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A pathogenic *COL7A1* variant highlights semi-dominant inheritance in dystrophic epidermolysis bullosa

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Abstract

Dystrophic epidermolysis bullosa is a rare subtype of inherited epidermolysis bullosa, caused by variants in the collagen type VII alpha 1 chain (*COL7A1*) gene (MIM120120). Both autosomal dominant and recessive inheritance has been reported with variable phenotype. We investigated a Pakistani family with dystrophic epidermolysis bullosa via exome sequencing and identified a pathogenic nonsense variant in *COL7A1* NM_000094 c.1573 C>T:p.(Arg525*). The inheritance pattern observed was consistent with a semi-dominant model, where heterozygous parents exhibited a mild phenotype, and homozygous children were more severely affected. For dystrophic epidermolysis bullosa, loss-of-function variants are typically associated with the autosomal recessive form, while missense variants are linked to the autosomal dominant form. A review of the literature suggests a semi-dominance pattern for some missense variants, particularly glycine substitutions, but this concept had not been formally recognized. This study highlights the importance of considering semi-dominant inheritance models for dystrophic epidermolysis bullosa and other Mendelian diseases with an autosomal recessive mode of inheritance, as it can significantly impact diagnosis and genetic counseling.

Introduction

Mendelian diseases are characterized by single-gene inheritance, while non-Mendelian patterns arise from more complex interactions involving genetic, environmental, or epigenetic factors. Even in seemingly straightforward Mendelian cases, considering non-Mendelian models is essential for achieving accurate diagnoses, risk assessments, and providing effective genetic counseling.

Hereditary epidermolysis bullosa (EB), is a wide group of rare genodermatoses characterized by mild to severe fragility of epithelial tissues which extends to erosions and blistering of the skin, skin fragility, milia, crusting and scarring of the skin, and nail dystrophy [1, 2]. Severe scarring on the hands and feet is frequently linked to the onset of aggressive squamous-cell carcinomas that spread quickly, potentially leading to early death [3].

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Based on the level of blistering, target proteins, and clinical symptoms, EB is divided into four broad groups [2], Epidermolysis Bullosa simplex (EBS) [1] junctional Epidermolysis Bullosa (JEB) (MIM226650) [4], dystrophica Epidermolysis Bullosa (DEB)(MIM226600), and Kindler Epidermolysis Bullosa (KEB) [5]. However, there are more than 30 distinct subtypes based on clinical heterogeneity, variant analysis and immunofluorescence mapping [4]. Dystrophic EB (DEB) is caused by variants in *COL7A1* (MIM120120) and affects ectodermal appendages, i.e., skin, hair, nail, and teeth. Segregation shows results with either an autosomal recessive (AR) or autosomal dominant (AD) modes of inheritance. With missense variant mainly associated with AD inheritance patterns and loss-of-function variants with AR disease [6]. For the DDEB, blistering tends to be relatively mild and localized while RDEB is more severe, with widespread blistering that can have complications [7, 8]. Both molecular diagnosis and genotype-phenotype correlations are DEB challenging due to phenotypic heterogeneity, the diverse range of variants which include de novo events.

A large study of 310 families with DDEB and RDEB, found that 48 families (15.5%) had DDEB, which was confirmed by family history or identification of known or de novo variants. The remaining 262 families (84.5%) had RDEB. Notably, a subset of seven patients (2.3%) which were member or RDEB families with unusual intrafamilial phenotypic heterogeneity were found to be compound heterozygous for an AR and AD variant [6]. Furthermore, certain glycine substitutions in *COL7A1* have been reported to cause both AD and AR disease [9].

Type VII collagen is mainly produced by epidermal keratinocytes, with dermal fibroblasts also contributing [10]. It forms a homotrimeric monomer intracellularly and assembles into antiparallel dimers after secretion. These dimers aggregate into anchoring fibrils that connect the epidermal basement membrane to the dermis, ensuring structural integrity [11–13].

EB, which primarily affects children, is observed in all genders and ancestries with an estimated prevalence of about half a million individuals worldwide [14, 15]. Symptoms typically appear at or shortly after birth and persist throughout life, though some may not manifest until early adulthood. Currently, there are no approved treatments for EB; management focuses on symptom control, wound care, and preventing complications, with additional support for feeding difficulties and overall care [2, 16].

