SHORT REPORT

Expanding the clinical spectrum of recessive truncating mutations of *KLHL7* to a Bohring-Opitz-like phenotype

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ABSTRACT

Background Bohring-Opitz syndrome (BOS) is a rare genetic disorder characterised by a recognisable craniofacial appearance and a typical 'BOS' posture. BOS is caused by sporadic mutations of*ASXL1*. However, several typical patients with BOS have no molecular diagnosis, suggesting clinical and genetic heterogeneity.

Objectives To expand the phenotypical spectrum of autosomal recessive variants of *KLHL7*, reported as causing Crisponi syndrome/cold-induced sweating syndrome type 1 (CS/CISS1)-like syndrome.

Methods We performed whole-exome sequencing in two families with a suspected recessive mode of inheritance. We used the Matchmaker Exchange initiative to identify additional patients.

Results Here, we report six patients with microcephaly, facial dysmorphism, including exophthalmos, nevus flammeus of the glabella and joint contractures with a suspected BOS posture in five out of six patients. We identified autosomal recessive truncating mutations in the *KLHL7* gene. *KLHL7* encodes a BTB–kelch protein implicated in the cell cycle and in protein degradation by the ubiquitin–proteasome pathway. Recently, biallelic mutations in the *KLHL7* gene were reported in four families and associated with CS/CISS1, characterised by clinical features overlapping with our patients.

Conclusion We have expanded the clinical spectrum of *KLHL7* autosomal recessive variants by describing a syndrome with features overlapping CS/CISS1 and BOS.

Bohring-Opitz syndrome (BOS) (MIM605039) is characterised by intrauterine growth retardation (IUGR), difficulty establishing feeding and severe reflux, severe developmental delay, internal rotation of the shoulders with fixed contractures of the elbows and ulnar deviation of the wrists and metacarpophalangeal joints, often referred to as the 'BOS posture'. This is associated with recognisable facial dysmorphism that includes exophthalmos, low-set and posteriorly rotated ears, anteverted nares and frontal nevus flammeus. Some patients display cerebral malformations, seizures, scoliosis and ophthalmological, cardiac and gastrointestinal abnormalities.[1] BOS is caused by a de novo truncating mutation in *ASXL1*.[2] Recently, de novo mutations in *ASXL2* (Shashi-Pena syndrome (MIM617190)) and *ASXL3* (Bainbridge-Ropers syndrome (MIM615485)) were identified in patients displaying a clinical spectrum overlapping BOS.[3–7]

However, numerous patients with a clinical diagnosis of BOS do not have mutations in ASXL genes.[1] *ASXL1/2/3* encode for three *additional sex combs-like* proteins involved in chromatin remodelling through interactions with the polycomb group protein complex (PRC).[8–10]

Here, we report on six patients from four unrelated families with clinical features overlapping BOS and carrying autosomal recessive truncating mutations of *KLHL7*. Biallelic *KLHL7* mutations were previously reported in Crisponi syndrome/cold-induced sweating syndrome type 1 (CS/CISS1)-like syndrome.[11] This report expands the clinical spectrum of *KLHL7*-related disorders.

PATIENTS

Patients 1 and 2

The first family included two affected children and one unaffected sibling. Patient 1 was a girl, born at full term with a birth weight of 2090 g (−3 SD), length 44 cm (−3.5 SD) and head circumference 32 cm (−2.5 SD). Patient 2 was a boy, born at full term with a birth weight of 2200 g (−2.5 SD), length 46 cm (−2.5 SD) and head circumference 32 cm (−2.5 SD). The two pregnancies were marked by IUGR and polyhydramnios. These siblings presented microcephaly, exophthalmos, nevus flammeus of the glabella, expressionless face, anteverted nares, hypertelorism, micrognathia and low-set ears (figure 1). Neurological defects included limb contractures, persistent flexion of the elbows, axial hypotonia and segmental hypertonia. Both siblings experienced non-specific seizures and

