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A Novel Homozygous Missense Variant in the BTB domain of KLHL7 Gene Causes PERCHING Syndrome in a Consanguineous Family

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Abstract

PERCHING Syndrome, is a condition that shares similarities with klhl7-related Bohring-Opitz-like and Crisponi/cold-induced sweating-like overlap syndrome, as well as hydrocephalus congenital. The Kelch Like Family Member 7 gene (KLHL7) plays a significant role in the development of PERCHING Syndrome. In this study, we utilized Whole-Exome Sequencing (WES) to identify disease-causing variations in a patient from an Iranian family affected by PERCHING Syndrome, who had consanguineous parents. In the proband, we detected a novel homozygous missense variation (c.254T>G, p.Val85Gly) in the gene. Confirmation of the variant's inheritance from parents was achieved through co-segregation analysis using Sanger sequencing. The identified variant underwent evaluation for its novelty and pathogenicity by utilizing multiple databases. Furthermore, bioinformatics tools were utilized to predict the three-dimensional structure of the mutant KLHL7 protein. Our report will contribute to broadening the understanding of the clinical and genetic manifestations of PERCHING syndrome. The diagnostic challenges associated with this syndrome are significant, and healthcare professionals often face difficulties in identifying the condition. Accurate and timely recognition of the syndrome requires specialized tests and collaborative diagnostic approaches, which underscores the intricate nature of the diagnosis.

Keywords: KLHL7; PERCHING syndrome; Whole Exome sequencing; Novel variant

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Introduction

The human genome is a complex and intricate puzzle comprising approximately 20,000 to 25,000 genes, each playing a unique role in the functioning of the human body. Among these genes, the KLHL7 gene has emerged as an intriguing subject of scientific inquiry. KLHL7, short for Kelch-like family member 7, is a gene that belongs to the Kelch-like family of proteins [1]. It is essential to explore its structure. The KLHL7 gene is on chromosome 7p15 and contains 12 exons. The gene encodes a protein with several Kelch-like domains, which are known for their involvement in protein-protein interactions. Its protein product's exact role is yet unknown, although it is known to have a role in substrate recognition when it forms E3 ubiquitin ligase complexes with Cullin-3 (CUL3), which mediate proteasome destruction. KLHL7 shares several Kelch motifs, a BACK domain, and a BTB/POZ domain with archetypal Kelch family members [2]. Evidence indicates that the BACK domain is necessary for CUL3 binding and ligase functionality. The BTB domain is known to aid binding to CUL3 to establish a functioning ubiquitin ligase complex. The active ligase's substrate recognition and specificity are determined by the Kelch motifs, which comprise a three-dimensional propeller structure and differ significantly between the members of the Kelch family. Its interactions with other proteins and signaling pathways are essential in these processes. Mutations in the KLHL7 gene have been linked to various genetic illnesses, including myopathy and Congenital Fibrosis of the Extraocular Muscles (CFEOM) [3].

Understanding the pathophysiology of these illnesses requires research into specific mutations and their effects on protein function. PERCHING syndrome (OMIM #617055) is brought on by truncating and missense homozygous or compound heterozygous mutations in the Kelch Like Family Member 7 gene (KLHL7) [3]. In the acronym, each letter represents two essential

phenotypic elements: P for Postural and Palatal abnormalities, E for Exophthalmos and Enteral-tube dependency/feeding issues, R for Respiratory distress and Retinitis pigmentosa, C for Contractures and Camptodactyly, H for Hypertelorism and Hirsutism, I for IUGR/growth failure and Intellectual disability/developmental delay, N for Nevus Flammeus and Neurologic [4]. Bringing attention to the signs and symptoms can help with timely treatment. This condition is also known as KLHL7-related Bohring-Opitz-like phenotype because of its similar characteristics. Despite the wide-ranging and multi-systemic symptoms associated with PERCHING syndrome, there appears to be a distinct physical presentation that can aid in its clinical identification. Since its initial documentation in 2016 [5], there have been reports of very few patients with this syndrome [3]. While there is no cure for PERCHING syndrome, there are numerous therapy options that can help to mitigate its effects. Medical research advances, providing hope to people suffering from PERCHING syndrome. This syndrome touches every aspect of an individual's life. The impact is far-reaching, from emotional well-being to social interactions and career choices [6].