We investigated a clinically well-characterized Pakistani family with DEB via exome sequencing, revealing a pathogenic nonsense variant c.1573 C > T:p.(Arg525*) in *COL7A1* that follows a semi-dominant inheritance pattern. For semi-dominant inheritance (also known as incomplete dominance or partial dominance) individuals who are heterozygous have a less serve phenotype than individuals who are either homozygous or compound heterozygous.

Materials and methods

Ethical approval

Institutional review board approval was obtained from Hazara University Mansehra, Pakistan (IF.No.HU/ORIC/2023/1230) and Columbia University New York, USA (AAAS3431). Informed consent was obtained from adult study subjects, and the parents/legal guardians of individuals under the age of 18.

Clinical assessment

The family under study (ED-5) is of Pashtun ancestry was ascertained in khyber Pakhtukhawa, Pakistan (Fig. 1). The consanguineous family has a total of four affected, parents and children. Parents (II:5 and II:6) have a mild phenotype and affected children (III:1 and III:2) are more severely affected. III:1, died a few days of after birth and was therefore not included in the present study. Affected member III:3 also died shortly after birth, but a DNA sample was obtained. All affected members were examined by a local dermatologist (Fig. 2). Phenotypic information for all affected family members is noted in detail (Table 1).

Exome sequencing and bioinformatic analysis

Blood samples were collected from the affected parents (II:5 and II:6) and the affected child (III:2). DNA extraction was done using phenol-chloroform. Genomic DNA of the affected member (III:2) was exome sequenced using the Agilent sure select Human All exon V6 kit (Agilent technologies, Santa Clara, CA, USA). Barcoded libraries were pooled, and sequencing was performed on the Illumina Novaseq6000 with an average targeted depth of 105.66x.

Filtered reads were aligned to GRCh38/ hg38 using the Burrows-Wheeler aligner (BWA) [17]. Picard was used for duplicate removal. Variant calling and quality recalibration were performed using the genome analysis toolkit (GATK) [18]. Variants were annotated using ANNOVAR [19]. The criteria for variant selection included being exonic or within the splice region (± 12 bp) and having a minor allele frequency (MAF) < 0.005 in every gnomAD population [20]. An AR model of inheritance was first considered due to the pedigree structure, but other models were also considered. Copy number variants were also investigated using CoNIFER [21].

Sanger sequencing

The *COL7A1* variant that was detected in the exome sequence data was validated and tested for segregation using DNA samples from all available family members

