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ARTICLE

Primrose syndrome: a phenotypic comparison of patients with a *ZBTB20* missense variant versus a 3q13.31 microdeletion including *ZBTB20*

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Abstract

Primrose syndrome is characterized by variable intellectual deficiency, behavior disorders, facial features with macrocephaly, and a progressive phenotype with hearing loss and ectopic calcifications, distal muscle wasting, and contractures. In 2014, *ZBTB20* variants were identified as responsible for this syndrome. Indeed, *ZBTB20* plays an important role in cognition, memory, learning processes, and has a transcription repressive effect on numerous genes. A more severe phenotype was discussed in patients with missense single nucleotide variants than in those with large deletions. Here, we report on the clinical and molecular results of 14 patients: 6 carrying *ZBTB20* missense SNVs, 1 carrying an early truncating indel, and 7 carrying 3q13.31 deletions, recruited through the AnDDI-Rares network. We compared their phenotypes and reviewed the data of the literature, in order to establish more powerful phenotype–genotype correlations. All 57 patients presented mild-to-severe ID and/or a psychomotor delay. Facial features were similar with macrocephaly, prominent forehead, downslanting palpebral fissures, ptosis, and large ears. Hearing loss was far more frequent in patients with missense SNVs ($p = 0.002$), ectopic calcification, progressive muscular wasting, and contractures were observed only in patients with missense SNVs (p nonsignificant). Corpus callosum dysgenesis ($p = 0.00004$), hypothyroidism ($p = 0.047$), and diabetes were also more frequent in this group. However, the median age was 9.4 years in patients with deletions and truncating variant compared with 15.1 years in those with missense SNVs. Longer follow-up will be necessary to determine whether the phenotype of patients with deletions is also progressive.

Introduction

Intellectual disability (ID) and multiple congenital anomalies (MCA) affect 1–3% of the population [1] and represent a large and heterogeneous group of disorders. The molecular bases still remain unresolved in a large proportion of cases due to this high heterogeneity, which makes their diagnosis challenging. Improvements in genomic investigation techniques in recent decades have allowed the identification of numerous genes in ID and MCA, first with array-comparative genomic hybridization (CGH), which gives a diagnostic yield of around 14% in ID/MCA [2], and more recently with exome sequencing (ES), which raised the diagnostic yield to around 28.8% [3]. For example, ES allowed the identification of about 555 genes between 2010 and 2015, including *ZBTB20* [4].

The first description of Primrose syndrome was made by Primrose in 1982, in a 33-year-old male with ID, muscle

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weakness of the lower limbs, calcified ear flaps, bone abnormalities, and a torus palatinus. Today, it is characterized by a possibly recognizable but largely underdiagnosed entity that associates ID, autism spectrum disorders, facial features, ectopic calcifications, and distal muscle wasting. Other findings, such as corpus callosum anomalies, tall stature, bilateral cataract, hearing loss, hypothyroidism, and diabetes mellitus have been reported, in particular in older patients [5–8]. The implication of the *ZBTB20* gene in this syndrome was established in [7], through the analysis of eight patients, all carrying heterozygous de novo missense variants in *ZBTB20*, detected by trio ES. Functional studies showed a dominant negative effect of missense variants affecting the DNA binding domain of this transcription factor. This gene plays a role in glucose metabolism, postnatal growth, and neurogenesis. It contains an N-terminal domain involved in protein–protein interactions and five zinc finger C_2H_2 domains binding the regulatory sites of α-fetoprotein promoters. It has been suggested that the *ZBTB20* gene was strongly implicated in the phenotype of 3q13.31 deletion syndrome, which associates ID, corpus callosum agenesis, skeletal malformations, and facial features [9].

Here, we report on 14 new patients: 6 patients with intragenic *ZBTB20* missense SNVs, 8 patients with either 3q13.31 microdeletions encompassing *ZBTB20*, and 1 with a *ZBTB20* truncating indel, collected through a national collaboration based on the French AnDDI-Rares network (Figs. 1 and 2). The aim of this study was to collect and compare the clinical features observed in patients carrying *ZBTB20* missense SNVs one the one hand, and *ZBTB20* microdeletions or truncating variant on the other hand, in our series and a review of 37 patients in the literature, and to determine whether patients with haploinsufficiency of *ZBTB20* are at risk of a progressive phenotype, as described in patients with missense variants.

Patients and methods

Patients

Two groups were defined according to whether the proband carried a *ZBTB20* missense SNV (dominant negative group) or a deletion involving *ZBTB20*/*ZBTB20* truncating variant (haploinsufficiency group). The medical history and clinical features of each patient are detailed in Supplemental data.

Molecular and cytogenetic analyses

Written and informed consent were obtained from all patients or legal guardians. Peripheral blood samples were provided in a diagnosis context for the proband and both parents, when available.

