Novel De Novo *TBL1XR1* Variant Causing PIERPONT Syndrome in an Indian Child: A Case Report and Genotype–Phenotype Review of Reported Patients

Shruti Bajaj[1] Pradnya Gadgil[2] Venu Seenappa[3] Phani N. Setty[3] Vaishali Joshi[2] Shailee Shah[2]

[1] Department of Clinical Genetics, The Purple Gene Clinic, Mumbai, Maharashtra, India
[2] Kokilaben Dhirubhai Ambani Hospital and Medical Research Centre, Mumbai, Maharashtra, India
[3] LifeCell International Pvt. Ltd., Chennai, Tamil Nadu, India

Address for correspondence Shruti Bajaj, MD, Department of Clinical Genetics, The Purple Gene Clinic, Mumbai 400064, Maharashtra, India (e-mail: drshru.a@gmail.com).

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Abstract

The transducin β-like-1 X-linked-receptor-1 gene (*TBL1XR1*) encodes for the TBL1XR1 protein which is involved in transcription. Single-nucleotide variants (SNVs) in the *TBL1XR1* gene have been reported to be associated with Pierpont's syndrome (PS) which exhibits numerous features including global developmental delay (GDD), intellectual disability (ID), varying neurobehavioral and psychiatric manifestations with/without autism spectrum disorder (ASD), abnormal fat distribution in the distal extremities, short stature (SS), head circumference abnormalities, hearing loss (HL), and facial dysmorphisms. Eight PS patients, having a de novo mutation resulting in p.Tyr446Cys, showed no manifestations of ASD. The three other PS patients, having mutations resulting in p.Tyr446His, p.Cys325Tyr and p.Gly237Asp, respectively, and without the p.Tyr446Cys alteration, were in addition associated with neurobehavioral abnormalities, including ASD, hyperactivity, and self-mutilation tendencies. Here, via trio whole exome sequencing, we describe a 12th PS patient, the first from the Indian subcontinent, reflecting a novel *TBL1XR1* p.His348Arg alteration. The proband is a 4.5-year-old male having GDD, speech delay, facial dysmorphisms, abnormal digital fat pads, hypotonia, microcephaly, patent ductus arteriosus, and ASD features. Our report strengthens the hypothesis that *TBL1XR1* variants coding for the TBL1XR1 protein other than p.Tyr446Cys might be more commonly associated with a neurobehavioral phenotype and autistic tendencies.

Keywords
- next-generation sequencing
- developmental delay disorders
- autism
- facial dysmorphism
- genotype–phenotype correlation

Introduction

The transducin β-like-1 X-linked-receptor-1 gene (*TBL1XR1*) encodes for the TBL1XR1 protein which plays a pivotal role in gene transcription.[1–6] A varied spectrum of variants has been described in *TBL1XR1*, ranging from microdeletions to single-nucleotide variations (SNVs; missense, frameshift, and nonsense).[5,6] Variants in *TBL1XR1* cause a pleiotropic spectrum of overlapping disorders, occurring in varying combinations of the following features: global developmental delay (GDD), nonsyndromic and syndromic intellectual disability (ID), neuropsychiatric and behavioral issues, seizures, facial dysmorphisms, tone abnormalities, and growth delays.[6,7] Among these, a recurrent, de novo, missense variant in

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TBL1XR1 has been described as being associated with a distinct syndrome called as Pierpont's syndrome (PS).[2]

PS is an ultrarare genetic disorder with just 11 cases reported in literature.[1–6] It is typified by growth and cognitive delays, hearing loss (HL), facial dysmorphisms, and abnormal fat distribution in the distal limbs.[1] Between 2016 and2017, eight unrelated individuals with PS were reported; all associated with a recurrent, de novo, missense, SNV; p.Tyr446Cys in *TBL1XR1*.[2–4] Later, three additional cases of PS, each associated with a variant other than p. Tyr446Cys were reported.[5,6] Analyzing the genotype and phenotype of 10 cases of PS (8 with p.Tyr446Cys and one each p.Tyr446His and p.Cys325Tyr), Quan et al postulated that the non-p.Tyr446Cys variant in *TBL1XR1* is more commonly associated with neurobehavioral and autistic spectrum.[7] This thought was backed by another recent report (2021), describing the association of ASD features in PS and non-p.Tyr446Cys missense variant in *TBL1XR1*, viz, p. Gly237Asp.[6]

We report a novel case of PS with features of ASD in addition to the typical spectrum without HL, deep palmar, and plantar grooves. The child was diagnosed by trio whole-exome sequencing (WES) as harboring a de novo, nonrecurrent *TBL1XR1* missense variant. This report expands the genotypic and the phenotypic spectrum of PS.