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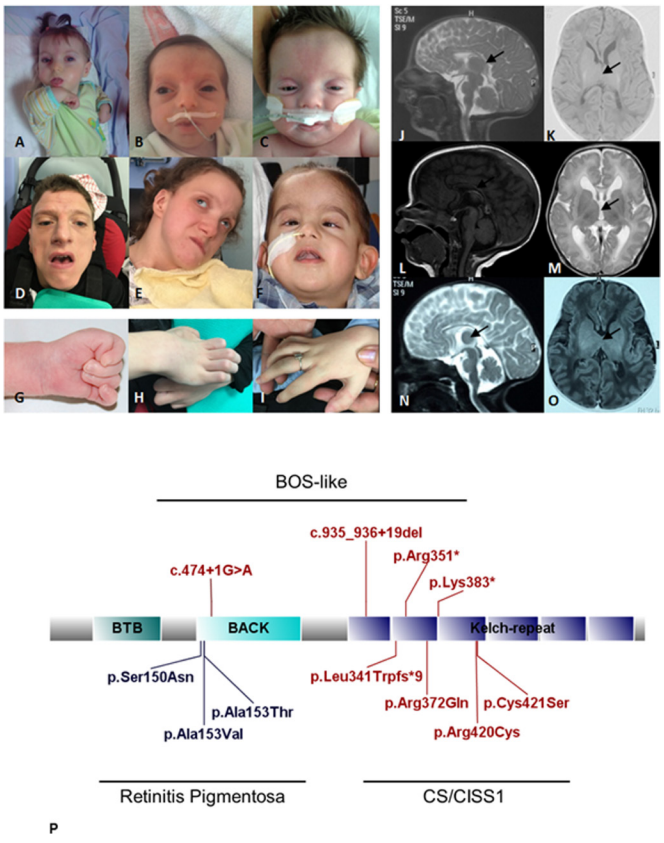

Figure 1 Clinical features of BOS-like patients and brain MRI. (A–F) Facial dysmorphism including microcephaly, exophthalmos, depressed nasal bridge and low-set ears. (G–I) Camptodactyly and fixed joint contractures of the hands. (J–O) Brain MRI shows a thin corpus callosum (J,K) or corpus callosum agenesis with fusion of thalami and frontal fusion (L–O) and cortical dysplasia (L,M). (A,J,K) Patient 1, (B,G,L,M) patient 2, (C,N,O) patient 3, (D,H) patient 4, (E,I) patient 5 and (F) patient 6. (P) Schematic representation of KLHL7 protein and reported mutations. Heterozygous and dominant mutations (in blue) in BACK domain cause retinitis pigmentosa. Recessive mutations (in red) in the Kelch domain are associated with CS/CISS1-like or BOS-like syndromes. Homozygous truncating variants identified in this study are located in BACK and Kelch regions. BOS, Bohring-Opitz syndrome; CS/CISS1, Crisponi syndrome/cold-induced sweating syndrome type 1.

had profound global developmental delay. Brain MRI revealed a thin corpus callosum. Patient 2 had an ostium secundum atrial septal defect. They were both fed via a nasogastric tube and presented with recurrent lung infections and respiratory distress. No episodes of cold-induced sweating were reported. They died in their first year of life (table 1). The diagnostic work-up included a normal array comparative genomic hybridisation (180k, Agilent), performed in patient 1.

Patient 3

Patient 3 was the first child of healthy unrelated parents (table 1, figure 1). The pregnancy was marked by IUGR and polyhydramnios. He was born at 39 weeks of gestation with a birth weight of 2850 g (−1 SD), length of 47 cm (−2 SD) and head circumference of 35 cm (−1 SD). Clinical examination revealed hypertelorism, exophthalmos and frontal nevus flammeus. He presented bilateral camptodactyly of the third finger, bilateral camptodactyly of the third finger and flexion of both elbows, a fixed position of the shoulders and stiffness in all joints, suggesting a BOS posture. Neurological investigations at birth revealed abnormal vagal activity with peripheral hypertonia and bilateral profound deafness. He had no swallowing reflex, poor facial expression, lack of head control and nearly absent pupillary light reflex. Brain MRI revealed a thin corpus callosum mainly in the posterior part and mild dilatation of the lateral ventricles. An investigation of the auditory brainstem response indicated bilateral profound deafness. He also had feeding troubles and frequent respiratory distress. Cardiac and renal ultrasound were normal. No skeletal defects were found on X-rays. At 10 months, he showed severe developmental delay and seizures. At 2 years and 10 months, no cold-induced sweating was reported. A clinical diagnosis of BOS was ascertained. Haematology, metabolic and biochemistry investigations were normal. The karyotype was 46, XY. Sanger sequencing and QPCR of the *ASXL1* gene remained negative.

