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Whole-Exome Sequencing Identified a Novel Mutation in an Iranian Patient with Epidermolysis Bullosa

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ABSTRACT

Background: Epidermolysis bullosa (EB) is a rare, genetically heterogeneous disorder characterized by skin fragility. EB is categorized into four types: simplex, junctional, dystrophic, and Kindler syndromes. The condition is caused by mutations in several genes that are important for skin integrity and dermal-epidermal adhesion. In the present study, we recruited a patient with EB from an Iranian pedigree for genetic evaluation.
Methods: Whole-exome sequencing (WES) and bioinformatics analysis were performed using genomic DNA from the patient (IV-3). The potential variant was confirmed by Sanger sequencing.
Results: We identified a novel likely pathogenic variant in exon 3 of the *COL17A1* gene: c.82dup (p.Thr28Asnfs*5). The patient's parents were heterozygous carriers of this mutation. In silico structural prediction suggested that the variant could cause premature termination of *COL17A1*. This variant is associated with intermediate junctional EB-4 (JEB4).
Conclusion: The study highlights that WES enhances our understanding of genetic diagnosis and it contributes to the expanded mutational spectrum of the *COL17A1* gene associated with JEB. ***DOI: 10.61882/ibj.5232***

Keywords: Epidermolysis bullosa, Exome sequencing, Mutation

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1. INTRODUCTION

Epidermolysis bullosa (EB) is a skin condition that can be inherited through both autosomal dominant and autosomal recessive patterns. It can affect several parts of the body, resulting in a range of symptoms[1]. The clinical manifestations of this disease vary in severity, from minor symptoms such as blisters, ulcers, and skin erosion on the hands and feet to more severe symptoms such as the formation of large mucous blisters at birth[2]. This condition is characterized by exceptionally fragile skin, which cannot withstand even minimal mechanical friction[1]. EB has a global prevalence of approximately 19.6 per million live births[3]. There are four main subtypes of EB: EB simplex, junctional EB (JEB), dystrophic EB, and Kindler syndrome[4].

Diagnosis of EB can be confirmed through several methods, including transmission electron microscopy to examine the skin's ultrastructure and immunofluorescence mapping to assess the degree of skin cleavage. Other methods include targeted next-generation sequencing or whole-exome sequencing (WES) in cases without a precise candidate gene, or when candidate genes have been ruled out. Additionally, direct genetic analysis of genes associated with EB using Sanger sequencing is the recommended approach[2].

Junctional epidermolysis bullosa (JEB) is associated with approximately eight genes that encode essential components of the hemidesmosome-anchoring filament complex. This complex connects the keratin

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cytoskeleton to the dermoepidermal junction lamina. JEB is classified into two main subtypes based on the type and severity of mutations. The first subtype, generalized severe JEB, is characterized by an extremely lethal condition with mucocutaneous blistering, recurrent infections, and a mean age of survival of 5.8 months. The second subtype is JEB generalized intermediate, a genetically heterogeneous group with a limited tendency for blistering, alopecia, and mucosal erosions[5].

In the present study, we conducted a mutational analysis of an Iranian patient with EB using WES.

2. MATERIALS AND METHODS

2.1. Family recruitment

One affected Iranian family member with EB participated in the study. The family pedigree of the patient with EB, which follows an autosomal recessive pattern of inheritance, is shown in Figure 1.

2.2. Whole-exome sequencing

The salting-out method was used to extract genomic DNA from 6 cc of peripheral blood from all subjects. The quality of the DNA extraction was evaluated using 1% agarose gel electrophoresis. Genomic DNA (1 µg) was sheared from the patient (Fig. 1, III-3). The NovaSeq 6000 platform was employed for WES of the proband's genomic DNA using the Twist-v2.0 Kit. The exomes were sequenced to achieve a target coverage of 100×. The sequencing reads were then aligned to the human reference genome using BWA (Burrows-Wheeler Aligner), followed by variant calling using the GATK best practices workflow. The identified variants were annotated using ANNOVAR. Subsequent filtering was performed using the gnomAD, 1000 Genomes, and Iranome population databases. Finally, variant pathogenicity was assessed using the in silico prediction tools, including SIFT, PolyPhen-2, and CADD, as previously described[6]. Sanger sequencing was used to confirm the mutation identified by WES in a patient with EB and its segregation in the parents. Primer design for exon 3 of the *COL17A1* gene and its flanking intronic regions was conducted using the Primer3 plus web-based server (www.primer3plus.com), resulting in the following primers: F-5'GCACAGTGGGTCAGA TCACA3′ and R-5′AGCCCCCAATCCTTGTTCAG3′, with a product size of 595 bp. The ABI 3500XL PE sequencer (Applied Biosystems, CA, USA) was used for Sanger sequencing. Finally, Chromas software was used to analyze the sequence traces. Additionally, Swiss-Model software (https://swissmodel.expasy.org/ interactive) was used to create three-dimensional protein models, as shown in Figure 2.