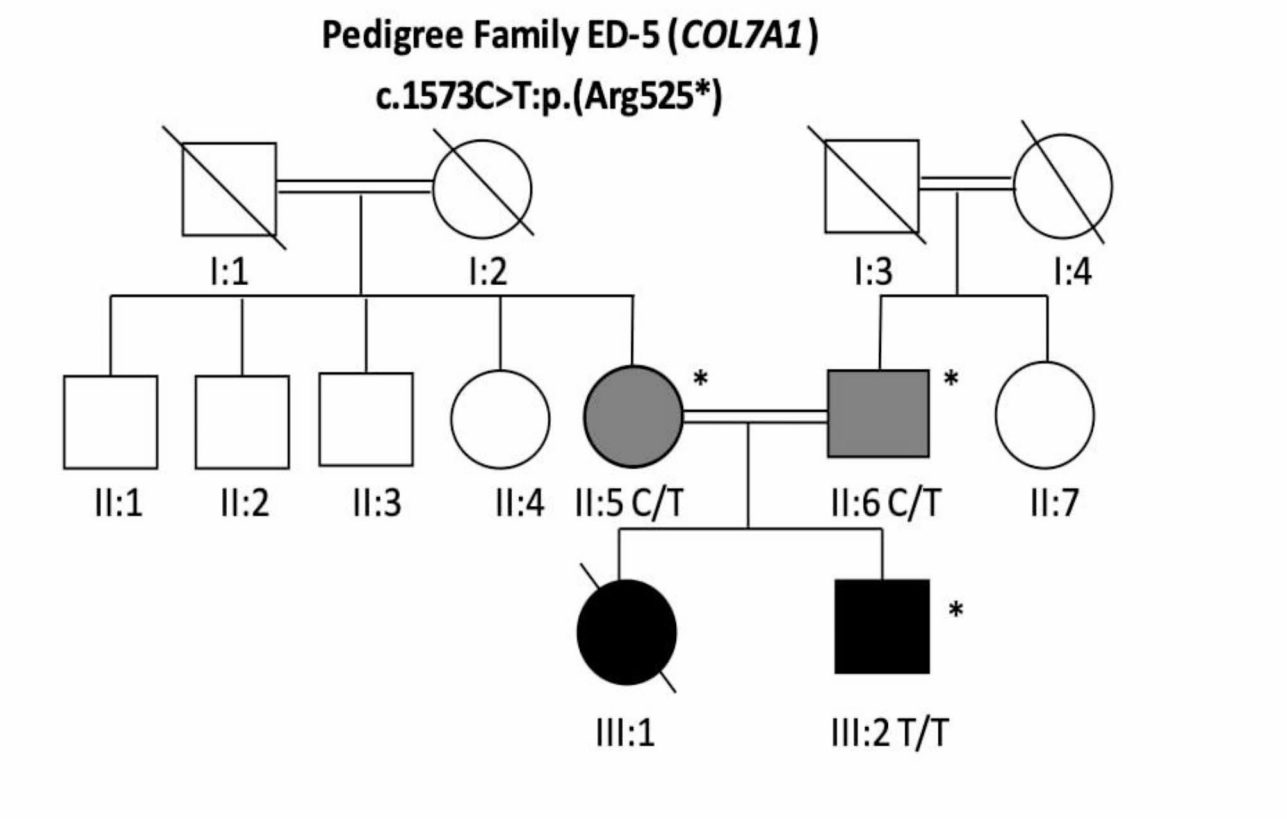

Fig. 1 Pedigree of family ED-5 (*COL7A1*). The consanguineous pedigree with segregation of the *COL7A1* [c.1573 C > T:p.(Arg525*)] variant. Squares indicate the males, circles female and filled black filed symbols represent individuals with severe dystrophic epidermolysis bullosa and grey filled symbols individuals with a milder form of dystrophic epidermolysis bullosa. Double lines indicate consanguinity and individuals with a line through the symbol indicates they are deceased

using Sanger sequencing. Primers were designed for the variant of *COL7A1* gene, where there putatively causal variant laid, using Primer3 [22]. PCR-amplified products were purified and sequenced. The DNA sequences were then aligned to the reference genome sequence using the Codon Code Aligner v7.1.2.

Results

Clinical investigation

An 8-day-old boy (III:2) from the family had a history of abnormal blisters covering his skin, along with erosions and erythema. He also had blisters in his mouth, making feeding difficult. Additionally, he exhibited milia on his hands, nail dystrophy, yellowish crusted lesions on his ears, and clubbed nails (Fig. 2I-L). He died a few days after birth. The child's mother exhibits a mild phenotype, including dry skin, black tartar on her teeth leading to edentulism, decay, and pain. She had several tartar-covered teeth extracted due to the pain (Fig. 2E, F). The child's father has dry skin and nail dystrophy, including clubbed nails, and Beau's lines (Fig. 2A-D).

Exome sequencing

Exome sequencing identified a known pathogenic variant with c.1573 C > T:p.(Arg525*) with CADD score of 36 in exon13 in the *COL7A1* gene, which was tested for segregation. Family members (II:5 and II:6) were heterozygous for the *COL7A1* variant and their child (III.2) carried homozygous alleles. Phenotypic analysis and segregation results indicate a semi-dominant inheritance pattern for the family. The variant was classified as pathogenic according to American College of Medical Genetics (ACMG) guidelines (PVS1, PS1) [23]. The nonsense variant p.(Arg525*) is predicted to lead to a loss-of-function via nonsense-mediated decay [24].

Discussion

EB is a group of genetic disorders that are characterized by mechanically induced blistering and fragility of the skin which includes DEB (MIM 226600) that typically manifests at birth and impacts the skin, nails, teeth and hairs. For DEB there is extensive genetic and phenotypic heterogeneity. Genetic interactions, cellular modifications, and environmental influences may vary phenotype expression [25].