Material and Method

Case presentation

At the age of 2 and a half years, the index patient was directed to the genetic clinic due to abnormal physical features and a delay in overall development. This young boy was born prematurely to first-cousin parents of Turkmen ethnicity and required treatment in the Neonatology Intensive Care Unit for respiratory issues. Additionally, he experienced multiple readmissions for pulmonary infections during the postnatal period and exhibited severe hypotonia for 4 months. Despite being two and a half years old, he was still unable to stand without assistance and had not yet developed speech. A Dysmorphic evaluation (Figure 1) revealed several physical abnormalities, including jaw contracture, sparse scalp hair, smooth philtrum, Hypertelorism, Hirsutism, Strabismus, broad nasal tip, low-set ears, wide internipple distance, knee contracture, overlapping fingers, tightly fisted hands and abnormal creases of palm. The presence of cleft lip and palate in the mother can also be identified through specific clinical symptoms in the parents. Conversely, no specific clinical symptoms with a genetic background were observed in the father.

DNA extraction

Collection of whole blood samples was carried out from the patient as well as their biological parents. To extract DNA samples, we utilized the Yekta Tajhiz DNA extraction kit (catalog number: FABGK001) from 1 µl of blood mononuclear cells. Subsequently, the concentration of the purified DNA samples was measured using a Nanodrop device.

Whole exome sequencing

Utilizing the Illumina HiSeq 6000 platform, the patient's DNA underwent WES analysis. The exome sequence was created by fragmenting the DNA sample, modifying it with barcodes, and subjecting it to hybridization in the solution phase using the Agilent SureSelect Human All Exon V7 Plus probe set. The sequencing was carried out with an average target coverage of 100X, with approximately 90% to 95% of bases having at least 20X coverage. To align the reads of the sequence to the human reference genome (GRCh37/hg19), we utilized the Burrows-Wheeler Aligner (BWA) [7]. Additionally, for sorting and indexing BAM files and removing duplicate and low-quality reads (QBase less than 20), we employed SAMtools software [8]. The Genome Analysis Tool Kit (GATK) software package 2 (available at: https://www.broadinstitute.org/gatk/) was utilized for variant calling of Single Nucleotide Variants (SNVs) and Indels variants. We selected variants with a less than 1 percent frequency based on various human population databases. The databases mentioned are the 1000 Genome Project (http://www.1000genomes.org/), Genome Aggregation Database (genomeAD) (https://gnomad.broadinstitute.org/), ExAC (http://exac.broadinstitute.org/), ESP6500 (http://evs.gs.washington.edu/EVS/), and Iranome (http://www.iranome.com/). First, synonymous and non-coding regions variants were excluded from consideration. Initially, the focus was on the known disease-causing genes [9], and the potential for pathogenicity of nonsynonymous, indels, deletions, splice-site homozygous, and compound heterozygous/homozygous variants was evaluated using in Silico prediction algorithms, including CADD (https://cadd.gs.washington.edu/), Mutation Taster (http://www.mutationtasetr.Org), and PolyPhen-2 (http:// http://genetics.bwh.harvard.edu/pph2/). The standards established by the ACMG, along with subsequent revisions, were employed to assess the prognosis of harmful effects and the classification of genetic variations.

Co-segregation study

The primary objective of the co-segregation analysis was to examine how the identified genetic variation is passed down through generations. By assessing the co-segregation of these variants with the disease phenotype, we can ascertain their potential involvement in the onset of the disease. In the initial phase, we conducted Polymerase Chain Reaction (PCR) amplification using custom-designed primers explicitly targeting the precise location of the identified variation within the KLHL7 gene. Validation and confirmation of the variation in the probands and their parents were achieved through the utilization of Sanger sequencing. The obtained sequence chromatograms were subsequently assessed using the Codon Code Aligner software.