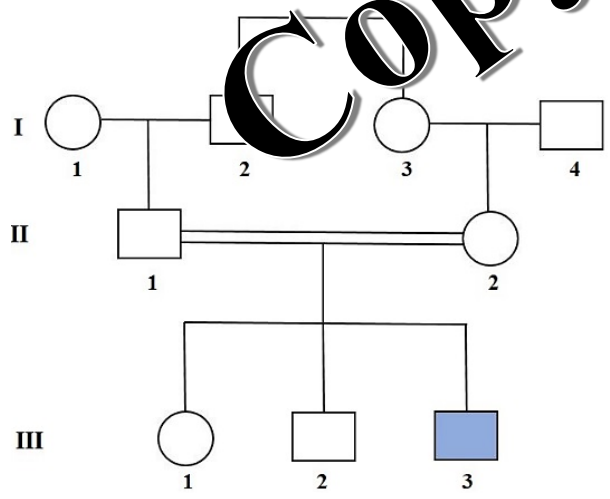

Fig. 1. Pedigree of EB patient. The index case (III-3) was affected with intermediate JEB4. III-3 is affected, and the parents are carriers (II-1 and II-2).

3. RESULTS

3.1. Case presentation

The 14-year-old patient born to a consanguineous marriage, had a history of erosions, blisters, crusted lesions, and hyperpigmentation patches on his face, trunk, and extremities. He also had alopecia, affecting his eyebrows, eyelashes, body hair, and scalp. Additionally, we observed nail dystrophy, fingernail loss (anonychia) and onychia (toenail). After examining his mouth and teeth, we observed excessive dental caries, discoloration, and tooth malformation. The patient also reported a medical history of reduced joint movement, dysphagia, hoarseness, and dry eyes.

3.2. Variant analysis

We identified a novel frameshift variant in exon 3 of the *COL17A1* gene, c.82dup (p.Thr28Asnfs15), which has not been reported previously. This mutation resulted in a premature stop codon at amino acid position 43. The allele frequency of this variant has not been reported in genome databases. The conservation score of the mutation location was 6.10, according to the phyloP100 site. This variant caused a frameshift effect, leading to an early stop of 15 codons after the mutation point. At the protein level, threonine was replaced by asparagine (Fig. 2). Sanger sequencing confirmed the presence of this variant in the patient with a homozygous pattern (Fig. 3A). Her parents (Fig. 1, II-1 and II-2) were heterozygous for the variant (Fig. 3B and 3C). The variant c.82dup (p.Thr28Asnfs15) in the *COL17A1* gene was classified as likely pathogenic according to the American College of Medical Genetics and Genomics criteria. These criteria include PVS1 (a null variant in a gene where loss of function is a known disease mechanism, PM2 is absent from population databases,