Patients 4 and 5

The third family was composed of two affected children born to non-consanguineous parents (table 1). Patient 4 is a 22-year-old man, born at 38 weeks with a birth weight of 3230 g (−0.5 SD) and head circumference of 33.8 cm (−1.5 SD). He presented severe respiratory distress at birth. Patient 5 was the second child and is now 19 years. She was born by emergency caesarean section at 27 weeks due to placenta praevia. Birth parameters were not available. Prematurity was complicated by severe respiratory distress, pulmonary hypertension and thrombocytopenia. Both siblings underwent gastrostomy in infancy for marked feeding difficulties and failure to thrive. They presented similar dysmorphisms including nevus flammeus, exophthalmos, hypertelorism, low-set ears, micrognathia and microcephaly. They also presented the BOS posture and rhizomelic upper limb shortening. Both had global developmental delay and absent speech. Patient 4 had movement disorders with permanent choreoathetosis. Patient 5 had absence seizures. Brain MRI revealed nodular heterotropia in both siblings. Cardiac (ventricular septal defect) and renal abnormalities (vesicoureteric reflux and proximal renal tubular acidosis leading to fragility fractures) were found.

Patient 6

Patient 6 was born to a consanguineous couple at 36 weeks after a normal pregnancy (table 1). The neonatal period was complicated by failure to thrive and persistent reflux requiring tube feeding and gastrostomy. Birth parameters were not available. In the neonatal period, he was found to have perineal hypospadias, a bifid scrotum and bilateral undescended testes, which were treated surgically. He had a small cardiac ventricular septal defect. Development was severely delayed; he started walking at 8 years of age. The evolution was complicated by recurrent and severe aspiration pneumonia. He had axial hypotonia, fixed contractures of the fingers and toes and ulnar deviation of the right wrist, suggesting the BOS posture. Facial dysmorphism included exophthalmos, hypertelorism, micrognathia, low-set posteriorly rotated ears and significant hirsutism. The MRI scan showed microcephaly and a thin corpus callosum and prominence of the sulci gyri, pericerebral cerebrospinal fluid spaces, ventricles and basal cisterns.

METHODS

Patient-only or trio-based whole-exome sequencing (WES) was performed (as previously detailed) for all patients, who either had a clinical diagnosis of BOS without mutations in the ASXL genes or lacked a clinical diagnosis.[12] Informed consent

Table 1 Detailed clinical features of patients with recessive variants in the *KLHL7* gene, compared with BOS and BCS series

Clinical	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	CS_144 Angius *et al*, 2016	CS_144 Angius *et al*, 2016	CS_258 Angius *et al*, 2016	CS_259 Angius *et al*, 2016	CS_169 Angius *et al*, 2016	Total	BOS series (%)	BCS series (%)
Ethnic origin	Syria	Syria	Italy	UK	UK	UK	Turkey	Turkey	Turkey	Turkey	Turkey			
Sex	F	M	M	M	F	M	F	F	F	M	M			
Age at death	2 years	1 year	–	–	–	–	21 months	–	24 months	–	5.5 months			
Feeding difficulties	+	+	+	+	+	+	+	+	+	+	+	11/11	28/28 (100)	12/12 (100)
Severe/profound LD	+	+	+	+	+	+	NA	NA	NA	NA	NA	6/6	24/24 (100)	NA
IUGR	+	+	+	+/–	–	+	NA	NA	NA	NA	NA	6/6	25/30 (83)	21/37 (57)
Recurrent infections	+	+	+	+	+	+	NA	NA	NA	NA	NA	5/6	16/26 (61)	NA
Seizures	+	+	+	_	+	–	–	–	–	–	–	4/11	13/25 (52)	5/39 (13)
Respiratory distress	+	+	–	+	+	+	+	+	+	+	+	10/11	11/26 (42)	8/12 (67)
Arrhythmia	–	–	–	NA	NA	NA	NA	NA	NA	NA	NA	0/3	4/25 (16)	0/36 (0)
Craniofacial														
Exophthalmos	+	+	+	+	+	+	–	–	–	–	–	6/11	28/30 (93)	0/36 (0)
Nevus flammeus	+	+	+	+	+	–	NA	NA	NA	NA	NA	5/6	28/30 (93)	0/36 (0)
Trigonocephaly	–	–	–	+	–	–	–	–	–	–	–	1/11	28/30 (93)	0/36 (0)
Microcephaly	+	+	+	+	+	+	NA	NA	NA	NA	NA	6/6	27/30 (90)	23/36 (64)
Micro/retrognathia	+	+	+	+	–	+	NA	NA	NA	NA	NA	5/6	25/30 (83)	39/39 (100)
Abnormal palate structure	–	+	–	+	+	+	+	+	+	–	+	8/11	25/30 (83)	5/39 (13)
Depressed nasal bridge	–	–	–	NA	NA	NA	+	+	+	+	+	5/8	23/30 (77)	NA
Low-set posteriorly rotated ears	–	–	–	+	–	+	NA	NA	NA	NA	NA	2/6	22/30 (73)	NA
Upslanting palpebral fissures	–	–	–	–	–	–	–	–	–	–	–	0/11	20/30 (67)	NA
Anteverted nares	+	+	+	+	+	+	NA	NA	NA	NA	NA	6/6	18/30 (60)	0/36 (0)
Hypertelorism	+	+	+	+	+	+	NA	NA	NA	NA	NA	6/6	17/30 (57)	0/36 (0)
Cleft/notch lips	–	–	–	–	–	–	–	–	–	–	–	0/11	11/30 (37)	NA
Epicanthal folds	–	–	–	–	–	–	–	–	–	–	–	0/11	6/30 (20)	NA
Buccal frenulae	–	–	–	–	–	–	–	–	–	–	–	0/11	4/30 (13)	0/36 (0)
Ophthalmic														
Retinal/optic nerve abnormalities	–	–	–	–	–	NA	–	+	NA	+	NA	2/8	18/30 (60)	0/36 (0)
Strabismus	–	–	–	+	+	+	–	–	–	–	–	3/11	15/30 (50)	NA
Myopia	–	–	–	NA	NA	+	–	–	–	–	–	1/9	10/30 (33)	NA
Hair/skin														
Low anterior hairline	–	–	–	–	–	–	–	–	–	–	–	0/11	21/30 (70)	NA
Hirsutism	+	+	+	_	+	+	NA	NA	NA	NA	NA	5/6	13/30 (43)	0/36 (0)
Neurological/skeletal														
BOS posture	–	+	+	+	+	+	–	–	–	–	–	5/11	30/30 (100)	–
Camptodactyly	–	–	+	+	NA	+	+	+	+	+	+	8/10	21/25 (84)	28/38 (74)
Brain abnormalities	+	+	+	+	+	+	NA	NA	NA	NA	NA	6/6	21/30 (70)	0/39 (0)
Joint contractures	+	+	+	+	+	+	+	+	+	NA	–	9/10	18/30 (60)	32/39 (82)

