REFINING THE PRIMROSE SYNDROME PHENOTYPE: A STUDY OF FIVE PATIENTS WITH ZBTB20 DE NOVO VARIANTS AND A REVIEW OF THE LITERATURE

Ruth Cleaver[1], Jonathan Berg[2], Emily Craft[3], Alison Foster[4], Richard Gibbons[5], Emma Hobson[6], Katherine Lachlan[7], Swati Naik[4] , Julian Sampson[8], Saba Sharif[4], Sarah Smithson[9], Deciphering Developmental Disorders Study[10], Michael J. Parker[11], Katrina Tatton-Brown[1,12]

1. South West Thames Regional Genetics Service, St. George's University Hospitals NHS Foundation Trust, London, UK
2. East of Scotland Regional Genetics Service, Dundee, UK
3. Department of Clinical Genetics, University Hospitals of Leicester NHS Trust, Leicester, UK
4. West Midlands Regional Genetics Service, Birmingham, UK
5. Oxford Centre for Genomic Medicine, Oxford, UK
6. Yorkshire Regional Genetics Service, Chapel Allerton Hospital, Leeds Teaching Hospitals NHS Trust, Leeds, UK
7. Wessex Clinical Genetics Service, Southampton, UK
8. All Wales Medical Genetics Service, Cardiff, UK
9. Bristol Regional Genetics Service, Bristol, UK
10. Deciphering Developmental Disorders Study, Wellcome Trust Sanger Institute, Cambridge, UK
11. Sheffield Children's NHS Foundation Trust, Sheffield Clinical Genetics Service, Sheffield, South Yorkshire, UK
12. St. George's University of London, UK

Abstract

Primrose syndrome is a rare autosomal dominant condition caused by heterozygous missense variants within *ZBTB20.* Through an exome sequencing approach (as part of the Deciphering Developmental Disorders (DDD) study) we have identified five unrelated individuals with previously unreported, *de novo ZBTB20* pathogenic missense variants. All five missense variants targeted the C2H2 zinc finger domains. This genotype-up approach has allowed further refinement of the Primrose syndrome phenotype. Major characteristics (>90% individuals) include an intellectual disability (most frequently in the moderate range), a recognizable facial appearance and brain MRI abnormalities, particularly abnormalities of the corpus callosum. Other frequent clinical associations (in 50-90% individuals) include sensorineural hearing loss (83%), hypotonia (78%), cryptorchidism in males (75%), macrocephaly (72%), behavioral issues (67%) and dysplastic/hypoplastic nails (53%). Based upon these clinical data we discuss our current management of patients with Primrose syndrome.

Introduction

Primrose Syndrome (OMIM 259050), first described in 1982 by David Primrose, has previously been associated with a moderate to severe intellectual disability, a recognisable facial appearance with deep set eyes, narrow, often down-slanting palpebral fissures, ptosis, depressed nasal bridge and macrocephaly with or without tall stature (1-12). Additional reported clinical features include muscle wasting, calcified pinnae, hearing loss, cataracts, hypothyroidism (6,9), torus palatinus (a benign osseous elevation usually found on the midline of the hard palate) (1,2,5,6), and sparse body and facial hair (1,2,3,4,5,8).

In 2014, heterozygous pathogenic variants within *ZBTB20* were shown to cause Primrose syndrome (12). *ZBTB20,* located at chromosome position 3q13.31, encodes one of a family of POK [POZ (pox virus and zinc finger) and Kruppel] proteins which acts as a transcriptional repressor and has a role in glucose metabolism, postnatal growth and neurogenesis (13,14,15). ZBTB20 has five C2H2 zinc finger domains (ZNFI-ZNFV) and an N-terminal BTB (Broad Complex, Tramtrack, Bric a brac) domain that mediate interaction with DNA (figure 1a, 13).

To date, 14 of 15 Primrose syndrome reported *ZBTB20* pathogenic variants are missense variants clustering within the first (three variants), second (six variants) and third (two variants) zinc finger domains and the linker region between the first two motifs (three variants, figure 1a, supplementary table 1, refs). Functional assays have supported a dominant negative mechanism of disease whereby missense variants result in a stable but dysfunctional protein with defective DNA binding (12). In contrast, haploinsufficiency of *ZBTB20* causing the 3q13.31 microdeletion syndrome (OMIM 615433), has been reported to cause a similar but distinct condition also characterized by increased growth but without many of the Primrose syndrome clinical associations including the recognizable facial appearance, calcified pinnae and muscle wasting (16,17).

Here we report five patients with *de novo ZBTB20* pathogenic missense variants, identified through trio-based exome sequencing. This genotype-up approach has replicated the previous finding that *ZBTB20* missense variants target the zinc finger domains and has allowed a non-biased refinement of the Primrose syndrome phenotype.