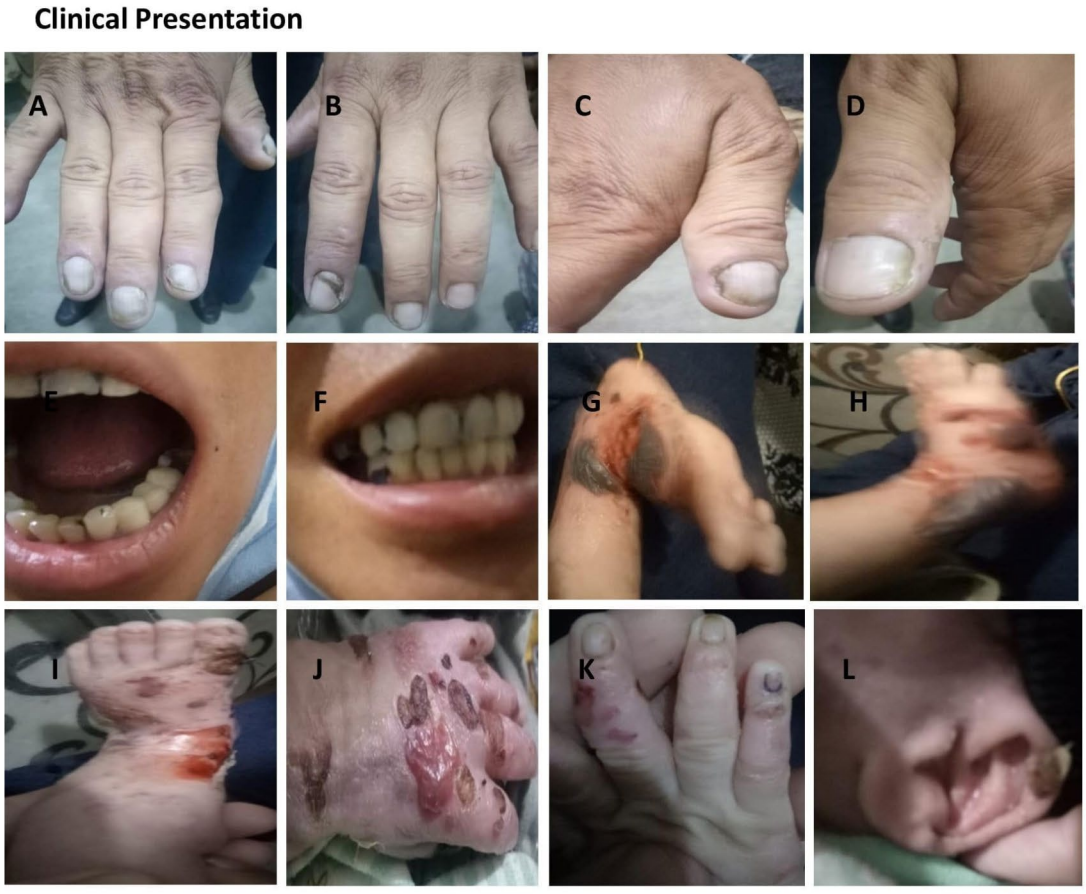

Fig. 2 Clinical presentation. Images (**A**-**D**) show nail dystrophy, Beau's lines, club nails and dry skin of the affected father (II:6), images (**E**) and (**F**) display the black tartar teeth of the affected mother (II:5) and images (**G**-**L**) show the phenotypes of affected child (II:2), image (**G**-**I**) shows blistering all over the left and right foot, erosions and erythematous skin, dryness, image (**J**) dryness and blisters on hands image (**K**) shows nail dystrophy and club nails of affected child, image (**L**) shows skin dryness, milia, and a yellowish crusted lesion present on the ear

Anchoring fibrils, composed of collagen VII, attach the epidermal basement membrane to the underlying dermal connective tissue and are synthesized by epidermal keratinocytes as procollagen VII, which is then processed into functional collagen VII [11, 26, 27]. This collagen VII undergoes proteolytic trimming, forms disulfide-bonded antiparallel dimers, aggregates into anchoring fibrils, and interacts with laminin 5 to stabilize dermo-epidermal adhesion, with further stabilization provided by cross-linking via transglutaminase-2 [28]. While severe forms of DEB may lack both collagen VII and anchoring fibrils, milder forms exhibit altered fibril morphology with presences of collagen VII [26]. Variants in *COL7A1*, that encodes collagen VII, are found in both DDEB and RDEB subtypes, leading to varying degrees of skin blistering and scarring. Despite identifying numerous variants, the precise biological consequences and pathogenic mechanisms remain unclear [29, 30].