Panels of different sizes were used for the diagnosis of patients 1, 3, 4, 5, 6, and 14. For patients 1, 3, 6, and 14, a panel comprising 456 genes involved in cognitive disorders was used (Strasbourg University Hospital). Sequence libraries preparation and coding region capture were performed [10] with in-solution enrichment methodology (Agilent XT2 or QXT SureSelect custom panel) and sequenced with an Illumina NextSeq 550 instrument (paired-end sequencing 2×150 bases). Reads were aligned on the hg19 human genome reference sequence, and SNVs and indels were called with the Genome Analysis Toolkit v.3.4.46 thanks to an in-house (Strasbourg Hospital University) pipeline (STARK) and following the Genome Analysis Toolkit (GATK) best practice. Annotation and analysis of the variants were performed using Varank [11]. For patients 4 and 5, the diagnostic panel test included 19 genes implicated in syndromes associating overgrowth and ID (Hôpital Necker, Paris). Sequencing was performed on a MiSeq instrument (Illumina), after multiplex PCR using the TruSeq Custom Amplicon (Illumina). Reads were aligned on the hg19 human genome reference sequence with the Burrows–Wheeler Aligner. Variant calling was done using the GATK. Annotation and analysis of the variants were performed via a local interface PolyWeb.

Exome capture and sequencing were performed for patient 2, in a research framework by the GAD team aiming to identify the molecular bases of marfanoid syndrome with ID. Sequence libraries preparation, coding region capture, and the in-house bioinformatics pipeline are detailed elsewhere [12].

In all cases, the presence of the *ZBTB20* variant in the proband and in the parents was validated by Sanger sequencing.

Array-CGH was performed using Agilent microarray 44 K for patients 7 and 9, 60 K for patients 8, 12, and 13, 180 K for patient 10 and 11. In all cases, the deletion was confirmed by fluorescence in situ hybridization experiments in the proband using bacterial artificial chromosome (BAC) clones containing chromosome 3 specific sequences, in accordance with publicly available genome resources (NCBI Map Viewer, Santa Cruz Human Genome Browser). The BACs were obtained from the RP library (BACPAC Resources Center, CHORI, Oakland, CA, USA) and selected according to their positions on chromosome 3. The same method was used for segregation in the parental samples.

Identified variants have been submitted to freely accessible public databases: SNVs and indel to ClinVar (IDs SCV000930642 to SCV000930648) and CNVs to Decipher (IDs 388596, 388597, 388611, 388612 to 388615).

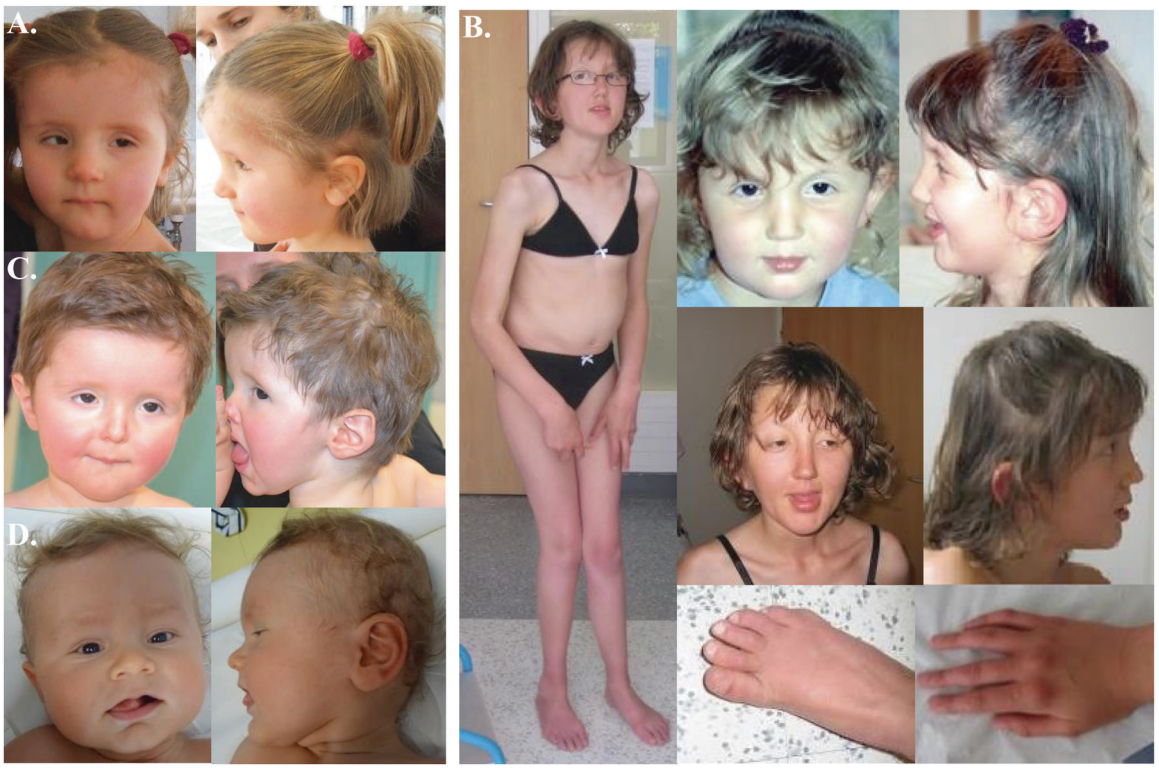

Fig. 1 Pictures of the patients with *ZBTB20* missense variants. a Patient 1. **b** Patient 2. **c** Patient 3. **d** Patient 5.