In Silico analysis of the identified variant

In order to determine the potential disease-causing effects of the identified variant, we performed an in-Silico analysis. This analysis utilized various computational tools such as ACMG, CADD, Mutation Taster, Polyphen-2, SIFT, Panther, FATHMM, MU pro, and I-Mutant. Furthermore, we manually assessed the most significant variations to validate their association with clinical outcomes.

Study of Protein-Protein interactions

In order to investigate the protein-protein interactions related to it, we utilized the online tool STRING (https://string-db.org/). This valuable resource integrates data from diverse datasets, including gene fusion, co-expression patterns, functional annotations, and experimental findings. It allows for predicting potential protein partners that could interact with KLHL7. Each identified interacting protein is assigned a comprehensive score ranging from 0 to 1, indicating the interaction's strength. A score of 0 suggests minimal interaction, while a score of 1 signifies significant interaction.

Prediction of protein three-dimensional structure

The I-TASSER server (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) was utilized to generate Three-Dimensional (3D) models of both the KLHL7 mutant and wild-type forms. This server uses the Protein Data Bank (PDB) to identify homologous sequences corresponding to different input sequence sections. These sequences

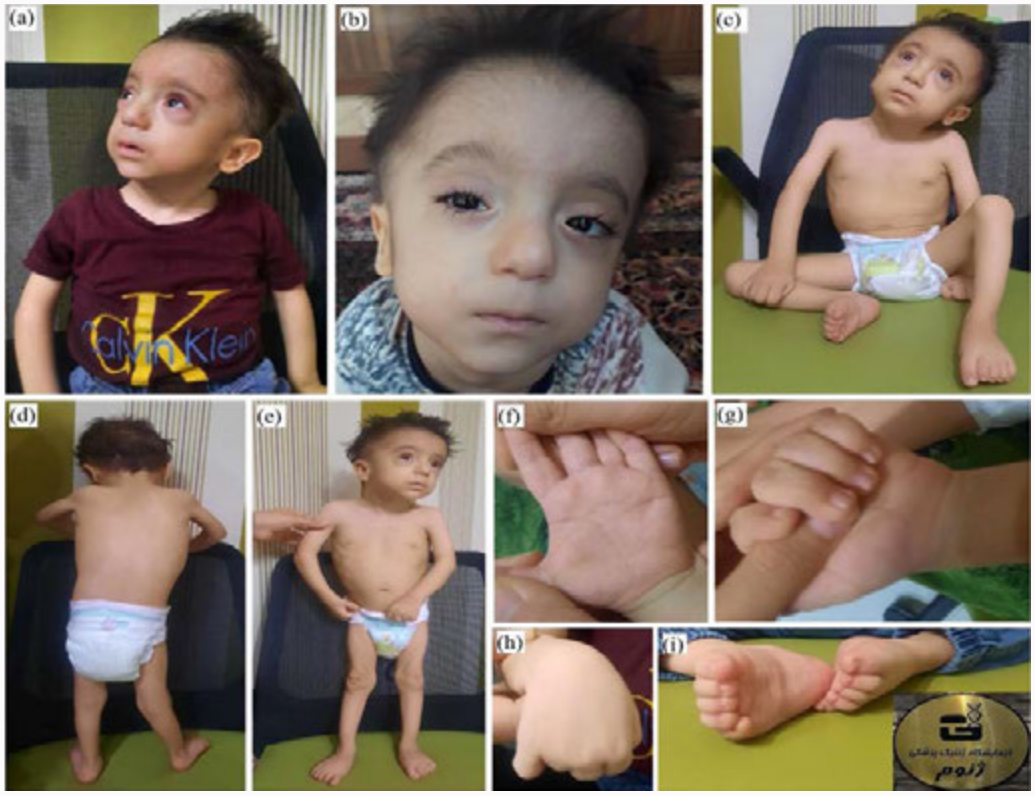

Figure 1: Clinical images of the patient. The pictures were taken at the age two and a half years showing jaw contracture, sparse scalp hair, smooth philtrum, Hypertelorism, Hirsutism, Strabismus, broad nasal tip, low-set ears (a-e), wide internipple distance (c, e), knee contracture (e), overlapping fingers (i), tightly fisted hands (c, g, h) and abnormal creases of palm (f).

are then combined to create a comprehensive 3D structure. Molecular simulations and modeling approaches are employed for further refinement to improve stability and optimize energy. The generated model was visualized using the UCSF Chimera software (https://www.cgl.ucsf.edu/chimera/).