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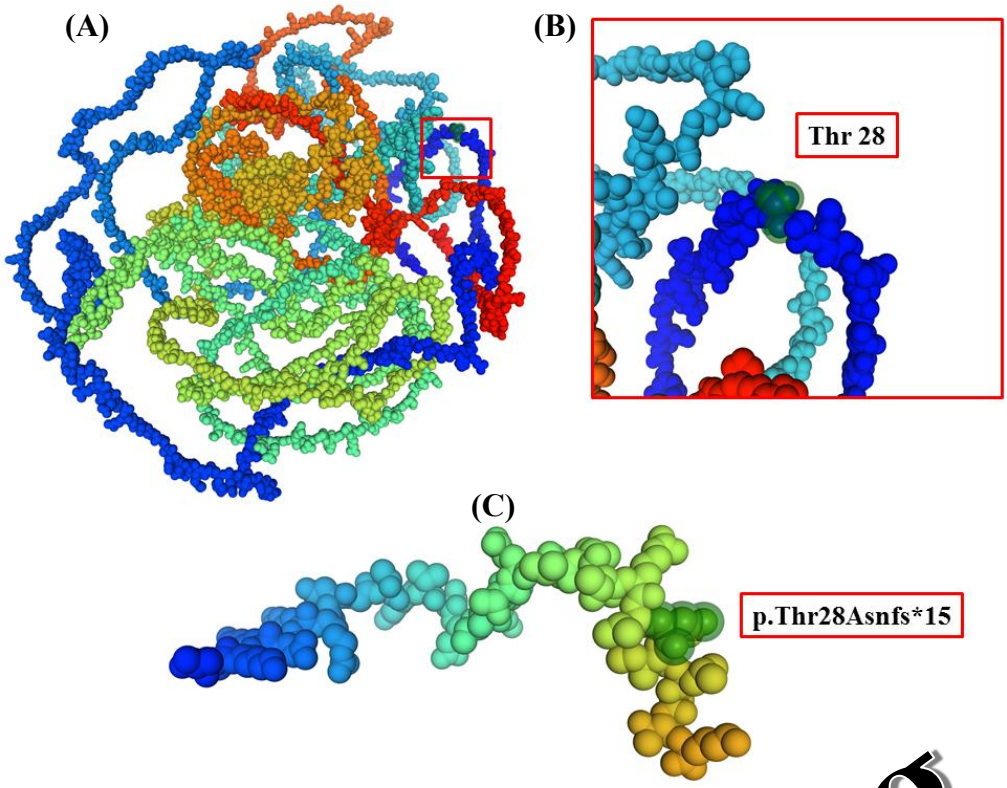

Fig. 2. Swiss-model and MutationTaster used to predict the arrangement of the wild-type (a and b) and the mutant c.82dup, p.Thr28Asnfs*15 (c) proteins of COL17A1.

PM3 is detected in trans with a known pathogenic variant, PPI is co-segregated with disease in the family, and PP4 is the patient phenotype, which is highly specific for JEB. Based on the segregation analysis within the family and the results from in silico prediction tools, this variant was predicted to be likely pathogenic. This mutation in the *COL17A1* gene causes intermediate JEB4.

4. DISCUSSION

As a result of healthcare facilities providing people with unusual skin disorders, such as those diagnosed with EB, continuously face challenges in managing their condition throughout their lives. In this study, we employed WES to molecularly diagnose EB in patients. WES has emerged as a leading genetic test for diagnosing most genetic diseases globally. We identified a novel frameshift variation in exon 3 of the *COL17A1* gene (c. 82dup. p.Thr28Asnfs15), which has not been previously reported. This variant was classified as "likely pathogenic" according to American College of Medical Genetics and Genomics criteria, and an autosomal recessive inheritance pattern was confirmed within the family.

EB is a genetically heterogeneous disease caused by mutations in several genes, including *LAMA3*, *LAMB3*, *LAMC2*, *COL17A1*, *ITGB4*, *ITGA3*, and *ITGA6*, which are typically inherited in an autosomal recessive manner[2]. The *COL17A1* gene, for instance, contains 56 exons located on chromosome 10q25.1[7]. Unlike most collagens, the alpha chain of type XVII collagen, which is encoded by COL17A1, is a membrane protein found in the specialized adhesion structures called hemi-desmosomes. These structures anchor intermediate filaments to the cell membrane and the underlying basement membrane[8]. COL17A1 plays important biological roles, including cell adhesion, morphogenesis, neuromuscular signaling, and host defense [3]. It also regulates cell migration, polarity, and follicular stem cell maintenance. In the absence of collagen XVII, interactions between the hemi-desmosome cytoskeleton and hemi-desmosome-anchoring filament are weakened. Hemidesmosomes serve as anchoring structures that attach keratin intermediate filaments of basal keratinocytes to the basement membrane at the dermal-epidermal junction[9]. Thus, collagen XVII is vital for the mechanical stability and integrity of adhesion. Loss-of-function mutations in this gene, such as the variant described in this study, which leads to a premature termination codon, disrupt hemidesmosome formation or function. This structural weakening makes the skin vulnerable to minor mechanical stress, leading to blister formation, tissue separation, and other clinical manifestations of JEB[9].