Methods

The study was approved by the UK Research Ethics Committee (10/H0305/83), granted by the Cambridge South Research Ethics Committee. Informed consent was obtained from all families. Seven patients with *de novo ZBTB20* variants were identified through the Deciphering Developmental Disorders (DDD) Study using a trio-based exome sequencing strategy and methods as previously described (18). Five patients had missense variants, predicted to be pathogenic according to American College of Medical Genetics and Genomics (ACMG criteria PS2, PM1, PM2, PP2 and PP3 (19)) with evidence detailed in supplementary table 2. These five missense variants targeted the first, second and third zinc fingers (ZNFI, ZNFII and ZNFIII). None had previously been reported.

The remaining two patients with *de novo ZBTB20* variants were not included in the current study: one patient because there was insufficient evidence to support pathogenicity of the variant and the other because the patient additionally had a maternally inherited *FLNA* likely pathogenic variant resulting in a compound phenotype. It was therefore unclear which clinical features were attributable to the *ZBTB20* variant and which to the *FLNA* variant.

Clinical data for the five patients with the single *de novo* pathogenic missense *ZBTB20* variants were obtained through face to face review by one of the authors, all experienced dysmorphologists, and a standardized proforma. Photographs were requested in all five individuals and received, with accompanying consent to publish, from ** families.

Results

Clinical details are summarized in table 1. Detailed case reports are shown below:

Patient 1 (DDD 273936)

Patient 1, female, had a *de novo ZBTB20* c.1749C>G p.(Cys583Trp) pathogenic variant. She was born at 38 weeks gestation following an uncomplicated pregnancy. Her birth weight was 2.9kg (0.3 standard deviations below the mean, -0.3SD) with a head circumference of 36cm (+2.1SD). At the age of 3 years, her height was 99cm (-0.5SD), weight was 15kg (-0.6SD) and head circumference was 51.5cm (+0.4SD). Patient 1 had a severe learning disability and was delayed in the attainment of her developmental milestones: she sat between 6 and 7 years of age, crawled aged 7, she was not walking when reviewed at the age of 9 years and remained non-verbal. She had hypothyroidism, hypotonia and brachycephaly. A brain MRI scan identified colpocephaly and agenesis of the corpus callosum.

Patient 2 (DDD 303448)

Patient 2, male, had a *de novo ZBTB20* c.1850T>C p.(Leu617Ser) pathogenic variant. He was one of twins born at 37+6 weeks gestation following an uncomplicated pregnancy. Birth weight was 2.3kg (-2.1SD) with a head circumference of 35.4cm (+0.4SD). At 3.9 years, his height was 97cm (-1.5SD), weight was 12.3kg (-2.5SD), and head circumference was 53.5cm (+1.9SD). Patient 2 sat unsupported at 21 months, was not able to walk (at 3.9 years) and began to babble at 34 months. He had a tendency to head bang when frustrated. Patient 2 had a moderate-severe congenital sensorineural hearing loss, a small patent foramen ovale and generalised hypotonia, more prominent in the lower limbs. He had had one focal seizure. He had hypermetropia, astigmatism and a convergent squint. Dysmorphic features included low-set ears, flat mid-face with prominent forehead, deep-set eyes with down slanting palpebral fissures and a thin upper lip (figure 1b). A brain MRI scan demonstrated partial agenesis of the corpus callosum.

Patient 3 (DDD 273033)

Patient 3, female, had a *de novo ZBTB20* c.1879A>G p.(Thr627Ala) pathogenic variant. She was born at 39 weeks gestation following a normal pregnancy with a birth weight of 2.98kg (-0.5SD). There were some difficulties establishing feeding in the early neonatal period but no other significant concerns. At the age of 2.3 years, her height was 85cm (-0.8SD), weight was 10.9kg (-1.2SD) and head circumference was 50.5 cm (+1SD). Patient 3 was slow to reach her developmental milestones; she sat at 6 months, walked aged 3-4 years, and spoke her first words between 2.5 and 3 years. She had a moderate learning disability. She had mild generalised hypotonia in infancy progressing to truncal hypotonia in adolescence with increased tone and reflexes in the left leg. Additional medical problems included kyphoscoliosis, mixed conductive and sensorineural hearing loss, hypermetropia with recurrent blepharoconjunctivitis and raised urinary calcium with possible nephrocalcinosis. Patient 3 had joint hypermobility with soft, doughy skin, cutis marmorata and dysplastic nails. She had a narrow mouth, pointed chin, and upslanting palpebral fissures (figure 1b). Dentition was poor with delayed secondary dentition. A brain MRI scan was unremarkable and bone age was delayed (the bone age was 2 years at chronological age of 3 years 4 months).