Conservation analysis

We utilized the Clustal Omega online service to examine the evolutionary conservation of the amino acid sequence of the KLHL7 protein. We aimed to assess the protein sequence and determine if it is conserved across different species. We obtained KLHL7 protein sequences from various species, including *Homo sapiens* (humans), *Bos Taurus* (domestic cattle), *Canis lupus familiaris* (dogs), *Pan troglodytes* (chimpanzees), *Macaca mulatta* (rhesus macaque), *Mus musculus* (house mice), *Gallus Gallus* (red jungle fowl), and *Xenopus tropicalis* (tropical clawed frog), from the NCBI. Clustal Omega was employed to align these sequences properly.

Result

Genetic results

WES was performed on the patient, taking into account their individual characteristics and the initial diagnosis as stated. Through genetic analysis, a novel missense variant in the KLHL7 gene was found in the patient: c.254T>G (p.Val85Gly) NM_001367178.1. This particular variant has been linked to the occurrence of PERCHING syndrome.

Co-segregation

In order to confirm the existence of the variant and determine its pattern of inheritance, Sanger sequencing was performed on the proband and his parents. The examination confirmed that both parents carried the variant in a heterozygous state, while the proband was homozygous for the identified variant. Figure 2b, 2c show the sequencing chromatograms and a visual depiction of the variant that was discovered.

Protein Stability and Pathogenicity

The variant c.254T>G (p.Val85Gly) is novel and has not been previously recorded. To evaluate its effect on the stability of the protein and its potential for causing disease, we utilized several prediction tools. The findings from the analysis using tools such as ACMG, I-Mutant, MUpro, SIFT, CADD, Mutation Taster, panther, Polyphen-2 and FATHMM are presented in Table 1.

3D Structure

Figure 3 presents the three-dimensional arrangement of the KLHL7 protein, encompassing both the natural form and the altered form containing the p.Val85Gly variation.

Conservation study

The Clustal Omega online tool was used to align the KLHL7 protein sequence across different species, and it revealed that the amino acid 85 (Valine), which is the point of variation, is highly conserved in all the species studied. This discovery indicates that this particular residue has played a crucial role in the evolutionary and functional history of the protein. The findings are emphasized in Figure 4.

Protein interaction network

We utilized the String tool to identify the proteins that closely interact with KLHL7. The closest interactors, referred to as the