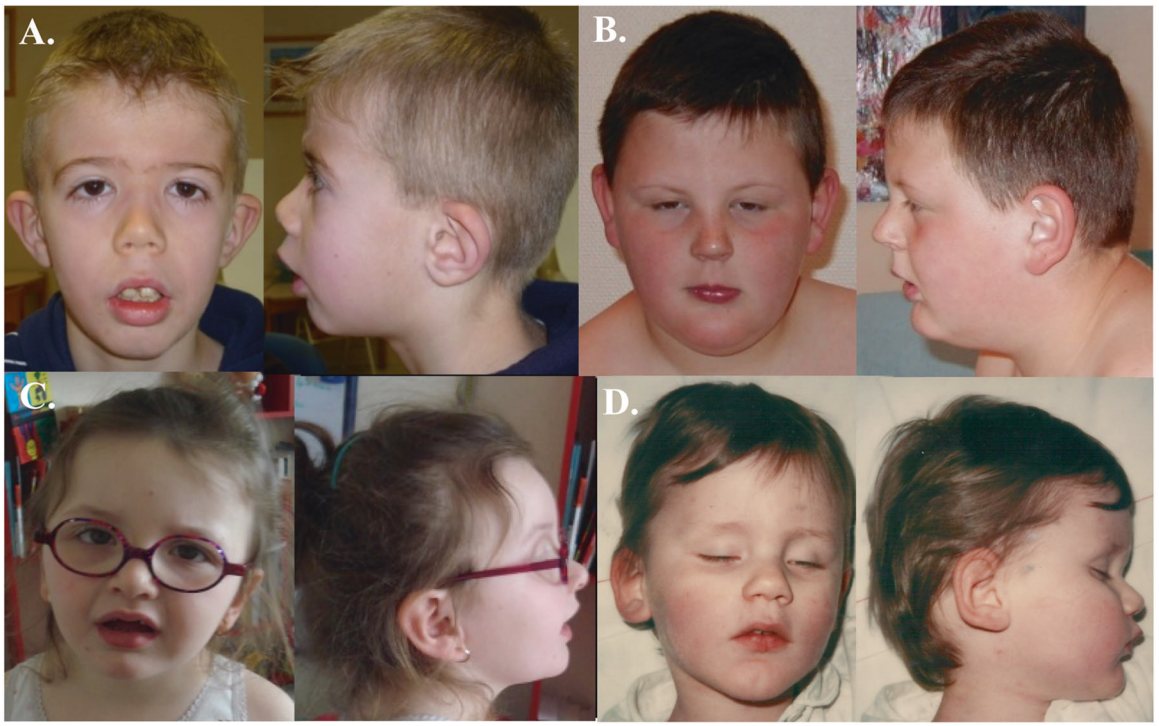

Fig. 2 Pictures of the patients with *ZBTB20* microdeletions. a Patient 7. **b** Patient 8. **c** Patient 9. **d** Patient 11.

Table 1 Molecular data of patients with *ZBTB20* SNVs/indel reported in this series.

Patient	Transcript	cDNA variant	Protein variation	Inheritance	ExAC-GnomAD
Missense					
1	NM_001164342.2	c.1939A>C	p.(Ser647Arg)	de novo	Absent
2	NM_001164342.2	c.1862T>C	p.(Leu621Pro)	de novo	Absent
3	NM_001164342.2	c.1760T>G	p.(Phe587Cys)	de novo	Absent
4	NM_001164342.2	c.1837C>T	p.(Arg613Cys)	de novo	Absent
5	NM_001164342.2	c.1955A>G	p.(His652Arg)	de novo	Absent
6	NM_001348803.2	c.1817A>C	p.(His606Pro)	de novo	Absent
Truncating					
14	NM_001164343.2	c.172_178delinsAA	p.(Asp58Asnfs*24)	de novo	Absent

Statistical analysis

Clinical features were compared within the missense SNV group, with an expected dominant negative effect, and the group comprising CNVs and the truncating *ZBTB20* variant, with an expected haploinsufficient effect, in patients gathered in this study and from the literature. χ^2 tests and Fisher's exact tests were used when appropriate. A p value below 0.05 was considered significant.

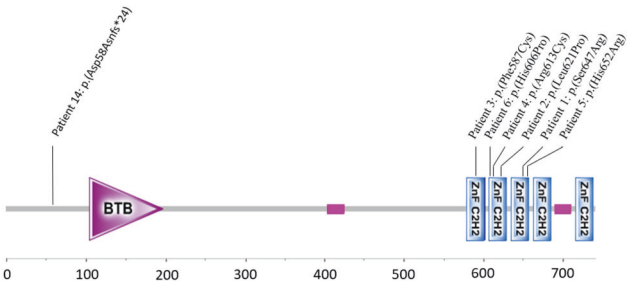

Fig. 3 Position of the *ZBTB20* intragenic SNVs and indel of the patients 1–6 and 14.