Patient 4 (DDD 263871)

Patient 4, male, had a *de novo* mosaic *ZBTB20* c.1943C>T p.(Ser648Phe) pathogenic variant. He was born at term following an uncomplicated pregnancy with a birth weight of 5.3kg (+3.4SD). At 11.25 years, his height was 154.5cm (+0.9SD), weight was 73.7kg (+2.6SD), and head

circumference was 61cm (+5SD). He sat at 7 months, walked at 12 months and developed speech at 3-4 years. He had a moderate learning difficulty. He was a poor sleeper, had a poor working memory and a tendency to temper tantrums. He had bilateral cryptorchidism, a convergent squint and sensorineural hearing loss. He had narrrow, downslanting palpebral fissures and a high arched palate (figure 1b). A brain MRI scan identified a Chiari malformation.

Patient 5 (DDD 280375)

Patient 5, male, had a *de novo ZBTB20* c.1967A>G p.(His656Arg) pathogenic variant. He was born at 41+5 weeks gestation following an uncomplicated pregnancy. He was delivered via an emergency caesarean section for fetal distress and had a birth weight of 2.6kg (-2.1SD). There were no immediate postnatal concerns. At 13.4 years of age, his height was 154cm (-1SD), weight was 68.3kg (+1.4SD), and head circumference was 59cm (+2.8SD). He sat at 10 months, walked aged 3-4 years, and developed his first words between 2.5 and 3 years of age. He had a moderate learning difficulty. He had moderate bilateral congenital sensorineural hearing loss, obesity (associated with hyperphagia), central and peripheral hypotonia and a marked lumbar lordosis. He had mild camptodactyly, hypoplastic 5th toe nails, and acrocephaly. A brain MRI scan demonstrated a thin corpus callosum.

Discussion

Through an exome sequencing approach, the current study has identified five patients with novel single *de novo ZBTB20* missense variants. These data replicate previous reports that Primrose syndrome missense variants cluster within the zinc finger domains and expand the clustering to include the third zinc finger domain where only two pathogenic variants had previously been reported (11). Stellachi et al. recently reported a frameshift variant outside of the zinc finger domains said to cause Primrose Syndrome (11). Given the findings both from the current study and previously reported studies, a frameshift variant would be an unusual cause of Primrose Syndrome. In addition, further evaluation of the phenotype in this patient suggests greater similarity with the 3q13.31 microdeletion syndrome than Primrose Syndrome with increased growth and a milder intellectual disability than that normally described in Primrose syndrome. In addition, this patient, facially, bears a significant resemblance to the patient reported by Rasmussen et al. with a 3q13.31 microdeletion, rather than patients with Primrose syndrome (21).

The identification of five additional patients with pathogenic *ZBTB20* missense variants has increased the total number of patients with *de novo ZBTB20* missense variants to 19 and has allowed further refinement of the Primrose syndrome phenotype (supplementary table 1). Major clinical features, reported in >90% of patients with Primrose syndrome, include an intellectual disability (most frequently in the moderate range), a characteristic facial appearance consisting of prominent forehead, deep set eyes, down slanting palpebral fissures, small mouth, thin upper lip and pointed chin and abnormal findings on brain MRI scan (most frequently abnormalities of the corpus callosum). Other likely clinical associations of Primrose syndrome, reported in 50-90% of patients with Primrose syndrome, include hearing loss (83%), hypotonia (78%), cryptorchidism in males (75%), macrocephaly (72%), behavioral issues (67%) and dysplastic or hypoplastic nails (53%). In addition, distal muscle wasting, abnormalities of glucose metabolism, contractures and ectopic calcification of the pinnae were reported in at least 80% of adult patients. None of these four clinical features were reported in the five patients in the current study. However, our oldest patient was only 13.4 years and so it is possible that our five patients may still develop these.

Based on our findings and data from the fourteen previously reported patients with missense variants, our practice is to ensure appropriate learning and behavior support is in place and to refer to physiotherapy for management of hypotonia and/or contractures. We undertake a hearing evaluation for all our patients. In addition, until there are longitudinal data, we are screening our patients for both abnormalities of glucose metabolism and thyroid abnormalities.

All five variants occurred *de novo.* One variant was mosaic. This has implications for the counselling of families with regard to recurrence risk. We would counsel a 1% recurrence risk where a child has a *de novo* constitutive variant, with no evidence of mosaicism, reflecting the possibility of germline mosaicism and we would offer prenatal testing in future pregnancies. In contrast, the risk of recurrence of a *de novo* mosaic variant is very low (background rate) and invasive prenatal testing for future pregnancies would not be recommended.

