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Bi-allelic c.181_183delTGT in BTB domain of KLHL7 is associated with overlapping phenotypes of Crisponi/CISS1-like and Bohring-Opitz like syndrome

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Abstract

Biallelic pathogenic variants in *KLHL7* are known to result in Crisponi syndrome (CS)/cold-induced sweating syndrome type 1 (CISS1) like phenotype and Bohring-Opitz-like syndrome. In this report, a trio whole-exome sequencing (WES) was performed in proband with cold-induced sweating, microcephaly, facial dysmorphism, spasticity, failure to thrive, pigmentary abnormalities of the retina, hypoplasia of corpus callosum and periventricular nodular heterotopia. A novel homozygous in-frame deletion was identified in exon 2 of *KLHL7*, affecting the BTB domain of the protein. Our findings expand the clinical and molecular spectrum of *KLHL7*-related disorders.

Keywords

Crisponi/CISS1-like syndrome; *KLHL7*; Exome sequencing; Bohring-Opitz-like syndrome

INTRODUCTION

Proteins encoded by kelch-like (KLHL) gene family have a BTB domain, a BACK domain and kelch repeats. Kelch like family member 7 (*KLHL7*) is one of the 42 currently defined KLHL genes which acts as a substrate recognition subunit of a CUL3-based ubiquitin ligase complex and mediates between E3 ubiquitin ligases and ubiquitinated substrates. Kelch Like Family Member 7 is involved in the ubiquitin proteasome system (UPS) and is part of the BCR (BTB-CUL3-RBX1) E3 ubiquitin ligase complex, with CUL3 and RBX1. It has also been shown to assemble with CUL3 through its BTB and BACK domains to carry out

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ubiquitin ligase (E3) activity. KLHL family has been conserved throughout evolution (Kigoshi et al., 2011). Heterozygous mutations have also been described in the gene to cause late-onset retinitis pigmentosa 42 (MIM #612943) with all the three mutations located in the BACK domain of the protein. Homozygous mutations in the kelch domains of KLHL7 have been reported Crisponi/CISS1-like phenotype (MIM #272430) in five individuals from four families of Turkish origin (Angius et al., 2016). Six more patients with pathogenic variants in BACK and kelch domains are reported with a phenotype described as Bohring-Opitz-like as they carried overlapping findings with Bohring-Opitz syndrome (Bruehl et al., 2017). Here we report an individual with a homozygous novel in-frame deletion in the BTB domain of KLHL7 resulting in a combined phenotype of Crisponi/CISS1-like phenotype and Bohring-Opitz like syndrome.

MATERIALS AND METHODS

Clinical summary

We evaluated a three-year-old male child with global developmental delay, facial dysmorphism, spasticity and failure to thrive. He was the first born to a consanguineous couple (Figure 1A). He was born at term by lower segment caesarean section and weighed 3.1 kg (+1 SD). He had delayed cry and was admitted in the intensive care unit for a month following his birth. He had marked feeding difficulty and received nasogastric feeding for 8 weeks. Recurrent episodes of fever and regurgitation of feeds were observed since birth. Choking during feeding solids and progressive tightening of the body were also noted in him. He had not achieved neck holding and social smile. He also had cold-induced sweating.

He measured 74 cm (−5 SD) in length, occipito-frontal circumference was 43 cm (−7 SD) and weighed 5.4 kg (−6 SD) at three years. He had a triangular face, sparse, hypopigmented hair, prominent forehead, facial nevus simplex (flammeus) on the forehead, hirsutism, arched eyebrows, dysplastic ears, downturned corners of mouth, gum hypertrophy, narrow palate. Spasticity, contractures at elbow, camptodactyly and absent deep tendon reflexes were also observed (Figure 1B and 1C). Magnetic resonance imaging of brain showed periventricular nodular heterotopia along frontal horn of right lateral ventricle, symmetrical supratentorial white matter volume loss with corpus callosum atrophy and delayed myelination (Figure 1D and 1E). Complete blood count and liver function tests returned normal results. Fundoscopy revealed retinal pigmentary abnormalities and mottling.

Written informed consents were obtained from the family. The study has the approval from Institutional Ethics Committee. The phenotypic information and the variant details can be accessed at ClinVar (Submission ID-SUB2844482).

Chromosomal microarray

Chromosomal microarray (CMA) was done on leukocyte-derived DNA of the patient using Illumina HumanCytoSNP-12 (Illumina, San Diego, California, USA). The genome-wide resolution of this array is ~30 kb. The data were analyzed using the software, KaryoStudio by Illumina.