We studied a Pakistani family via exome sequencing and detected a pathogenic nonsense variant c.1573 C > T:p.(Arg525*) in the *COL7A1* gene that inherits with a semi-dominant mode of inheritance. Semi-dominance, (aka incomplete dominance or partial dominance) is a condition where the phenotype for heterozygote carriers is intermediate between the homozygous or compound heterozygous phenotypes. This raises awareness in the medical community, as it formally acknowledges semi-dominant inheritance in DEB for the first time. This is crucial for accurate genetic counseling, diagnosis, and patient management, particularly in populations where consanguinity may impact the inheritance patterns of rare genetic conditions.

The *COL7A1* nonsense variant, c.1573 C > T:p.(Arg525*), was previously identified in two Pakistani families with severe DEB. They were reported to have a similar phenotype including skin blisters, erosions, erythematous skin, oral blisters, and nail dystrophy [Table 1]

Table 1 Phenotype of individuals with homozygous and heterozygous COL7A1 c.1573 C > T:p.(Arg525*) variants

	This study			Khan et al., 2021	Fozia et al., 2022
Family ID/Patient ID	**ED-5/(II:5)**	**ED-5/(II:6)**	**ED-5/(III:2)**	**Family7/(III:6)**	**Family C/(V-2)**
Zygosity status	0/1	0/1	1/1	1/1	1/1
Phenotypes					
Blisters	-	-	+	+	+
Erosions & erythematous skin	-	-	+	**+**	**+**
Black tartar on teeth\edentulism	+	-	-	-	-
Skin dryness	+	+	+	**-**	**-**
Oral Blisters	-	-	+	+	+
Milia	-	-	+	**+**	**-**
Nail dystrophy (onychogryphosis)	-	+	+	+	+
Yellowish crusted lesion ear	-	-	**+**	**+**	**-**
Beau's lines	-	+	-	-	-
Club Nails	-	+	+	**-**	**-**
Joint contractures	-	-	-	-	+
Severe anemia	-	-	-	**-**	**+**
Growth delay	-	-	-	-	+
Frequent corneal & conjunctival erosions	-	-	-	**-**	**+**
Symblepharon	-	-	-	-	+
Exposure Keratitis	-	-	-	**-**	**+**

Clinical details of the affected individuals and comparison with previously reported cases with the same c.1573 C > T:p.(Arg525*) variant. 0/1: heterozygous; 1/1: homozygous.

Table 2 Variants in COL7A1 consistent with semi-dominant inheritance in additional families

McGrath et al., 2011		**Christiano et al., 1996**
***p*.(Gly1770Ser)**		***p*.(Gly2351Arg)**
Two affected Brothers (1/1)	**Affected distant cousin (1/1)**	**Affected (0/1)+(0/1)***
Skin fragility	Skin fragility	Scarring along the trunk
Skin scarring	Skin scarring	Extremities, face and scalp
Oral erosions	Oral erosions	Synechias (webbing)
Poor wound healing	Poor wound healing	Mutilations of hands
Mother (0/1)	**Mother (0/1)**	**Mother (0/1)**
Inflammation on shins	No reported clinical phenotype	Erosions on forearm
Nail dystrophy		Shading of the toenails
Blistering		
Father (0/1)	**Father (0/1)**	**Maternal grandfather (0/1)**
Nail dystrophy	No reported clinical phenotype	Blisters on hands
		Nail loss

Clinical demonstration of additional families with *COL7A1* variants showing semi-dominant inheritance. 0/1: heterozygous; 1/1: homozygous.

*This case is compound heterozygous with variant p.(Pro1701Argfs*9), inherited from the unaffected father. This second variant does not show a semi-dominant inheritance pattern

[31, 32]. One of the proband's elder brothers had also died eight days after his birth due to the same severe clinical features as was observed in our family, ED-5. However, unaffected heterozygous family members were not reported to have any phenotypes related to DEB in these previous studies. In our study, heterozygous parents show a milder phenotype but with some overlap with the phenotype of the homozygous case, including dry skin, nail dystrophy, and club nails. These mild features may be easily overlooked in clinical investigations of heterozygous individuals believed to be unaffected carriers.

We next performed a review of the literature to determine if potentially other semi-dominant DEB cases have been reported (Table 2). One *COL7A1* variant, p.(Gly1770Ser), was reported as homozygous in two brothers with a severe DEB phenotype, while the phenotype for their heterozygous parents was mild. The father had nail dystrophy without skin blistering, while the mother had nail dystrophy, blistering, and inflammation on her shins, consistent with pretibial DEB. No DEB phenotypes were reported for the parents of a distant affected cousin.