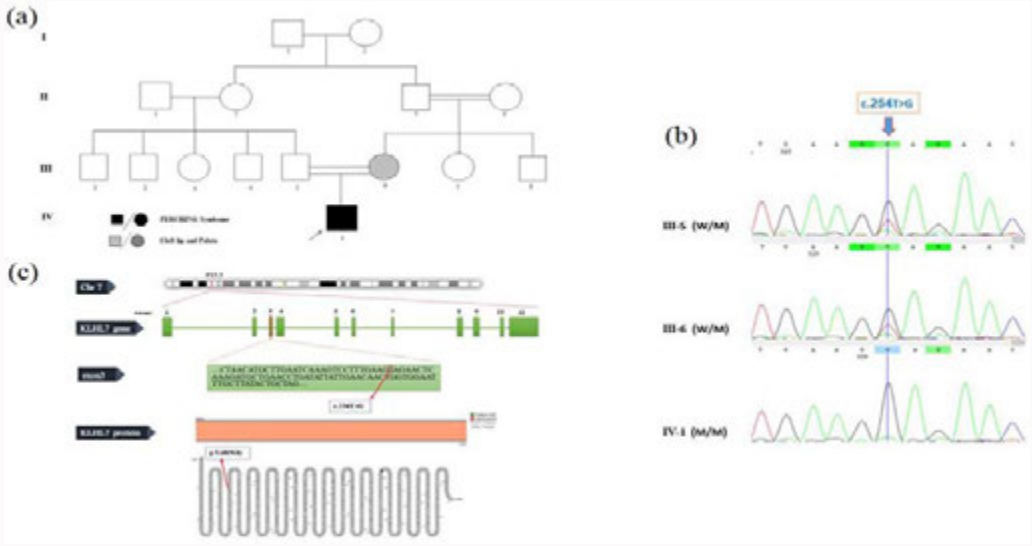

Figure 2: (a) The pedigree diagrams depict an Iranian family that is impacted by PERCHING syndrome, which is linked to the KLHL7 gene. (b) The sequencing chromatograms showcase the genetic information of the proband and their family members. (c) A graphical representation highlights the precise location of the identified variant within the KLHL7 gene.

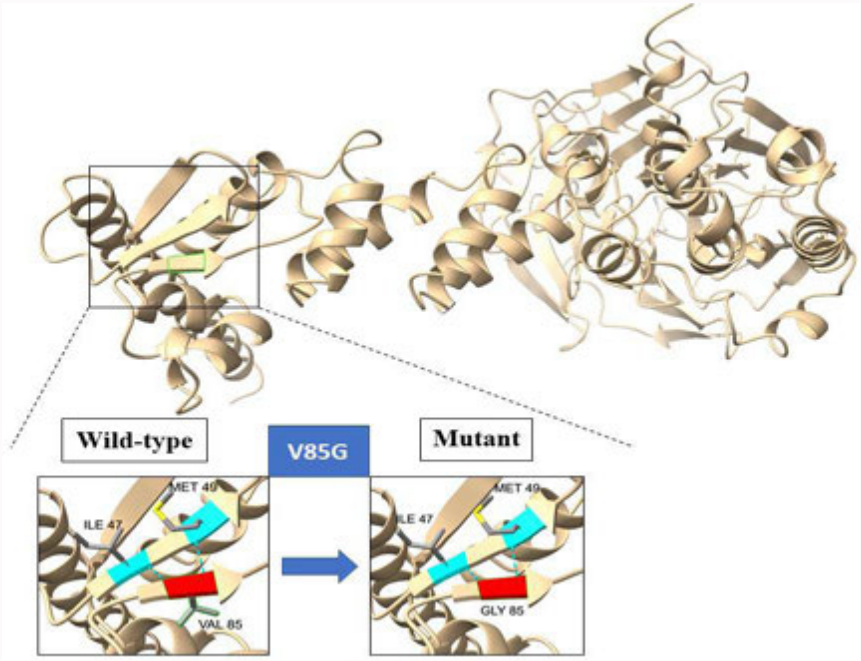

Figure 3: Tertiary structure of wild-type, and mutant forms of KLHL7 protein.

first shell interactors, are displayed in Figure 5 and consist of the following proteins: CUL3, RBX1, KLHL9, GAN, ENC1, KLHL12, KLHL13, KLHL20, KLHL21, and KEAP1. Among these proteins, it is predicted that CUL3 has the highest level of interaction with KLHL7, with a score of 0.985. Additionally, there are second shell interactors, including NEDD8, UBE2M, CRBN, DDB2, VHL, SKP1, ELOB, SKP2, CDC34 and SPOPL.

Discussion

This report presents the initial clinical description of the PERCHING syndrome observed in an Iranian patient. This disorder is classified as a highly uncommon autosomal recessive condition, with 19 additional cases reported. Notably, the majority of these cases have been identified among individuals of Turkish descent [1-5,10-13].