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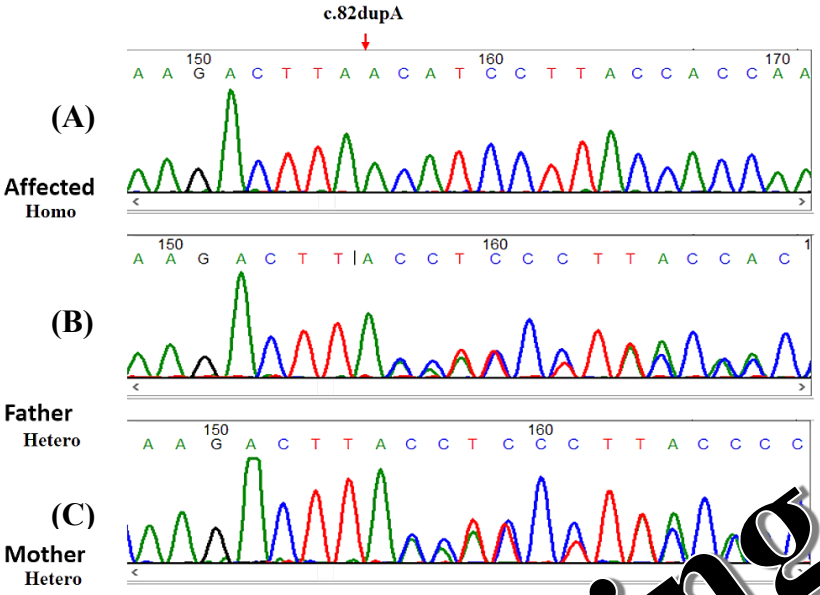

Fig. 3. Chromatograms of Sanger sequencing displaying the c.82dupA mutation in the patient in a homozygous state (A), while his parents exhibit the mutation in a heterozygous state (B and C).

The clinical manifestations observed in our patient, including blisters, erosions, alopecia, nail dystrophy, hyperpigmentation, and dental involvement are consistent with the pathogenic spectrum of *COL17A1* mutations. Specifically, milder mutations in this gene, such as missense or splice-site variants that may produce partially functional proteins, often associated with the "intermediate" or generalized non-Herlitz" form of JEB, characterized by longer survival and more localized skin involvement[5]. Conversely, null mutations, such as deletions, nonsense, or frameshift variants that lead to transcript degradation via nonsense-mediated decay or the production of severely truncated proteins, are typically linked to the more severe "Herlitz" form (JEB-H), which has high mortality in infancy[10]. However, there are reports on intermediate phenotypes occurring even with pure null mutations in the homozygous state, which may be influenced by various genetic or environmental factors[8]. The variant identified in this study (c.82dup), which causes a frameshift and a premature stop codon at position 43, falls into the category of null mutations. The observation of a relatively intermediate phenotype in our patient suggests that compensatory molecular mechanisms or the existence of alternative transcripts bypassing the mutated region, could attenuate phenotypic severity. This aspect permits further investigation at the protein and transcript levels.

According to the Human Gene Mutation Database, there are currently about 208 known mutations in the *COL17A1* gene (https://www.hgmd.cf.ac.uk/ac/gene.php?gene=COL17A1&accession=CM001970). Most reported variants, caused by nonsense or small insertion/deletion mutations, result in premature termination codons[10], which is consistent with the pathogenic variant that we identified. The c.82dup variant reported in this study is described for the first time globally, underscoring the importance of investigating diverse ethnic populations to fully characterize the spectrum of genetic variations associated with rare diseases. Several studies in Asian populations, including those in India[5], China[2], Indonesia[4], and Pakistan[3], have utilized WES to identify novel variants in EB-related genes. Our findings, similar to those of Yenamandra et al.[5] in India, confirm the high efficacy of WES in the molecular diagnosis of JEB, particularly in cases with nonspecific phenotypes or ambiguous family histories.