Results

Molecular and cytogenetic results

Molecular results of the SNV group are summarized in Table 1 and Fig. 3. All missense SNVs occurred de novo and impacted the zinc finger domains of the protein, at its C-terminal extremity. Of note, the variant of patient 2 (c.1862T>C; p.(Leu621Pro)), presented an amino-acid substitution in the same position as that in subject 11D5028 reported in 2014 [7] (c.1861C>T; p.(Leu621Phe)).

The extents of the CNVs are summarized in Table 2 and Fig. 4. The size of the 3q13.31 microdeletions ranged from 2.78 to 20.35 Mb, and contained 15 to 56 RefSeq genes, including 3–11 OMIM genes. The CNVs start and end positions listed in Table 2 only represent the minimal extent of the deletions detected by using CGH-array. All CNVs were found to be de novo.

Genotype–phenotype correlations

The clinical features of all patients of this study are presented in Table 3.

The comparison between the missense SNV and CNVs/truncating variant groups was extended to patients reported in the literature (Table 4). The early truncating variant, located in the first exon above the BTB domain, prevents the synthesis of a functional protein. Therefore, this variant is expected to lead to haploinsufficiency. The median age was 15.1 years in patients with missense SNVs and 9.4 years in patients with CNVs/truncating variant. All the patients presented ID and/or mild-to-severe psychomotor delay. Behavioral features, stereotypies, and anxiety were found in both groups. One patient of each group developed schizophrenia, 2/23 with a missense SNV, and 5/34 patients in the CNVs/truncating variant group were diagnosed with autism. ADHD was found only in the CNVs/truncating variant group. Facial features of the two groups overlapped, with macrocephaly, prominent forehead, downslanting palpebral fissures, ptosis, and large ears. Ectopic calcifications, progressive muscle wasting, and contractures were observed only in patients with missense SNVs although clinical significance is reached for hearing loss ($p = 0.002$), observed in 18/23 in missense SNV group versus 1/8 in CNV/truncating variant group. In addition, corpus callosum anomalies, diabetes, and hypothyroidism were more frequent in this group. Again, clinical significance is reached only for corpus callosum anomalies ($p = 0.00004$) and hypothyroidism ($p = 0.047$). Skeletal malformation, mainly deformations of the knees, feet, and spine were present with no significant difference between the two groups (65.2% in SNVs and 56.3% in CNVs). However, the differential median age between the two groups requires caution in the interpretation of results.

Table 2 Cytogenetic and molecular data of patients with *ZBTB20* CNVs.

Patient	Chromosome coordinates (GRCh37/hg19)	Size (Mb)	Array	Inheritance	Genes	DGV
7	chr3:g.(?_112152400)_(115507949_?)del	3.35	Agilent 44 K	de novo	16 genes 4 OMIM: *ATP6V1A, CFAP44, DRD3, ZBTB20*	Absent
8	chr3:g.(?_ 112198129)_(114983673_?)del	2.78	Agilent 44 K	de novo	15 genes 4 OMIM: *ATP6V1A, CFAP44, DRD3, ZBTB20*	Absent
9	chr3:g.(?_114522462)_(121094268_?)del	6.6	Agilent 60 K	de novo	22 genes 4 OMIM *ZBTB20, ARHGAPS1, POGLUT1, HGD*	Absent
10	chr3:g.(?_104884356)_(116229200_?)del	11.34	Agilent 180 K	de novo	42 genes 5 OMIM: *CD96, CFAP44, ATP6V1A, DRD3, ZBTB20*	Absent
11	chr3:g.(?_109439522)_(117042142_?)del	7.6	Agilent 180 K	de novo	29 genes 5 OMIM: *CD96, CFAP44, ATP6V1A, DRD3, ZBTB20*	Absent
12	chr3:g.(?_94473675)_(114825050_?)del	20.35	Agilent 60 K	de novo	56 genes 11 OMIM: *ARL6, CPOX, TBC1D23, TFG, IMPG2, TRMT10C, CD96, CFAP44, ATP6V1A, DRD3, ZBTB20*	Absent
13	chr3:g.(?_94473675)_(114825050_?)del	20.35	Agilent 60 K	de novo	56 genes 11 OMIM: *ARL6, CPOX, TBC1D23, TFG, IMPG2, TRMT10C, CD96, CFAP44, ATP6V1A, DRD3, ZBTB20*	Absent

