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ORIGINAL ARTICLE

# Novel and very rare causative variants in the *COL7A1* gene of Vietnamese patients with recessive dystrophic epidermolysis bullosa revealed by whole-exome sequencing

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## Abstract

**Background:** Dystrophic epidermolysis bullosa (DEB) is a rare inherited disorder characterized by skin fragility leading to trauma-induced subepidermal blisters and healing with scarring. DEB is caused by mutations in *COL7A1*, the gene encoding for type VII collagen (COLVII). The DEB inheritance trait is divided into dominant dystrophic epidermolysis bullosa (DDEB) and recessive dystrophic epidermolysis bullosa (RDEB).

**Methods:** Whole-exome sequencing (WES) was performed for identifying mutations in six affected individuals of five Vietnamese families.

**Results:** Three novel variants in total of eight variants were found in five families. The first novel variant causing glycine substitution (c.8279G>A, p.G2760E), the remaining two novel variants resulted in splice site affecting (c.4518+2delT and c.5821-2A>G). Functional analysis indicated that the splice site at c.4518+2delT resulted in a skipping of exon 43, leading to an in-frame deletion of 12 amino acids.

**Conclusion:** Our finding expands the spectrum of *COL7A1* mutations and reports altered splicing at c.4518+2delT during the processing of the pre-mRNA. This study provides an additional scientific basis for diagnosis, genetic counseling, and prognosis purposes of EB patients.

**KEYWORDS**
COL7A1, dystrophic epidermolysis bullosa, novel variants, whole-exome sequencing

## 1 | INTRODUCTION

Epidermolysis bullosa (EB) is a rare hereditary skin disorder defined by moderate to excessive fragility of epithelial tissues and blister formation. The disease can manifest in many different organ systems, and in severe cases, blisters may occur inside the body, such as the lining of the mouth or the stomach. The human skin is made up of an outer layer

Thi Huyen Thuong Ma and Lan Anh Luong Thi are contributed equally to this work.

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(epidermis) and an underlying layer (dermis). The basement membrane is the area where these two layers meet. In people affected with EB, the two skin layers (epidermis and dermis) lack anchoring protein that holds them together, causing extremely fragile skin that is not able to tolerate even minor mechanical friction/trauma. In updated recommendations on diagnosis and classification, four major EB subtypes were classified as follows: epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JBS), dystrophic epidermolysis bullosa (DEB), and Kindler syndrome (Fine et al., 2014). The different types of EB are characterized by a certain layer in which the blisters form. Until now, mutations in at least 21 genes encoding components of keratin, adhesion contracts, and desmosomes were described (Vahidnezhad, Youssefian, Saeidian, & Uitto, 2019).

The advent of high throughput next-generation sequencing (NGS) provides massively parallel or high deep data in a rapid and cost-effective manner. To date, whole-exome sequencing (WES) has become the standard of care for the identification of the functional genomic variants associated with rare disorders (Trujillano et al., 2017). In 2015, the pathogenic mutation identification of nine EB patients, failed by skin biopsy analysis and Sanger sequencing, was performed successfully by WES (Takeichi et al., 2015). The implementation of WES as a diagnostic tool in EB was referred to in several published studies to this point (Gong, Liu, Li, & Xu, 2019; Mahajan et al., 2018; Yenamandra et al., 2017, 2018).

The aim of this study is to identify mutations/genetic variants that might be pathogenic by WES in six EB individuals of five Vietnamese families. In the future, these data could have implications for genetic counseling and prognosis.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

Six affected individuals of five families with the clinical diagnosis of EB and their parents were recruited from Hanoi Medical University Hospital, Hanoi, Vietnam. Patients present mechano-bulluos lesions of the skin with blistering and scarring. The parents of all families were not consanguine and healthy in clinical features. Written informed consent was obtained from all family members before sample collection. This study was approved by the Institute of Genome Research Institutional Review Board, Vietnam Academy of Science and Technology.

For patients and their parents, 2 ml of whole blood was collected, preserved in EDTA-containing tubes, frozen, and stored at −20°C until use. Genomic DNA was extracted from the peripheral blood samples using Exgene™ Blood SV (GeneAll Biotechnology) according to the manufacturer's protocol. DNA quantification was performed by using Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific) and Eppendorf BioSpectrometer® fluorescence (Eppendorf AG).

### 2.2 | Methods

#### 2.2.1 | Whole-exome sequencing

For the affected individuals (Figures 2a–6a), the DNA library construction was performed by using Sure Select V6-Post (Agilent Technologies) following the manufacturer's guidelines. In brief, the sequencing library was prepared by random fragmentation of DNA, following by 5′ and 3′ adapter ligation. Fragments ligated with adapter were subsequently amplified by PCR and gel purified. The Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific) was used for enriched library quantification. The library size distribution was checked by bioanalyzer using high sensitivity DNA chip (Agilent Technologies) with an expected size ranging from 200 to 400 bp. The sequencing was performed by using an Illumina NovaSeq 6000 platform (Illumina) with paired reads of 150 bp.

#### 2.2.2 | Sanger Sequencing

The candidate variants were validated by direct Sanger sequencing in patients as well as their parents. Primers for PCR and sequencing were provided by PHU SA Biochem Company. For PCR amplification, 10 ng of total genomic DNA was used as a template in 20 µl of reaction mixture containing 1× Neb Master mix (New England Biolabs, Ipswich), 0.8 µl of each primer (10 pmole), and 8.4 µl of deionized water. The thermocycling was 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, 68°C for 20 s, and a final extension at 68°C for 5 min. The PCR products were purified using Multiscreen PCR 96 Filter Plate (Merck-Millipore), and sequenced by ABI Prism BigDye Terminator Cycle Sequencing Kit, Version 3.1 (Applied BioSystems) on ABI 3500 Genetic Analyzer (Applied BioSystems).