Clinical Details

A nonconsanguineous couple of South Asian descent presented to our clinic with developmental concerns about their male infant. The proband was delivered electively at full term, following an uneventful antenatal period by caesarean section in view of breech presentation with a birth weight of 2.8 kg. The APGAR score were normal. He underwent device closure for patent ductus arteriosus (PDA) at 7 months of age in view of recurrent chest infections and failure to gain weight.

Motor-predominant developmental delay was noted by 6 months age of age. On assessment, he had no head control and inability to roll over. He was able to fix and follow, as well as had developed eye contact. His growth parameters plotted on the World Health Organization chart were: weight: 4.5 kg (<-3Z score), length: 64 cm (between 0 and -2Z score), and head circumference: 39 cm (-3Z score) at 4.5 months. He had dysmorphic facial features; microcephaly, brachycephaly, fleshy, low-set and posteriorly rotated ears, bulbous nose with short columella, esotropia, elongated philtrum, thin vermilion of the upper lip, microretrognathia, and small widely spaced teeth. He also had striking fetal fat pads on the hands and the feet. He was hypotonic with preserved deep-tendon reflexes.

He started undergoing neurodevelopmental therapy, specifically physiotherapy. By 15 months of age, his social interaction improved in the form of good eye contact, social smile, and responsive laughing. By 2.5 years of age, he was able to pull to stand, as well as stand with support. However, his social interaction seemed to regress; his eye contact was poor and he would not follow gaze. He had not developed any nonverbal communication skills.

By 4 years of age, motor improvement continued; he started walking independently with a broad-based gait. However, he seemed to have developed autistic features. Eye contact continued to be poor. He had repetitive behaviors. He had virtually no communication skills, neither verbal nor nonverbal. The Childhood Autism Rating Scale-II (CARS II) was conducted. It is a 15-item behavioral rating scale developed to identify children with autism and to categorize these behaviors on the basis of severity from mild to severe. CARS II is robust diagnostic tool for ASD in children more than 2 years of age, recommended by the Centre for Disease Control and Prevention (CDC).[8] CARS II has been proven to be a helpful tool in this regard irrespective of the underlying developmental delay in the child.[9] Results in our case, indicated presence of severe symptoms of ASD (raw score: 35.5 and percentile: 38). The family was sensitized to this slow, but worrying autistic regression and occupational therapy was introduced. In about 6 months, he showed significant improvement. He regained consistent eye contact and a social smile. He developed stranger anxiety. He developed gestures and started indicating for the toilet.

Ophthalmic evaluation at 3 years indicated compound hyperopic astigmatism. The following investigations tested normal: complete blood count, thyroid function tests, magnetic resonance imaging of the brain, electroencephalogram, visual evoked potentials, electroretinogram, brainstem evoked audiometry response, fluorescence in situ hybridization to rule out William's syndrome, chromosomal microarray, and karyotype.

We suspected an underlying genetic causation for the child's syndromic GDD with ASD. When the child was nearly 4.5 years old, we ordered trio (i.e., proband and both parents) WES with copy-number-variant (CNV) coverage. The WES test includes sequencing of the human core exome on the Illumina next-generation sequencing (NGS) platform. Sequencing is done at mean depth of 80–100X and more than 90% of the bases in the exome are covered at 20X depth. The test identified a missense variant (NM_001321193.1:c.1043A$>$G; p.His348Arg) in heterozygous state in *TBL1XR1* gene. This variant was classified as likely-pathogenic by the American College of Medical Genetics (ACMG) guidelines which satisfies the definition of PS2 (de novo with confirmed biological parenthood), PM2 (rare), PP2 (constraint $Z=4.2$), and PP3 (predicted damaging by multiple algorithms)[10] (**Supplementary Material S1**; available in the online version). Further segregation of this variant in the parents confirmed its de novo status as parents were found to be negative for this variant. Subsequent analysis revealed that the variant was not reported in the 1000 Genomes database (*https://www.internationalgenome.org*) neither in gnomAD nor in the general South Asian population. This confirmed the diagnosis of PS (OMIM no.: 602342) in the proband, known to follow an autosomal dominant inheritance pattern. The distinctive syndromic profile in our patient matched the