Whole exome sequencing

Trio whole exome sequencing (WES) was carried out for the family as described previously (Shukla et al., 2017). The strategy implemented to filter down the list of called variants is outlined in Table S1. Final list of homozygous variants which were absent in population databases are given in Table S2. The effect of the deletion on the KLHL7 protein was assessed by various *in-silico* tools. The deletion was predicted to be damaging by CADD (22.5), DDIG (0.849532), PaPI (0.904), PinPor (0.522), VEST-Indel (0.45), MutationTaster (0.99) and PROVEAN (−11.324) (Choi and Chan, 2015; Douville et al., 2016; Kircher et al., 2014; Limongelli et al., 2015; Schwarz et al., 2010; Zhang et al., 2014; Zhao et al., 2013). Conservation analysis was carried out by Clustal Omega and domain mapping was done using MutationMapper (Vohra and Biggin, 2013). Validation of the variant and segregation analysis in the trio was performed by Sanger sequencing (Figure 1A).

Homology modeling

The crystal structure available from the RCSB Protein Data Bank archive (PDB ID: 3II7) for human KLHL7 consists of only the kelch-repeat domain. In the proband, since the homozygous in-frame deletion is at residue 61 in the BTB domain of KLHL7 (Figure 2B), the homology modeling of the wild-type and mutant BTB domain (residues spanning 44–111) was carried out using SWISS-MODEL (Biasini et al., 2014). Protein model was built from the template of “Crystal structure of human CUL3 N-terminal domain bound to KEAP1 BTB and 3-box” (PDB ID: 5NLB.1.A), having a resolution of 3.45 Å. The sequence identity obtained with the template was 44.78% and 43.94% for the wild-type and mutant structure respectively, with high coverage. YASARA package was used for the visualization of the modeled structures (Krieger and Vriend, 2014).

RESULTS

Retrospectively, the phenotype was found to match with Crisponi/CISS1-like and Bohring-Opitz like syndrome. A 3-bp novel homozygous in-frame deletion c.181_183delTGT (NM_001031710, p.(Val61del)) was identified in *KLHL7* gene for the proband. The variant was not present in the 1000 Genomes Project, ESP, gnomAD and in our in-house exome data of 481 individuals (Auton et al., 2015; Lek et al., 2016). CMA revealed no copy number variants and *KLHL7* was found to be present in a 13.6 Mb region of homozygosity block on chromosome 7 (Table S3). The site of the in-frame deletion has a high GERP score of 5.65 and is seen to be well conserved from *Caenorhabditis remanei* to *Homo sapiens* (Figure 2A) (Davydov et al., 2010). Sanger sequencing confirmed the segregation of the variant in the proband, parents being healthy carriers (Figure 1A).

In the wild-type modeled protein structure, V61 residue has a H-bonding with A65 (Figure 2C). Due to the in-frame deletion p.(Val61del), along with the loss of the hydrogen bonding with A65, structural disruption was also observed in the mutant structure where the α -helix in the region was seen to be altered (Figure 2D).

DISCUSSION

Crisponi/CISS1-like phenotype is a rare recessive disorder with severely affected patients presenting with hyperthermia, feeding difficulties, facial dysmorphism and posturing (Angius et al., 2016). Facial dysmorphism and posturing consistent with Bohring-Opitz like syndrome was noted in another six families with recessive truncating variants in *KLHL7* (Bruel et al., 2017). The individuals previously reported by Bruel *et al.* had exophthalmos, facial nevus simplex (flammeus), palate defects, microretrognathia, camptodactyly and a typical posture with internal rotation of shoulders, fixed contraction of elbows and ulnar deviation of the wrists and metacarpophalangeal joints, often referred to as the 'BOS posture' (Bruel et al., 2017). It was observed that feeding difficulties, severe learning disability, intrauterine growth retardation, seizures, microcephaly and brain malformations (thin corpus callosum and cortical dysplasia) were also present in patients with pathogenic variants in *KLHL7* (Bruel et al., 2017). By thorough phenotype analysis, Bruel *et al.* concluded that these subjects with pathogenic variants in *KLHL7* presented like Bohring-Opitz like syndrome (BOS). However, some of the patients reported earlier diverged in the phenotype from BOS (Angius et al., 2016). They showed contraction of oropharyngeal muscles, hyperthermia, retinitis pigmentosa and a depressed nasal bridge which were not observed in patients with BOS-like phenotype (Table 1). All the patients showed camptodactyly and BOS posturing. Paradoxical sweating induced by cold was not observed in any of the reported cases. We observed overlapping findings from both the reports. Our patient has triangular face, feeding difficulties, severe learning disability, recurrent infections, facial nevus simplex (flammeus), hirsutism, narrow palate, camptodactyly, BOS-like posture, hypoplasia of corpus callosum and pigmentary changes of the retina. In addition, he also had cold-induced sweating, periventricular nodular heterotopia which are reported for the first time in patients with pathogenic variants in *KLHL7*.