Continued

Table 1 Continued

Clinical	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	CS_144 Angius *et al*, 2016	CS_144 Angius *et al*, 2016	CS_258 Angius *et al*, 2016	CS_259 Angius *et al*, 2016	CS_169 Angius *et al*, 2016	Total	BOS series (%)	BCS series (%)
Congenital dislocations	–	–	–	–	–	–	–	–	–	–	–	0/11	10/30 (33)	NA
Hypotonia	+	+	+	+	–	+	NA	NA	NA	NA	NA	5/6	8/30 (27)	NA
Hypertonia	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	3/3	7/30 (23)	NA
Other														
Cardiac abnormalities	–	+	–	–	+	+	NA	NA	NA	NA	NA	3/6	14/30 (47)	8/39 (21)
Genital abnormalities	–	–	–	+	–	+	NA	NA	NA	NA	NA	2/6	6/30 (20)	13/23 (57)
Renal abnormalities	–	–	–	NA	NA	–	NA	NA	NA	NA	NA	0/4	4/30 (13)	0/36 (0)

Additional details are in online supplemental data[11 17 18]

BCS, Bowen-Conradi syndrome; BOS, Bohring-Opitz syndrome; F, female; IUGR, intrauterine growth retardation; LD, learning difficulty; M, male.

was obtained from the families. Rare variants were prioritised according to the suspected recessive mode of inheritance and the clinical similarity between these patients. Homozygosity mapping was performed on the raw variant call format file using homozygosity mapper (http://www.homozygositymapper.org/) with default settings in the consanguineous family. The splice defects were predicted in silico (http://www.umd.be/HSF/). The familial segregation was confirmed by Sanger sequencing.

After identifying the candidate gene by WES in patients 1 and 2, data sharing, which included informal exchanges between collaborators, presentations of case reports in international congresses and PhenomeCentral data sharing, allowed the recruitment of four additional patients from three families. All four had homozygous mutations in *KLHL7* with significant clinical similarities.