Also reported in the literature was a family with compound heterozygous *COL7A1* variants [p.(Pro1701Argfs*9) and p.(Gly2351Arg)] with a mild to severe DEB phenotypes: severely affected twins that were compound heterozygous for both variants (Table 2). The mother and maternal grandfather who were heterozygous for the missense variant p.(Gly2351Arg) showed a semi-dominant profile, while the father who carried the frameshift variant did not present with any DEB phenotypes. The heterozygous mother had erosions on the forearm and shedding of the toenails while the maternal grandfather had clinically abnormal nail loss and blisters on the hand (Table 2) [33].

Glycine substitutions within the type VII collagen triple helix have previously been reported to lead to either AD or AR phenotypes in unrelated patients, causing challenges in molecular diagnosis. Only investigation of larger families provided the evidence that the variants [p.(Gly1770Ser) and p.(Gly2351Arg)] are inherited with a semi-dominant mode of inheritance. These glycine substitutions can disrupt the biosynthesis of collagen VII in a dominant-negative manner. The biological consequences of substitutions likely depend on the specific position of glycine within the triple helix [9].

This study not only enhances the understanding of DEB but also emphasizes the need to consider a semi-dominant inheritance model. This inheritance pattern might be present in other Mendelian diseases, potentially leading to new insights and more effective management approaches. Expanding research on the phenotypic and genotypic spectrum of DEB is crucial for developing effective clinical and genetic therapies.

Consanguineous marriages significantly increase the likelihood of inheriting rare recessive variants due to shared ancestry, raising the prevalence of conditions like DEB. In Pakistan, where EB is prevalent and consanguineous marriages are common, patients face significant treatment challenges due to limited healthcare resources. This highlights the importance of genetic counseling for DEB families within the specific cultural and social context of Pakistan. Tailoring counseling efforts to address the social norms, perceptions of genetic diseases, and concerns about genetic testing can significantly improve patient understanding and adherence to treatment plans.

In conclusion, this study empowers individuals and families to understand and address the potential genetic implications of cousin marriages, ultimately help in reducing the genetic disorders in future generations, enhances the understanding of DEB but also emphasizes the need to consider complex inheritance patterns in genetic diseases. This approach can lead to better diagnostic accuracy, more informed genetic counseling, and improved patient care.

Abbreviations

BWA	Burrows-wheeler aligner
CADD	Combined annotation dependent depletion
COL7A1	Collagen type VII alpha 1 chain
DNA	Deoxyribonucleic acid
DDEB	Dominant dystrophic epidermolysis bullosa
DEB	Dystrophic epidermolysis bullosa
EB	Epidermolysis bullosa
EBS	Epidermolysis bullosa simplex
JEB	Junctional epidermolysis bullosa
KEB	Kindler epidermolysis bullosa
GATK	Genome analysis tool kit
MAF	Minor allele frequency
PCR	Polymerase chain reaction
RDEB	Recessive dystrophic epidermolysis bullosa

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Author contributions
SS: collect blood sample and obtain consent from the affected family, conducted research, analyzed data and wrote the main manuscript text. UmK is the co-corresponding author, design study, research supervisor. SK: Co-Supervised the research work, IS: is the co-host supervisor, corresponding author and senior board member of BMC Medical Genomics, help in manuscript text, images and table designing and review literature. SL: is the senior host supervisors of the research project, keenly evaluate the whole project and manuscript text, images and tables. AA and TB help in variant selection and lab work. All authors evaluate the manuscript.

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Data availability
The variant was deposited in ClinVar under accession number SCV005328388.

Declarations

Ethics approval and consent to participate
The research study was approved by Institutional review board of Hazara University, Mansehra, Pakistan (IF.No.HU/ORIC/2023/1230) and Columbia University New York, USA (AAAS3431). Informed written consent was taken from participating members of family. Blood sampling from unaffected and affected individuals were carried out according to guidelines provided by Declaration of Helsinki.

Consent for publication
We hereby provide our explicit consent for the publication of our research materials, including clinical details and images, on behalf of patients who have given written consent for publication, subject to approval by the Board of BMC Medical Genomics.

Competing interests
The authors declare no competing interests.

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