The present study focuses on a single family and case. Further research is needed to investigate the precise functional impact of the identified variant at the protein level through methods such as immunofluorescence or Western blotting and to explore potential mechanisms modifying phenotypic severity.

5. CONCLUSION

We report a new mutation resulting from consanguineous marriage for the first time. This finding demonstrates the importance of identifying family carriers, as EB is a severe condition that can impact life and impose financial and psychological burdens on families, the government, and society. Fortunately, EB is preventable through techniques such as prenatal diagnosis or preimplantation genetic diagnosis. Our findings expand our understanding of the mutational spectrum associated with JEB.

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DECLARATIONS

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Generative AI and AI-assisted technologies

In this study, no artificial intelligence technology was used in the production of the submitted work.

Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Arak University of Medical Sciences, Arak, Iran (IR.ARAKMU.REC.1403.188).

Consent to participate

The patient and his parents provided their written informed consent to participate.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

All authors contributed to the study design. RM: performed the experiments, analyzed the data, assisted in reviewing and editing the manuscript; SHFK: performed the experiments, wrote the original draft of the article; MM: performed the experiments, wrote the original draft of the article; SB: had responsibility for sample collection and data gathering, wrote the original draft of the article; MS: had responsibility for sample collection and data gathering, assisted in reviewing and editing the manuscript; AK: had responsibility for sample collection and data, assisted in reviewing and editing the manuscript analyzed the data gathering; MG: performed the experiments, analyzed the data.

Data availability

The data supporting the findings of this study are accessible to the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Supplementary information

The online version does not contain supplementary material.

REFERENCES

1. Ma THT, Hong LA, Hoang TL, Nguyen TTH, Vu TH, Tran VK, et al. Novel and very rare causative variants in COL17A1 gene of Vietnamese patients with recessive hereditary epidermolysis bullosa revealed by whole-exome sequencing. Mol Genet Genomic Med. 2021;9(8):1748.
2. Yueqian Y, Zhenzhen W, Zihao M, Lele S, Xi'an F, Gongqi Y, et al. Epidermolysis bullosa in Chinese patients: Genetic analysis and mutation landscape in 57 pedigrees and sporadic cases. Acta Derm Venereol. 2021;101(7):503.
3. Fozia F, Nazli R, Bibi N, Khan SA, Muhammad N, Shakeeb N, et al. Whole exome sequencing confirms molecular diagnostics of three pakhtun families with autosomal recessive epidermolysis Bullosa. Front Pediatr. 2021;9:727288.
4. Widhiati S, Danarti R, Trisnowati N, Purnomosari D, Wibawa T, Soebono H. Novel mutations of epidermolysis bullosa identified using whole-exome sequencing in indonesian javanese patients. Intractable Rare Dis Res. 2021;10(2):88-94.
5. Yenamandra VK, Vellarikkal SK, Kumar M, Chowdhury MR, Jayarajan R, Verma A, et al. Application of whole exome sequencing in elucidating the phenotype and genotype spectrum of junctional epidermolysis bullosa: A preliminary experience of a tertiary care centre in India. J Dermatol Sci. 2017;86(1):30-6.
6. Rostampour D, Zolfaghari MR, Gholami M. Novel insertion mutation in the PLA2G6 gene in an Iranian family with infantile neuroaxonal dystrophy. J Clin Lab Anal. 2022;36(3):24253.
7. Hany U, Watson CM, Liu L, Smith CEL, Harfoush A, Poulter JA, et al. Heterozygous COL17A1 variants are a frequent cause of amelogenesis imperfecta. J Med Genet. 2024;61(4):347-55.
8. Hoffmann J, Casetti F, Reimer A, Leppert J, Grüninger G, Has C. A silent COL17A1 variant alters splicing and causes junctional epidermolysis bullosa. Acta Derm Venereol. 2019;99(4):460-1.
9. Nishie W. Collagen XVII processing and blistering skin diseases. Acta Derm Venereol. 2020;100(5):54.
10. Nakamura H, Sawamura D, Goto M, Nakamura H, Kida M, Ariga T, et al. Analysis of the COL17A1 in non-Herlitz junctional epidermolysis bullosa and amelogenesis imperfecta. Int J Mol Med. 2006;18(2):333-7.

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