RESULTS

We identified a homozygous variant in the *KLHL7* gene in patient 1 (NM_018846.4:c.474+1G>A), predicted to affect the splice site (figure 1P). Variants were absent from public genomic databases. *KLHL7* truncating events are rare in Exome Aggregation Consortium (ExAC) databases (0.02%), and there are no homozygous individuals. Based on ExAC data, the probability of carrying autosomal recessive truncating variants would be approximately one in 8.9×10^{-7} non-consanguineous individuals. Four additional patients were gathered through the MatchMaker Exchange initiative. A homozygous nonsense mutation (NM_018846.4:p.Lys383*) in patient 3 and patients 4 and 5 (NM_018846.4: p.Arg351*) was identified. In patient 6, a deletion in exon 7 of *KLHL7* was detected and generated a splice donor variant according to predictive algorithms (NM_001031710.2:c.935_936+19del). Parental segregation by Sanger sequencing confirmed the heterozygous state of the unaffected parents and siblings of each reported patient, confirming the recessive mode of inheritance.

DISCUSSION

This article reports on six patients carrying autosomal recessive truncating variants of *KLHL7* with phenotypes strongly overlapping with BOS. Dominant missense mutations in the *KLHL7* gene have previously been reported in autosomal dominant retinitis pigmentosa.[13] More recently, homozygous mutations associated with CS/CISS1-like syndrome were reported in four families[11] (table 1).

Gathering additional patients with autosomal recessive truncating variants of KLHL7 expanded this clinical spectrum to a BOS-like phenotype. The patients previously reported presented exophthalmos, facial nevus flammeus, microretrognathia, palate defects, camptodactyly and the BOS posture. We also observed feeding difficulties, severe learning disability, IUGR and microcephaly in our cases, all of which are associated with BOS (table 1). However, we noted some differences in the clinical features compared with previously reported patients with CS/CISS1, who also had homozygous mutations in the *KLHL7* gene. Patients with CS/CISS1 are reported to have contraction of oropharyngeal muscles, hyperthermia (3/5), inconstant retinitis pigmentosa (2/5) and a depressed nasal bridge (5/5), none of which were found here. In addition, learning difficulties, seizures, microretrognathia, exophthalmos and brain abnormalities, which were reported here, were not described in the CS/CISS1 cohort. However, we noted that patients with CS/CISS1 showed camptodactyly and joint contracture similar to the BOS posture, even though these features were not discussed by Angius *et al*.[11] The phenotype observed in the four families presented here expands the clinical spectrum of CS/CISS1, which has a considerable

overlap with BOS. The differential diagnoses put forward during the diagnostic work-up in families 1 and 2 included trisomy 18, Bowen-Conradi syndrome (BCS) and BOS. These disorders have an overlapping spectrum of manifestations that include joint contractures and camptodactyly, retrognathia, microcephaly and IUGR. Feeding and swallowing difficulties and respiratory distress are also frequently observed in BCS and observed in *KLHL7*-mutated patients. However, additional clinical reports of patients are needed to further delineate the clinical spectrum of recessive variants of *KLHL7*.

The *KLHL7* gene encodes a 75 kDa Kelch-like protein and is ubiquitously expressed, but its functions are largely unknown. It has been shown that KLHL7 forms a homodimer, binds with two Cullin-RING ligase complexes (CUL3 and ROC1) with its BTB and BACK domains and recruits the substrates for polyubiquitination through the Kelch region.[14] A potential target of KLHL7 is histone 2A, suggesting a role in chromatin remodelling. CUL3/ROC1 is known to interact with PRC1, a multisubunit complex that is able to recognise epigenetic marks and enable the ubiquitination to block the repressive state of chromatin.[15] Interestingly, the PRC1 complex seems to contain another subunit, ASXL1, which is mutated in BOS.[16] We hypothesise that KLHL7 is involved in the transcriptional inhibition driven by the PRC1 complex through its binding with CUL3/ROC1. ASXL3 and ASXL2 are also epigenetic regulators and interact with PRCs.[6 10] This hypothesis may explain the clinical overlap between patients with mutations in ASXL1/2/3 and KLHL7 (online supplementary table s1). However, further studies are required to confirm the colocalisation and the interaction of these proteins.

In summary, we have ascertained four families (six patients) affected by a syndrome strongly overlapping BOS and carrying autosomal recessive mutations of *KLHL7*. Although overlapping with the BOS phenotype, patients showed similarities to BCS and CS/CISS1. A clinical continuum between the CS/CISS1 and BOS-like phenotype was noted. The reported variants affected BACK and Kelch domains and probably their interaction with CUL3 or its substrates, respectively. Our current understanding of the molecular action of KLHL7 suggests a common pathway with ASXL1, which may explain the similarity with BOS .

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