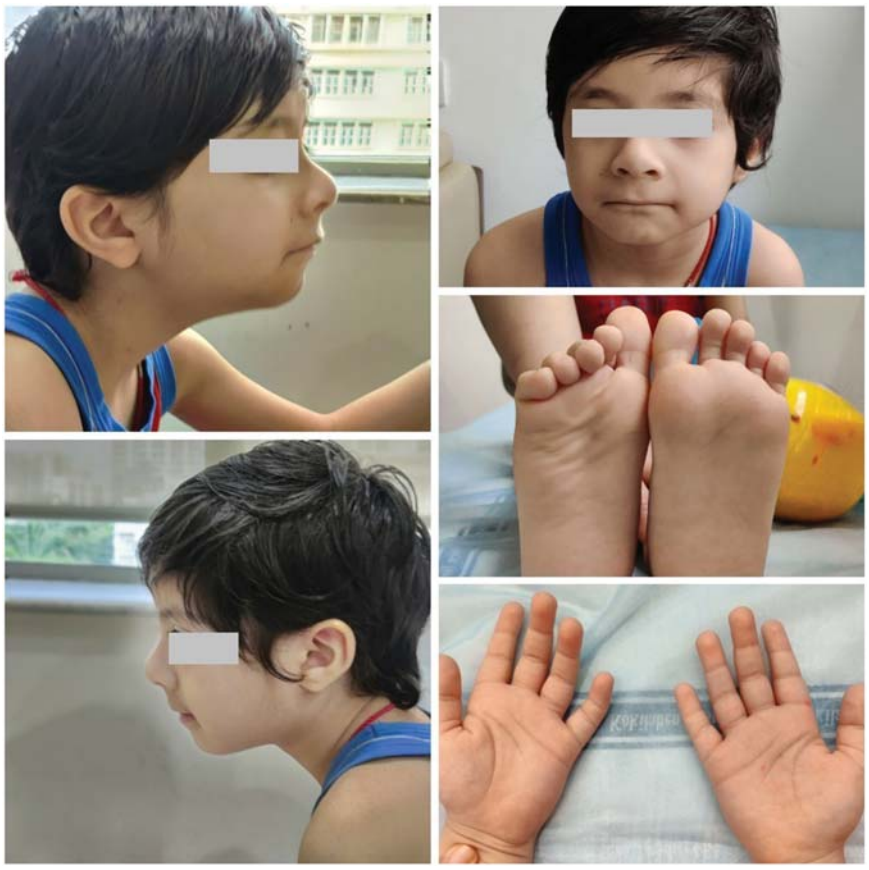

Fig. 1 Clinical photographs of the child. Note the following features: brachycephaly, fleshy, low-set and posteriorly rotated ears, bulbous nose with short columella, esotropia, elongated philtrum, thin vermilion of the upper lip, microretrognathia, and fetal fat pads on the hands and the feet.

syndrome diagnosed (►**Fig. 1**). The pictures are shared herewith after due consent from his parents.

The precise molecular diagnosis did not alter the existing line of management. It, however, provided the family a closure regarding the etiology. They were reassured that the risk of recurrence of PS in the proband's siblings is <1%,[11] assuming a remote possibility of germline mosaicism for the *TBL1XR1* variant. The available reproductive options were explained to the parents, with regard to future pregnancies.

Discussion

PS, an ultrarare genetic disorder, is typically associated with growth and cognitive delays, HL, facial dysmorphisms, and abnormal fat distribution in the distal limbs.[1,2] It was first clinically described in 1998 in two unrelated boys by Pierpont et al,[1] although its genetic elucidation became clear only in 2016.[2]

On reverse phenotyping (reevaluation of the patient after the genetic report), we appreciated our patient to have features of PS[1–6] including distinct crescent moon-shaped eyes when the child smiled. Our patient however did not have HL and the classic deep palmar and plantar grooves described in other case reports of PS (►**Table 1**). It is appreciated in the review of the cases reported so far (►**Table 1**) that PS is associated with phenotypic variability; the core features described by Pierpont et al are not necessarily present in all the cases, and vice versa (i.e., expansion of phenotype as more cases get elucidated).