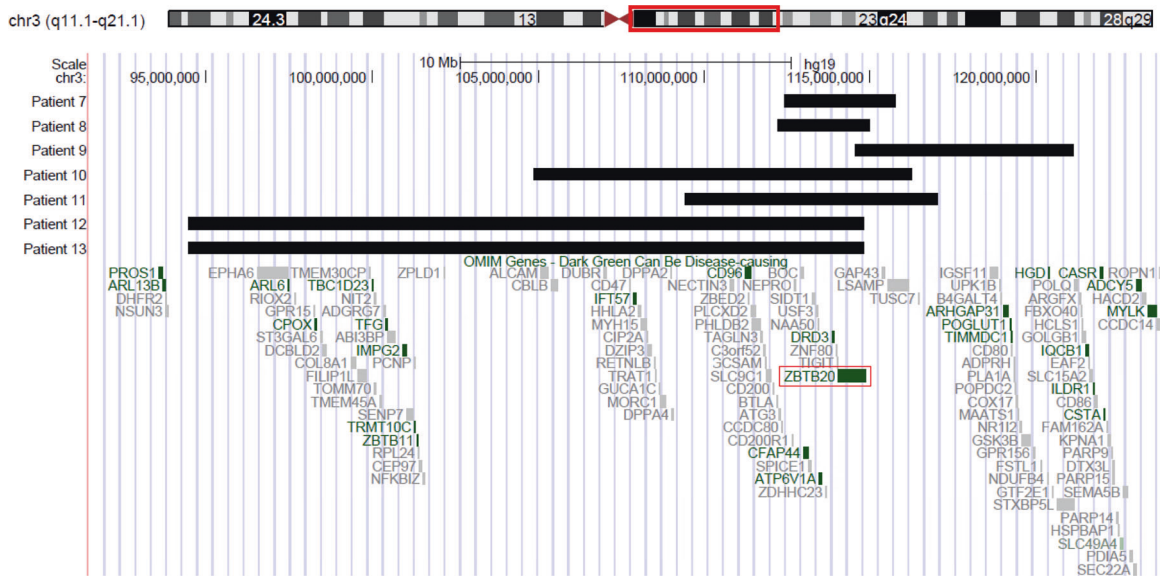

Fig. 4 *ZBTB20* deletions of patients 7–13 showed in UCSC Genome Browser.

Discussion

Primrose syndrome is a rare syndrome, usually unrecognized in childhood. Indeed, patients display a specific association in late childhood or adulthood, with the emergence of progressive distinctive features, such as hearing loss, pinnae calcification, endocrine manifestations, and muscle wasting. Previous authors have mentioned that patients with missense SNVs may be more severely affected than patients with CNVs [7], but a large genotype–phenotype correlation was needed.

To date, the progression of the symptoms has not been described in patients with CNVs, but this finding has to be interpreted with caution since the mean age at the time of reports in patients with missense SNVs is significantly higher than the mean age of patients with CNVs (15.1 years for SNV patients and 9.4 years for CNVs/early truncating variant patients, including this series, and data from the literature). Indeed, patients with CNVs are now rapidly diagnosed since array-CGH is widely available and considered as a first-intention genetic test in developmental disorders. In the future, easier accessibility to large panels

Table 3 Clinical features of the 14 reported patients.

Patient *ZBTB20* variant type	1 Mis. SNV	2 Mis. SNV	3 Mis. SNV	4 Mis. SNV	5 Mis. SNV	6 Mis. SNV	7 Del	8 Del	9 Del	10 Del	11 Del	12 Del	13 Del	14 Trunc. variant
Sex	F	F	M	F	M	F	M	M	F	F	M	M	M	M
Age (years)	12	24	2	40	3	6	15	20	5	4	38	3	3	5
Measurements														
Birth weight (kg)	2.93	3.5	3	3.6	3.74	3.33	3.45	3.85	3.34	2.7	3.9	2.19	2.39	3.84
Birth length (cm)	48	52	54	50	53	51	52.5	51	50	50	53	48	48	53
Birth OFC (cm)	36	NA	36	40	39	37	36.5	35	36	34	37	34	34	38.5
Weight at last visit (SD)	NA	−3	+0	+0	+1.5	−2	+0	+3	+3	+1	NA	+0	−0.5	+0
Length at last visit (SD)	−3	−3	+0	−0.5	+2	−1.5	+0	+2	+3.5	+1	+2	+1	+0.5	+1
OFC at last visit (SD)	NA	+0.5	+2.5	+2	+3	+2.5	−2	+0	+2	+2	+1.5	+0.5	+0.5	+3
Neurological														
Developmental delay/ID	S	S	S	Mod	S	S	Mod	Mod	Mod	S	S	Mod	Mod	Mi
Ataxia	+	−	−	−	−	−	+	−	−	−	−	−	−	−
Fine motor disorders	−	−	−	+	−	−	−	−	−	+	−	−	−	−
Chronic pain	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Corpus callosum anomaly	+	−	+	+	+	+	−	−	−	−	+	−	−	+
Cerebral calcifications	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Behavior disorders	−	−	−	−	−	+	−	−	+	−	+	−	−	+
Aggressivity	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Anxiety	−	−	−	−	−	−	−	+	−	+	−	+	+	−
Motor stereotypies	+	−	−	−	+	+	−	−	−	−	+	+	+	+
Autism	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Schizophrenia	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Facial features														
Plagiocephaly	−	−	−	−	−	−	−	−	+	+	−	+	−	+
Prominent forehead	−	−	+	+	−	+	−	−	+	+	−	−	−	−
Strabismus	+	−	+	−	−	+	−	−	−	−	−	−	−	+
Downslanted palpebral fissures	+	+	−	+	−	+	+	−	+	+	−	+	+	−
Ptosis	−	−	+	−	−	+	−	−	−	−	−	−	−	−
Epicanthus	+	−	+	+	+	+	−	−	+	+	−	−	−	−
Large ears	−	+	+	−	+	+	−	−	+	−	−	−	−	−
Depressed nasal bridge	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Anteverted nares	−	−	−	+	−	+	+	−	−	+	−	−	−	−
Downturned corners of the mouth	+	−	−	−	−	+	−	−	−	+	−	−	−	−

