's speech development was mildly delayed. He used a few single words. Neurological examination was normal apart from persistent central hypotonia. Tendon jerks were brisk but symmetrical with no abnormal movements. At 17 months of age he had bilateral adenotonsillectomy. His breathing and sleep study results improved postoperatively, and a hearing test was normal. His growth parameters at 18 months of age were height 78.6 cm (13th centile), weight 10.4 kg (18th centile) and OFC 51.5 cm (96th centile). At 20 months of age the patient presented with vomiting and focal seizures. CT brain scan showed multiple bilateral intracerebral abscesses which required repeated burr hole drainage. Cultures grew *Streptococcus milleri*. He was loaded with intravenous phenytoin before being started on regular sodium valproate. He had a brief focal seizure a few days after admission but has subsequently been seizure free. HbF level at 22 months was 11.1%.

The patient had recurrent ear infections. At 2 years of age detailed speech therapy evaluation showed his verbal comprehension was normal for age but he had a delay in the motor component of speech. His swallow was normal. His motor development was at the lower end of normal for age. There were no concerns about his social development. On examination at 25 months of age his height was 91.2 cm (88th centile), weight 16.9 kg (99.5th centile), OFC 55.0 cm (99.98th centile). He had epicanthic folds, mild frontal bossing, flat nasal bridge and midface, and pectus excavatum. At 27 months of age he was occasionally putting two words together. Echocardiography was normal with no evidence of pulmonary hypertension or ventricular hypertrophy. Routine karyotype, array CGH and testing for Fragile X syndrome were normal. He was enrolled in the DDD research study.

At two and a half years of age the child began having increasing airway problems again with desaturations at night to below 90%. He snored loudly and used several bibs per day for drooling. Sleep studies found evidence of worsening obstruction with desaturation during active sleep. It was suspected that his adenoids had regrown. At three years of age his height was 98.3cm (79th centile) and weight 19.7kg (99.4th centile). The patient had repeat adenoidectomy at 3 years and 3 months. His dribbling improved postoperatively.

At four years of age the boy was not toilet trained. He had constipation that was treated with Movicol. He remained mildly hypotonic. The sodium valproate was stopped at four years and one month. MRI brain scan at four years and two months showed mature damage within both parietal lobes (more marked on the right) at the site of the previous abscesses. The ventricles were normal in size and configuration with no evidence of hydrocephalus. The myelination pattern was appropriate for age.

At 5 years of age the boy attended a mainstream school with one-to-one support. He was diagnosed with developmental co-ordination disorder (dyspraxia). His planning and spatial awareness skills were reasonable, but he had delayed learning and gross motor skills. His fine motor skills were good. His dribbling and mild constipation persisted. On examination at five years and two months of age he had nasal speech. His height was 114 cm (76th centile), weight 24 kg (96th centile) and OFC 56.5 cm (99.3rd centile). Upper airway obstruction remained a problem and a third adenoidectomy was considered around six years of age. However, lateral neck X-ray found little adenoid tissue remained and his nasal airway was clear. His breathing, dribbling and hypotonia slowly improved as he got older. Eye examination at six years of age found mild hypermetropia (+1.00 diopter)

The child had several years of immunology follow-up and investigation following the intracerebral abscesses. No major immunological problems were found. His naïve T cells and IgM levels were sometimes low. Total T cell numbers were normal. Pneumococcal antibody titers were low initially but later these and other vaccine responses were normal.

The boy is now 10 years old. He attends a mainstream school with 25 hours one-to-one support. He is within 12 months of meeting age-appropriate expectations for reading writing, and at the lower end of age-appropriate expectations for mathematics. His speech remains nasal, and he still snores, especially if he has a cold. He is relatively immature socially and emotionally. He enjoys interacting with younger children but finds interaction with his peers more difficult. There are no concerns with his vision or hearing. His motor function is reasonable, but he struggles with dressing. His regular medication includes montelukast, azithromycin prophylaxis and fluticasone nasal spray. He has numerous retained deciduous teeth in the upper and lower jaw, and recently had several removed. His height is 148 cm (94th centile), weight 49 kg (98th centile) and OFC 59 cm (99.8th centile).

Individual 12

This 17-year-old male was the third child of healthy non-consanguineous parents. He had a familial history of isolated ASD. He was born after an uneventful pregnancy with normal birth parameters. His medical history was significant for recurrent upper airways infections. He underwent a surgical intervention for adenoids and tonsils hypertrophy. Sleep apnea has been suspected but polysomnography has not been performed to confirm the diagnosis. Early developmental milestones were mildly delayed. He had learning difficulties requiring special education. Neuropsychiatric evaluations revealed multiple specific learning disorders

including developmental coordination disorder associated with social interactions deficit and language development disorder. At the age of 13, intellectual quotient was within the normal ranges. Nevertheless, subtests were heterogeneous, mainly within the verbal scale. On physical examination, he had mild macrocephaly (+2.5 SD). Head circumferences of his mother and father were normal, +0.5 SD and +1.7 SD respectively. He also had a mild pectus excavatum, short palpebral fissures and epicanthal folds. Standard diagnostic work-up, including array-CGH, was normal. Genome sequencing identified a *de novo* missense variant in *ZBTB7A* (NM_001317990.1:c.1214C>A [p.Thr405Lys]). This variant was absent in the control population database Genome Aggregation Databases (gnomAD v.2.1.1, v.3.1), and was predicted to be pathogenic by several *in silico* prediction programs.