The missense variant (NM_001321193.1:c.1043A > G and chr3:176756105T > C) detected in this case is located at the C-terminus tryptophan-aspartic acid (W-D) domain of the TBL1XR1 protein. This domain comprises a total of eight WD40 repeats and the detected variant is found within the fifth WD40 repeat region. With respect to PS, this is the first reported variant in this domain (►**Fig. 2A and B**). The Combined Annotation Dependent Depletion (CADD) score of the detected variant is 26.3 and the Missense Variants Z-Score for the *TBL1XR1* gene is 4.2. The higher/positive *Z*-scores indicate the transcript is more intolerant to variation (increased constraint) and therefore the gene had fewer missense variants than expected. In silico computational analysis to assess the variant penetrance effect by bioinformatic tools, such as BayesDel_addAF, DANN, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MVP, Mutation Assessor, MutationTaster, PrimateAI, and SIFT, predicted a damaging effect of the reported c.1043A > G variant in *TBL1XR1*. Protein–protein interaction analysis revealed primary association of TBL1XR1 with multiple proteins, such as NCOR1,

Table 1 Comparison of genotype and the phenotype of Pierpont syndrome cases reported to date[2–6]

Serial number	Age (y), sex	Phenotype	Genotype (*TBL1XR1*)	Reference
1	28, M	DD, FD, HL, SS, hypotonia, abnormal distal fat distribution[a]	p.Tyr446Cys	2
2	20, M	DD, FD, HL, SS, hypotonia, abnormal distal fat distribution[a]		
3	12, M	DD, FD, HL, SS, hypotonia, abnormal distal fat distribution[a]		
4	5.7, M	DD, FD, HL, SS, hypotonia, abnormal distal fat distribution[a]		
5	10, F	DD, FD, HL, SS, hypotonia, abnormal distal fat distribution[a]		
6	19, F	DD, FD, hypotonia, SS, abnormal distal fat distribution[a]		
		No HL		
7	3.4, M	DD, FD, hypotonia, abnormal distal fat distribution[a]		3
		No HL Additionally present- cryptorchidism, microphthalmia, nystagmus		
8	7, M	DD, FD, hypotonia, abnormal distal fat distribution[a]		4
		No HL Additionally present- submucous CP, hydronephrosis		
9	14, M	DD, FD, hypotonia, SS, abnormal distal fat distribution[a]	p.Tyr446His	5
		No HL Additionally present- autistic features, hyperphagia, sleep disturbances, aortic valve dysplasia, mitral valve prolapse		
10	21, F	DD, FD, hypotonia, deep plantar and palmar grooves (no fetal or subcalcaneal fat pads)	p.Cys325Tyr	
		No HL Additionally present hyperactivity, hyperphagia, self-mutilation, sleep disturbances, unmotivated laughing and crying episodes		
11	4, M	DD, FD, hypotonia, SS, fetal fat pads on fingers and toes	p.Gly237Asp	6
		No deep plantar and palmar grooves Additionally present genitourinary abnormalities: (glandular hypospadias, crossed left renal ectopia, reduced renal function, both kidneys showed vesicoureteral reflux) and ASD		
12	4.5, M	DD, FD, hypotonia, SS, fetal fat pads on fingers and toes	p.His348Arg	Current case
		No HL, deep palmar/plantar grooves Additionally present ASD, PDA, compound hyperopic astigmatism		

Abbreviations: ASD, autism spectrum disorder; CP, cleft palate; DD, developmental delay; F: female; FD, facial dysmorphisms; HL, hearing loss; M, male; PDA, patent ductus arteriosus; SS, short stature.
[a]Deep palmar and plantar grooves/pillowing, marked fetal finger and toe pads, and subcalcaneal fat pads.

HDAC1/2/3, GPS2 (nuclear receptor corepressors complex, deacetylation process), CTNNB1 (cell adhesion and signaling protein), and CDC5L (cell cycle regulator; ►**Fig. 2C**; *String. org*).

We performed protein modeling to check the impact of mutant *TBL1XR1* variant. Although the overall protein confirmation remained intact, interestingly, it was noted that the backbone of the mutant protein (►**Fig. 2D**) was minimally influenced by the missense variant in concern, wherein a hydrogen bond was lost with an increased single turn in comparison to wild type protein (►**Fig. 2E**). We postulate that the detected variant in our case resulted in arginine replacing the original histidine within the protein backbone. Arginine methylation is a posttranslational modification which can potentially result in altered protein–protein interaction, impacting normal protein function.[12–16] Another possibility is that the variant in concern, being close to the intron–exon boundary, may possibly create aberrant splicing due to the creation of a GT donor splice site.[10–14] It is also food for thought whether similar altered protein–protein interactions involving arginine, in other genes alike, could be an important pathomechanism clue for other cases of syndromic and nonsyndromic autism.