Table 3 (continued)

Small mouth	−	+	+	−	−	+	−	−	−	−	−	−	−	−
High-arched palate	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Retrognathia	−	−	−	−	−	−	−	−	+	−	+	−	−	−
Muscular														
Hypotonia	+	+	+	−	+	+	+	−	−	+	+	+	+	+
Muscle wasting	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Muscle contracture	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Sensory														
Bilateral hearing loss, perceptive or mixed	+	+	−	+	+	+	−	−	−	−	+	−	−	−
Orthopedic														
Ossification delay	+	+	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA
Joint hyperlaxity	−	+	+	−	−	+	+	+	−	+	+	−	−	−
Knee malposition	−	valg	−	valg	−	−	−	recurv	valg	recurv	−	−	−	−
Hip dysplasia	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spine anomalies	−	scol	−	−	−	−	−	kyphosis	−	−	scol	−	−	−
Long fingers	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Feet malposition	valg	varus	valg	−	−	−	−	−	pes planus	valg, pes planus	−	−	−	−
Endocrinology														
Hypothyroidism	−	−	+	−	−	+	−	−	−	−	−	−	−	−
Puberty delay	NC	+	NC	−	NC	NC	+	−	NC	NC	−	NC	NC	NC
Others														
Constipation	+	+	+	−	−	+	−	−	−	+	−	+	−	−
Calcification of the external auditory canal	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pinnae calcification	NA	NA	NA	+	NA	−	NA	NA	NA	NA	NA	NA	NA	NA
Bilateral cryptorchidism	NC	NC	−	NC	−	NC	−	+	NC	NC	−	−	−	−
Congenital cardiopathy	−	−	+	−	−	−	−	−	+	−	−	−	−	−

Del deletion, *Mi* mild, *Mis* missense, *Mod* moderate, *NA* not available, *NC* not concerned, *S* severe, *Trunc* truncating, *Recurv* recurvatum, *Valg* valgus, *Scol* scoliosis.

Table 4 Genotype–phenotype correlations in this series and patients of the literature.