The observed characteristics in our patient align with the documented presentations in PERCHING patients for P (typical resting posture), for E (feeding difficulties), for R (respiratory distress and recurrent infections), for C (jaw contracture, joint contracture, Camptodactyly), for I (failure to thrive, Birth weight 3.200 kilos Now (2 and a half years) 8 kilos, global developmental delay, seizures), for G (microcephaly, long face, down-slanted palpebral fissures, broad nasal tip, smooth philtrum, tented upper lip, narrow mouth, macrotia, broad inferior crus of antihelix at left, underdeveloped inferior crus of antihelix of the right ear) and additional phenotypic manifestations

Figure 4: The Clustal Omega service was utilized to conduct an evolutionary conservation analysis of the KLHL7 protein, with specific emphasis on the identified variant (p. Val85Gly) identified in this study.

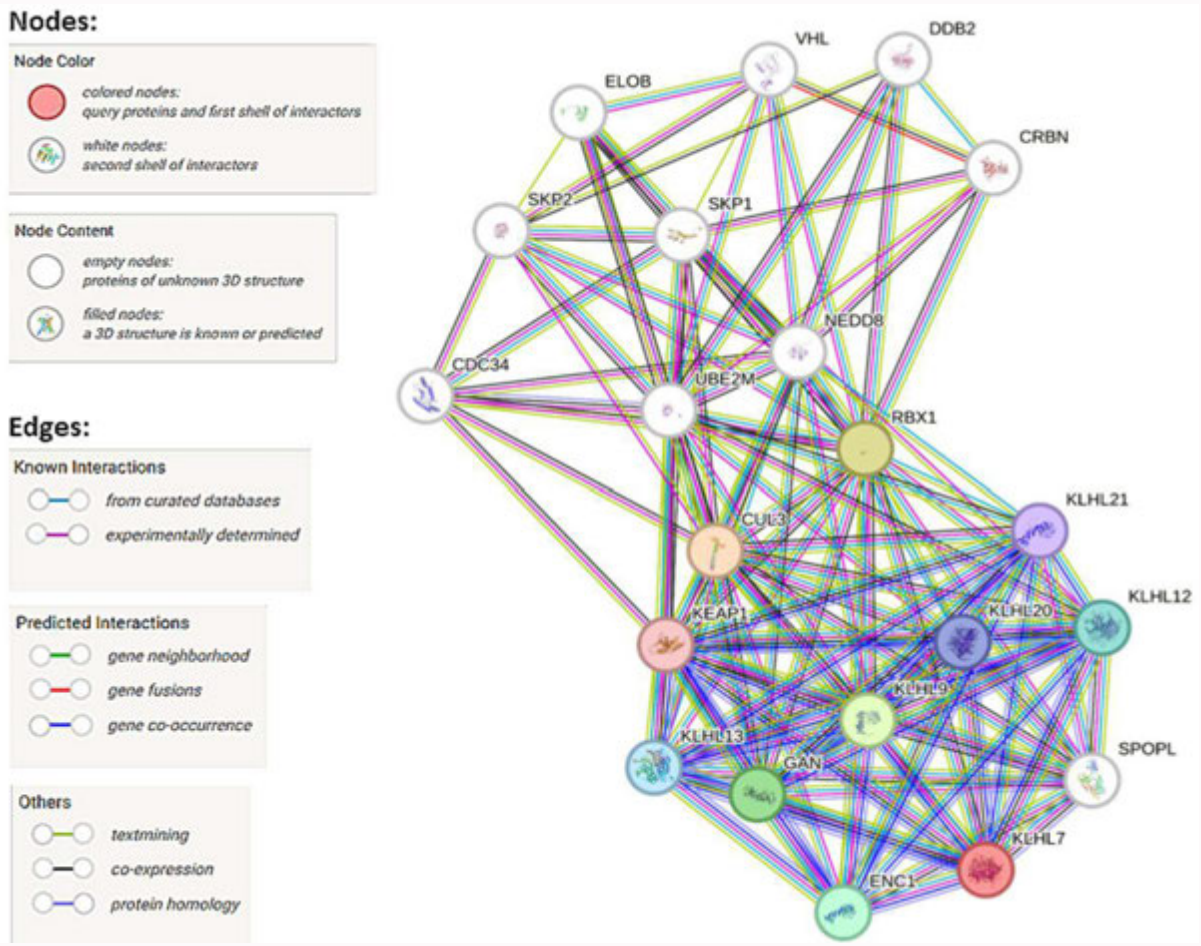

Figure 5: The outcomes derived from the analysis of protein-protein interactions carried out using the STRING database.