Whole-exome sequencing led us to the identification of a novel homozygous in-frame deletion of 3-bp in *KLHL7* in the proband. In the wild-type *KLHL7* protein, binding of CUL3 occurs through the BTB/BACK domains followed by proteasome ubiquitination and degradation of the target substrate(s) which bind to the kelch domain. Homozygous variants reported for Crisponi/CISS1-like phenotype in Angius et al. (2016), are located in the kelch domain of *KLHL7* and might have an influence on the binding of target substrate(s) without ubiquitination taking place, leading to its accumulation (Angius et al., 2016). Pathogenic variants are also observed in the BACK domain in patients with BOS-like phenotype (Bruel et al., 2017). In a study by Kigoshi et al. (2011), it was shown that CUL3 binding takes place in mutants lacking the kelch domain. The variant found in our study is located in the BTB domain of the protein, which is necessary for CUL3 binding to exert E3 activity. All *in-silico* tools used to determine the pathogenicity of the variants, predict the in-frame deletion to have a damaging effect on the protein. As CUL3-BTB binding is affected, the normal functioning of *KLHL7* in ubiquitin ligase activity is likely to be impaired leading to further accumulation of the target substrate(s) resulting in a severe phenotype. The protein modeling displays structural alteration in the mutant as compared to the wild-type, indicating that this could destabilize the proteins structure. Further functional validation could not be performed

to determine the precise underlying pathogenic mechanism involved with in-frame deletion of 3-bp in *KLHL7* in the proband.

This is the first report of a child with biallelic mutations in *KLHL7* manifesting cold induced sweating. Overlapping clinical phenotype of cold-induced sweating syndrome 1-like and Bohring-Opitz like syndrome was observed in him. In addition, he also had a cortical malformation, periventricular nodular heterotopia which was not reported earlier in patients with variants in *KLHL7*. The variant we observed is in the BTB domain of the protein unlike the previously reported individuals with Crisponi/CISS1-like phenotype due to variants in kelch repeats and BACK domain. Our findings expand the clinical and molecular spectrum of *KLHL7*-related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Pedigree and Sanger chromatograms show the segregation of the variant, c.181_183delTGT in the family (A). Alphabets in the chromatogram indicate the following nucleotide changes; 'S' indicates (G>C), Y indicates (C>T), and W indicates (A>T). Triangular face, sparse, hypopigmented hair, prominent forehead, facial nevus simplex (flammeus) on the forehead, hirsutism, arched eyebrows, dysplastic ears, downturned corners of mouth, contractures at elbow and camptodactyly (B, C). T1 weighted axial section of the brain at 3 years of age shows periventricular nodular heterotopia along frontal horn of right lateral ventricle (white arrow) and delayed myelination, sagittal section shows hypoplasia of corpus callosum and white matter volume loss (D, E).

Figure 2:
Multiple sequence alignment shows that the highlighted variant position is conserved across species (A). Representation of the variant site on KLHL7 protein in the BTB domain (B). Protein modeling of the wild-type and mutant BTB domain shows deletion of V61 residue leading to loss of hydrogen bonding with A65 and deformation of BTB domain in the mutant structure as compared to wild-type KLHL7 (C, D).

Table 1:

Comparison of clinical findings observed in Crisponi syndrome (CS)/cold-induced sweating syndrome type 1 (CISS1)-like syndrome and Bohring-Opitz-like syndrome

Clinical feature	CS/CISS1-like syndrome	Bohring-Opitz-like syndrome
References	Angius <i>et al.</i> , 2016	Bruehl <i>et al.</i> , 2017
Intrauterine growth retardation	NA	+
Recurrent infections	NA	+
Learning disability	NA	+
Feeding difficulty	+	+
Contraction of oropharyngeal muscles	+	–
Palatal defects	–	+
Seizures	–	+
Depressed nasal bridge	+	–
Anteverted nares	–	+
Hypertelorism	–	+
Retinitis pigmentosa	+	–
Hirsutism	NA	+
Joint contractures	+	+
Camptodactyly	+	+
Hyperthermia	+	–
Microcephaly	NA	+
Exophthalmos	–	+
Nevus flammeus	NA	+
BOS posturing	–	+
Hypotonia	NA	+
Hypertonia	NA	+
Brain abnormalities	NA	+

+ Present, – Absent, NA not available