		Missense SNV		CNV (and truncating variant[a])			
Patient/article	Patients study 1–6	Mathijssen et al. [19] Dalal et al. [6] Carvalho et al. [21] Posmyk et al. [22] Mattioli et al. [8] Casertano et al. [14] Alby et al. [13] Stellacci et al. [24] Grímsdóttir et al. [25] Cleaver et al. [26]	Total missense SNV	Patients study 7–14	Molin et al. [9] Vuillaume et al. [20] Lowther et al. [18] Quintela et al. [23]	Total CNV and truncating variant	*p* value SNV/CNV and truncating variant (Fisher's test)
General							
Number of patients	6	17	23	8	26	34	
M/F	2/4	10/7	12/11	6/2	14/12	20/14	$p = NS$
Mean age (years)	14.5	15.4	15.1	11.6	8.7	9.4	$p = NS$
Extreme ages (years)	(2–40)	(1.5–43)	(1.5–43)	(3–38)	(0–41)	(0–41)	
Measurement							
Birth weight > +2 SD	0/5	1/15	1/20 (5%)	0/8	0/18	0/26 (0%)	$p = NS$
Birth length > +2 SD	1/5	2/11	3/16 (18.8%)	0/8	1/13	1/21 (4.8%)	$p = NS$
Birth OFC > +2 SD	3/5	4/10	7/15 (46.7%)	1/8	4/11	5/19 (26.3%)	$p = NS$
Last weight > +2 SD	0/5	4/16	4/21 (19%)	2/7	6/20	8/27 (29.6%)	$p = NS$
Last length > +2 SD	0/6	2/17	2/23 (8.7%)	3/8	6/21	9/29 (31.0%)	$p = NS$
Last OFC > +2 SD	4/5	10/17	14/22 (63.6%)	3/8	7/20	10/28 (39.7%)	$p = NS$
Neurological							
Developmental delay/ID	6/6	17/17	23/23 (100%)	8/8	21/21	29/29 (100%)	$p = NS$
Language delay	6/6	17/17	23/23 (100%)	8/8	17/19	25/27 (92.6%)	$p = NS$
Behavioral disorder	1/6	7/17	8/23 (34.8%)	3/8	2/3	5/11 (45.5%)	$p = NS$
Ataxia	2/6	0/17	2/23 (8.7%)	1/8	1/3	2/11 (18.2%)	$p = NS$
Epilepsy	0/6	0/17	0/23 (0%)	0/8	2/26	2/34 (5.9%)	$p = NS$
Corpus callosum anomalies	5/6	14/17	19/23 (82.6%)	2/8	7/26	9/34 (26.5%)	$p = 0.00004$
Ventriculomegaly	3/6	1/17	4/23 (17.4%)	0/8	3/26	3/34 (8.8%)	$p = NS$
Cerebral calcification	0/NA	3/NA	NA	0/8	0/26	0/34 (0%)	NA
Anxiety	2/6	1/17	3/23 (13%)	4/8	1/3	5/11 (45.5%)	$p = NS$
Motor stereotypies	4/6	1/17	5/23 (21.7%)	4/8	1/3	5/11 (45.5%)	$p = NS$
Autism	0/6	2/17	2/23 (8.7%)	1/8	4/26	5/34 (14.7%)	$p = NS$
ADHD	0/6	1/17	1/23 (4.3%)	0/8	6/26	6/34 (17.6%)	$p = NS$
Schizophrenia	1/6	0/17	1/23 (4.3%)	0/8	1/26	1/34 (2.9%)	$p = NS$
Sleep disturbances	0/6	1/17	1/23 (4.3%)	1/8	1/3	2/11 (18.2%)	$p = NS$
Facial features							
Skull malformation	0/6	3/17	3/23 (13%)	3/8	7/26	10/34 (29.4%)	$p = NS$
Hypertelorism	0/6	2/17	2/23 (8.7%)	0/8	8/17	8/25 (32%)	$p = NS$
Prominent forehead	3/6	8/17	11/23 (47.8%)	2/8	10/14	12/22 (54.5%)	$p = NS$
Strabismus	3/6	4/17	7/23 (30,4%)	0/8	6/15	6/23 (26.1%)	$p = NS$
Downslanting palpebral fissures	4/6	9/17	13/23 (56.5%)	5/8	7/13	12/21 (57.1%)	$p = NS$
Ptosis	2/6	5/17	7/23 (30.4%)	1/8	5/11	6/19 (31.6%)	$p = NS$
Epicanthus	5/6	4/17	9/23 (39.1%)	2/8	9/15	11/23 (47.8%)	$p = NS$
Larges ears	4/6	0/17	4/23 (17.4%)	1/8	5/15	6/23 (26.1%)	$p = NS$
Depressed nasal bridge	0/6	5/17	5/23 (21.7%)	1/8	0/4	1/12 (8.3%)	$p = NS$
Broad nasal bridge	0/6	6/17	6/23 (26.1%)	0/8	2/13	2/21 (9.5%)	$p = NS$
Anteverted nares	2/6	2/17	4/23 (17.4%)	2/8	3/10	5/18 (27.8%)	$p = NS$
Downturned corners of the mouth	2/6	4/17	6/23 (26.1%)	1/8	ND	1/8 (12.5%)	$p = NS$
Small mouth	3/6	4/11	7/17 (41.2%)	0/8	ND	0/8 (0%)	$p = NS$

Table 4 (continued)

		Missense SNV		CNV (and truncating variant[a])			
High-arched palate	1/6	3/17	4/23 (17.4%)	0/8	ND	0/8 (0%)	$p = NS$
Micrognathia/retrognathia	0/6	2/17	2/23 (8.7%)	2/8	ND	2/8 (25%)	$p = NS$
Muscular							
Hypotonia	5/6	10/17	15/23 (65.2%)	6/8	14/18	20/26 (76.9%)	$p = NS$
Muscle wasting	1/6	5/17	6/23 (26.1%)	0/8	0/2	0/10 (0%)	$p = NS$
Muscle contractures	1/6	4/17	5/23 (21.7%)	0/8	ND	0/8 (0%)	$p = NS$
Sensory							
Bilateral hearing loss (perceptive or mixed)	5/6	13/17	18/23 (78.3%)	1/8	ND	1/8 (12.5%)	$p = 0.002$
Cataract	0/6	1/17	1/23 (4.3%)	0/8	1/25	1/33 (3%)	$p = NS$
Calcification of the external auditory canal	0/6	1/17	1/23 (4.3%)	0/8	ND	0/8 (0%)	NA
Orthopedic							
Ossification delay	3/3	3/NA	NA	NA	NA	NA	NA
Joint hyperlaxity	3/6	3/17	6/23 (26.1%)	3/8	1/24	4/32 (12.5%)	$p = NS$
Knee deformation	2/6	2/17	4/23 (17.4%)	3/8	0/24	3/32 (9.4%)	$p = NS$
Hip dysplasia	0/6	1/17	1/23 (4.3%)	0/8	1/24	1/32 (3.1%)	$p = NS$
Spine deformation	1/6	5/17	6/23 (26.1%)	2/8	5/24	7/32 (21.9%)	$p = NS$
Long fingers	1/6	0/17	1/23 (4.3%)	0/8	2/24	2/32 (6.3%)	$p = NS$
Feet deformation	3/6	2/17	5/23 (21.7%)	2/8	7/24	9/32 (28.1%)	$p = NS$
Total skeletal malformation	5/6	10/17	15/23 (65.2%)	4/8	14/24	18/32 (56.3%)	$p = NS$
Endocrinology							
Hypothyroidism	2/6	3/17	5/23 (21.7%)	0/8	1/23	1/31 (3.2%)	$p = 0.047$
Diabetes mellitus	0/6	2/14	2/20 (10%)	0/8	1/23	1/31 (3.2%)	$p = NS$
Delayed puberty	1/2	0/6	1/8 (12.5%)	1/3	ND	1/3 (33.3%)	$p = NS$
Others							
Pinnae calcification	2/NA	3/NA	NA	NA	NA	NA	NA
Inguinal hernia (males)	0/2	3/10	3/12 (25%)	1/6	10/17	11/23 (47.8%)	$p = NS$
Congenital cardiopathy	1/6	3/17	4/23 (17.4%)	1/8	2/26	3/34 (8.8%)	$p = NS$