(abnormal palmar crease, overlapping toes, sparse scalp hair and hyperthermia). Regrettably, we could not carry out ophthalmologists' assessments and imaging evaluations to examine eye and brain abnormalities in our patients due to financial limitations. Moreover, this instance serves as a clear example of how neonatal distress, a common occurrence in low- and middle-income countries, can mask the phenotypic traits associated with congenital disorders. Genetic investigations must include these patients and not exclude them. Furthermore, insufficient training in dysmorphology may contribute to the under diagnosis of some instances. Owing to the paucity of cases, there is a lack of information about the prenatal manifestations of PERCHING syndrome. It is noteworthy that, to the best of our knowledge, there are no published reports of PERCHING syndrome being diagnosed prenatally. In this research, we present a novel discovered autosomal recessive missense variant in the BTB domain (situated between residue positions 44 and 111) of the KLHL7 gene within a non-consanguineous Iranian family. The BTB domain, also called the POZ (Poxvirus and Zinc finger), is a well-known protein-protein interaction motif. It is commonly found at the N-termini of various C2H2-type transcription factors and Shaw-type potassium channels. The structural analysis of this domain has revealed a tightly intertwined dimer, which is formed through interactions between the N-terminal strand and helix structures. However, in certain BTB/POZ domains, these two secondary structures seem to

Table 1: Results of in Silico analysis conducted using predictive algorithms for the c.254T>G variant in KLHL7.

Gene	Mutation	Protein	ACMG	Sift	CADD	Mutation Taster	Polyphon-2	panther	MUpro	I-Mutant 2	FATHMM
KLHL7	c.254T>G	p.V85G	Uncertain significance	Damaging	26.9	Disease causing	Probably damaging (score: 0.996)	Probably damaging (Pdel: 0.74)	Decrease (DDG: -2.54)	Decrease (-2.37)	Tolerated (score: -0.48)

be absent. SMART, a bioinformatics tool, predicts the presence of BTB/POZ domains even in the absence of the beta1- and alpha1-secondary structures. A significant number of BTB proteins are considered to be transcriptional regulators, hypothesized to exert their regulatory functions by modulating chromatin structure. The X-ray crystallography technique has been utilized to determine the structure of the BTB domain of the PLZF protein, which is associated with promyelocytic leukemia. The structure of the protein reveals a tightly intertwined dimer with a significant hydrophobic interface. Additionally, a groove that is exposed on the surface and lined with conserved amino acids is formed at the dimer interface, indicating a peptide-binding site's presence. The mutated residue is located this domain that is important for binding of other molecules and in contact with residues in a domain that is also important for binding. The mutation might disturb the interaction between these two domains and as such affect the function of the protein. In addition, the c.254T>G variant in the KLHL7 gene by applying a missense change leads to the substitution of the highly conserved amino acid Valine (nonpolar) at protein position 85 to Glycine (polar uncharged) and changes in the protein structure. The mutant amino acid is less hydrophobic and smaller than the wild type. This difference in hydrophobicity can affect hydrophobic interactions with membrane lipids, and differences in amino acid size may result in loss of interactions. Glycine's are very flexible and can disturb the required rigidity of the protein at this position.

In conclusion, additional experiments are required to gain a deeper understanding of how the KLHL7 gene contributes to the PERCHING syndrome. Crucially, these discoveries not only enhance the accuracy of diagnoses but also offer valuable knowledge in the realms of genetic and neurological research. Conducting functional analysis would validate the significance of the gene variant, ultimately enhancing medical care, genetic guidance, and preventive measures for individuals and families who are susceptible to this condition.

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