Thus, the current study expands the genotypic and the phenotypic landscape of Pierpont's syndrome, while

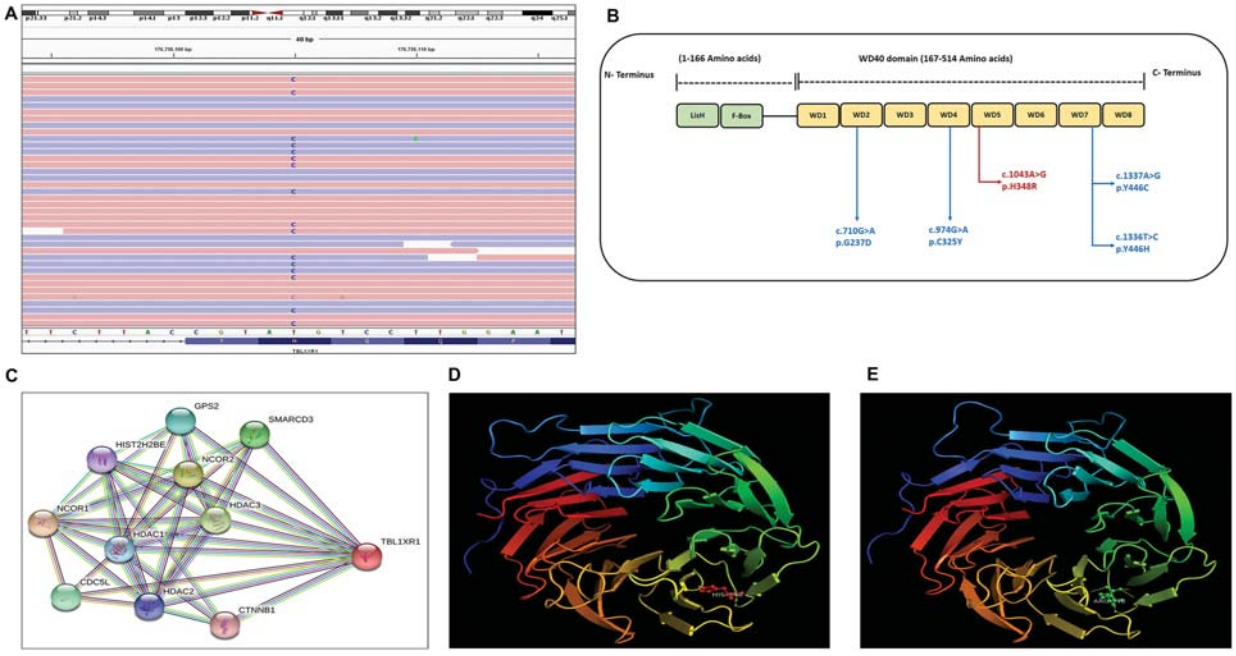

Fig. 2 Integrative genomics viewer (IGV) image of the detected variant, schematic representation of the protein domains, protein–protein interaction map and protein models image of the *TBL1XR1* gene: **(A)** A heterozygous missense variant c.1043A > G (chr3:176756105T > C) in *TBL1XR1* gene is shown in the above image for child against human genome reference sequence GRCh37/hg19. **(B)** TBL1XR1 protein domains (Lish, F-Box and WD) and previously reported variants in association with Pierpont syndrome is depicted in blue lines which are distributed across different WD domains, and the current case variant is depicted in red line in 5th WD domain. **(C)** Protein–protein interaction map showing the primary association of TBL1XR1 protein with nuclear receptor corepressors complex proteins, deacetylation process proteins, cell cycle, cell adhesion and cell signaling proteins. Protein interaction map was generated using String.org. **(D, E)** The protein model was built using Swiss model for both wild **(D)** and mutant **(E)** variant using PDB 4LG9 structure obtained from *Uniprot.org*. The visualization and comparison of the models built (Wild & Mutant) was performed using PyMol and Rasmol respectively. The wild-type protein showed 231 hydrogen bonds and number of turns 39, whereas the mutant protein showed 230 hydrogen bonds with a loss of one hydrogen bond and number of turns increased to 40 without any changes in number of strands. Wild type protein amino acid Histidine is showed in red color **(D)** and Arginine amino acid is shown is green color **(E)**. CDC5L, cell division cycle 5 like; CTNNB1, catenin β 1; GPS2, G protein pathway suppressor 2; HDAC1/2/3, histone deacetylase 1/2/3; NCOR1, nuclear receptor corepressor 1; SMARCD3, SWI/SNF-related, matrix associated, actin dependent regulator of chromatin, subfamily D member 3.

highlighting the variability associated with it too. It also proposes likely pathomechanisms explaining the deleterious effect of the detected missense variant.

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Conflict of Interest
None declared.

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