ND no data, *NC* not concerned, *NS* not significant.

[a]Only one truncating SNV.

and ES should decrease the time to diagnosis for patients with SNVs.

In our study, ID and/or psychomotor delay were observed in all patients and ranged from mild to severe in both groups. Patients in the two groups shared the same facial features. Birth weight was generally normal in both groups. Overgrowth was a rare feature, but observed in both groups. The most distinctive difference was the presence of perceptive hearing loss, diagnosed in the majority of affected patients from the missense SNVs group, whereas in patients with CNVs only one patient developed hearing loss at the age of 14 months. The mean age at the diagnosis of the hearing loss cannot be determined from the literature data, but the youngest patient of the missense SNVs group, aged 2 years, had not yet been diagnosed with hearing loss. However, not all CNV patients had been screened, and this could make it difficult to draw definitive conclusions. Similarly, cerebral and pinnae calcifications have never been reported in *ZBTB20*-CNV patients and they therefore appear to be specific to patients carrying *ZBTB20* missense SNVs. The age at onset of ear calcifications is unknown. In our study, it has been reported only in a 40-year-old female patient. An 11-year-old patient had been reported in 2018 with pinnae calcification [13]. However, this feature had not been explored by specific imaging in all cases. A higher rate of corpus callosum anomalies was found in the missense SNVs group, even though anomalies were observed in both groups [9]. Concerning endocrine complications, hypothyroidism was also more frequent in the missense SNVs group (21.7% vs 3.2%), with variable age of onset, from 2 years and 7 months in the present series to 43 years in the literature [6]. In addition, diabetes mellitus was mostly reported in patients with missense SNVs (10% vs 3.2% in the CNVs group). Diabetes mellitus was usually reported in adulthood (in a 43 and 23-year-old patients with missense SNV and in a 41-year-old patient with CNV), although an 8-year-old patient with glucose intolerance has been described [14]. Muscle wasting is often reported in association with missense SNVs (6/23), but never reported in patients with CNVs.

Among patients with *ZBTB20* CNVs, the phenotype could also be explained by the deletion of other adjacent

genes in the 3q13.31 region. Patients 9, 10, and 11 also had a deletion comprising the *LSAMP* gene. This gene encodes a limbic system-associated membrane protein. Significant associations between the *LSAMP* gene and schizophrenia or neuropsychiatric features have been found [15]. *DRD3* is close to *ZBTB20* and encodes a dopamine receptor. Polymorphisms in this gene are involved in susceptibility to schizophrenia and essential tremor [16, 17]. Altogether, 32 patients from the CNV group had a *DRD3* deletion, and only one 41-year-old patient with a *ZBTB20* and *DRD3* complete deletion and partial deletion of *LSAMP* had schizophrenia [18]. Since one 40-year-old patient was also diagnosed with schizophrenia in the missense SNVs group, we cannot exclude that *ZBTB20* could contribute to schizophrenia.

In data from the study and the literature, there were no findings present in patients with CNVs and absent in patients with missense SNVs, in favor of the major role of *ZBTB20* in the 3q13.31 CNV phenotype. Functional studies performed by transfection experiments were in favor of a dominant negative impact of missense *ZBTB20* SNVs [7], compatible with a more severe phenotype in patients with a missense *ZBTB20* SNV than in patients with a *ZBTB20* CNV. The difference in the mean age of patients remains an obstacle and makes it difficult to draw definitive conclusions since the majority of the differential features is progressive. The long-term follow-up of audiometric and endocrine parameters in patients with *ZBTB20* CNVs is needed in order to determine risk and monitoring recommendations for these patients.

In conclusion, we compared the clinical features of six patients with missense *ZBTB20* SNVs and eight patients with *ZBTB20* CNVs or *ZBTB20* early truncating variant, associated with a review of the literature. We highlighted the existence of a genotype–phenotype correlation between *ZBTB20* missense SNVs and haploinsufficient variants, with a tendency toward a more severe and progressive clinical presentation in missense SNVs, although a longer follow-up of patients with CNVs is needed to draw further conclusions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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