Although the current study has allowed further refinement of the Primrose syndrome phenotype, the total number of patients and, in particular, the number of adults with Primrose syndrome remains small. However, as exome/genome sequencing becomes more accessible, it is likely that additional individuals, of all ages, will be identified with Primrose syndrome. This will result in an improving understanding of the Primrose syndrome phenotype, a greater knowledge of the long term syndrome complications and the implementation of optimal, consistent, evidence-based management.

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Web resources

Online Mendelian Inheritance in Man (OMIM); https://www.omim.org
Decipher; https://decipher.sanger.ac.uk
ExAC; http://exac.broadinstitute.org
GnomAD; http://gnomad.broadinstitute.org

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Table 1: Clinical features of the five patient reported in the current study with de novo pathogenic *ZBTB20* variants

Patient details	**Patient identifier**	**1**	**2**	**3**	**4**	**5**
	Sex	Female	Male	Female	Male	Male
	Age/years	3.0	3.9	2.3	11.3	13.4
Variant details	Nucleotide	c.1749C>G	c.1850T>C	c.1879A>G	c.1943C>T	c.1967A>G
	Amino acid	p.(Cys583Trp)	p.(Leu617Ser)	p.(Thr627Ala)	p.(Ser648Phe)	p.(His656Arg)
	Inheritance	de novo	de novo	de novo	de novo mosaic	de novo
Growth	Ht/cm (SD)	99 (-0.5)	97 (-1.5)	85 (-0.8)	154.5 (+0.9)	154 (-0.1)
	Wt/kg (SD)	15 (-0.6)	12.3 (-2.5)	10.9 (-1.2)	73.7 (+2.6)	68.3 (+1.4)
	HC/cm (SD)	51.5 (+0.4)	53.5 (+1.9)	50.5 (+1)	61 (+5)	59 (+2.8)
	BW/kg (SD)	2.9 (-0.3)	2.3 (-2.1)	3.0 (-0.5)	5.3 (+3.4)	2.58 (-2.1)
	BHC/cm (SD)	36 (+2.1)	35.4 (+0.4)	nk	nk	nk
Learning and behavior	Learning difficulties			Moderate	Moderate	Moderate
	Behavioral issues	-	Head banging	-	Poor sleep, tantrums, poor memory	-
Additional medical problems	Hearing loss	-	+	+	+	+
	Hypothyroid	+	-	-	-	-
	Hypotonia	+	+	+	-	+
	Muscle wasting	-	-	-	-	-
	Contractures	nk	-	-	-	+
	Dysplastic nails	-	nk	+	-	+
	Eyes	-	Hypermetropia, astigmatism, strabismus	Hypermetropia, recurrent blepharoconjunctivitis	Strabismus	-
	Cryptorchidism	na	+	na	+	-
	Error glucose metabolism	nk	nk	-	nk	-
	Ectopic calcification pinna	-	-	-	-	-
	Other	-	Patent foramen ovale, afebrile seizure	Joint hypermobility, poor/delayed secondary dentition, raised urinary calcium, kyphoscoliosis	-	Hyperphagia, lumbar lordosis
Brain imaging	Brain MRI	Agenesis of the corpus callosum, colpocephaly	Agenesis of the corpus callosum (partial)	Normal	Chiari malformation	Hypoplastic corpus callosum

Abbreviations: +, present; -, absent; nk, not known; na, not applicable; Ht height, Wt, weight; HC, head circumference; BW, birth weight; BHC, birth head circumference; SD, standard deviations ; GTT, abnormal glucose tolerance; DM, diabetes mellitus

Supplementary table 1: Table of clinical features reported in 19 patients with *ZBTB20* missense variants including five patients included in the current study and 14 patients reported in the literature.

Supplementary table 2. Pathogenicity evidence and ACMG classification for variants in this study

Variant	Inheritance	Domain	Polyphen	SIFT	GnomAD	ACMG classification
c.1879A>G, p.(Thr627Ala)	De novo	ZnF II	Probably damaging	Deleterious	Absent	Pathogenic
c.1749C>G, p.(Cys583Trp)	De novo	ZnF I	Probably damaging	Deleterious	Absent	Pathogenic
c.1850T>C, p.(Leu617Ser)	De novo	ZnF II	Probably damaging	Deleterious	Absent	Pathogenic
c.1943C>T, p.(Ser648Phe)	De novo mosaic	ZnF III	Probably damaging	Deleterious	Absent	Pathogenic
c.1967A>G, p.(His656Arg)	De novo	ZnF III	Probably damaging	Deleterious	Absent	Pathogenic

Figure 1 a) schematic of *ZBTB20* showing the BTB domain (blue) and the four C2H2 zinc finger domains (red). Variants identified in the current study are shown above the line and previously reported variants are shown below the line; **b)** facial appearance of ** individuals with *ZBTB20* variants.