#### 2.2.3 | Variant calling

The reads were mapped to hg19/GRCh37 human reference genome by BWA.v0.7.12 tool (Li & Durbin, 2009), and Picard was used to marking the duplicates. Genome Analysis Tool Kit (GATK) and Samtools were used to detect single nucleotide variants (SNVs) and short insertions/deletions (Indels). To exclude false positive, all variants with depth read lower than 20× were removed. Short Indels in the repeat regions and within the 10 bp range from the start and

end of the read were also excluded. After that, the remaining variants were filtered from the public databases comprising 1000 Genomes and gnomAD. Because the prevalence of EB is about 8.2 per 1,000,000 live births in the global population (Fine, 2010), all variants with a frequency above 0.5% were eliminated.

#### 2.2.4 | Variant annotation and prediction

The variants were annotated with the ANNOVAR program (Wang, Li, & Hakonarson, 2010). The in silico analysis was performed by SIFT (Hu & Ng, 2013), Polyphen-2 (Adzhubei et al., 2010) and Mutation Taster (Schwarz, Cooper, Schuelke, & Seelow, 2014) to anticipate the functional effect of missense, nonsense variants. For splicing prediction, Human Splicing Finder Professional (HSF Pro) and Alamut Visual (http://www.interactive-biosoftware.com) were used.

#### 2.2.5 | Functional analysis of splice site (c.4518+2delT)

Total RNA of patient 4, patient 5, and their parents were isolated from the fresh blood samples using Monarch Total RNA Miniprep Kit (New England Biolabs). Reverse transcription (RT) was performed by ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs) following the manufacturer's instruction. The cDNA obtained was amplified using the forward primer (5′-GGTGACCGGGGCTTTCCA-3′) and reverse primer (5′-TTCAGGACCCTTGGCTCCAG-3′) located in exons 42 and 44 of *COL7A1*, respectively. PCR products were separated by electrophoresis in a 3% agarose gel.

## 3 | RESULTS

### 3.1 | Clinical characteristics of patients

Patient 1 was a 4-year-old male and presented with moderate blisters, erosions, and scarring in the body, such as the shoulder, back, hands, feet, elbows, buttocks, and knees. He had contracted toes and dystrophic nails (Figure 1a). Patient 2 was an 8-years-old girl, who reported severe blistering on the left face and peeling skin on her feet at birth. Pseudosyndactyly, contractures and complete absent nails were observed in hands and feet. In particular, the mitten deformity was developed in feet. The blistering was extended and formed erosions, exudate, crusting, and scarring as she increased in age (Figure 1b). Immunofluorescence antigen mapping showed the reduced stain of type VII collagen (COLVII) at the epidermal side of the blister (data not shown). Patient 3 was about 1-month-old baby girl with blisters occurring in hands, feet, and inside the mouth (Figure 1c). The severe phenotype was also found in two affected brothers, patients 4 (7 years old) and 5 (15 years old). They presented with widespread blistering, extensive erosions, and scarring. The skin was dry and winkled in hands and feet, with loss of finger and toenails (Figure 1d). The milder phenotype in patient 6 (Figure 1e) was associated with lesions in the lower back and groin.

### 3.2 | Molecular findings

Analysis of the WES data revealed eight different *COL7A1* (NM_000094.4, MIM:120120) variants, comprising of three novel mutations and five very rare variants ($3.99 \times 10^{-6}$–$1.65 \times 10^{-5}$, Table 1). Three novel variants, including one glycine substitution (c. 8279G>A, p.G2760E) and two splicing variants (c.4518+2delT and c.5821-2A>G), were not found in any public database (1000 Genomes, dbSNP, gnomAD, HGMD, and *COL7A1* database) and in the inhouse database of 500 healthy Vietnamese. Five very rare changes included one missense variant (c.6205C>T, p.R2069C), two stop-gain variants (c.5047C>T, p.R1683* and c.8233C>T, p.R2745*), and two indels (c.2858_2859del and c.6081delC). Analysis by multialignments (www.genome.ucsc.edu) displayed conservation of p.G2760, p.R2745, and p.R2069 among several vertebrates (human, rhesus, mouse, dog, elephant, opossum, and chicken), indicating the critical contribution of these amino acids for normal protein function (Figures 2c, 4c and 6c).

In patient 1, a homozygous change in c.8279G>A in exon 111 was found, leading to the substitution of conserved amino acid glycine by glutamic acid (p.G2760E). This variant was predicted as probably damaging/deleterious by SIFT, Polyphen2, and MutationTaster (Table 1). The heterozygous c.8279G>A variant was observed in both parents by Sanger sequencing (Figure 2b).

A compound heterozygote frameshift deletion in the *COL7A1* gene was identified in patient 2. The first variant was c.2858_2859del on exon 22, which has not been found in 1000 Genomes, dbSNP, gnomAD, and HGMD. However, this variant was reported in two patients with generalized severe RDEB (1-year-old girl and 3-year-old boy) and submitted in *COL7A1* gene variant database (http://www.col7a1-database.info). The c.2858_2859del resulted in a premature termination codon (PTC) at eighth amino acid downstream of exon 22 (p.E953fs*8) and leading to a deletion of 1984 amino acids downstream. The second variant was c.6081delC in exon 73 and created a PTC at codon 2206 (p.P2029fs*177), which has been reported previously with autosomal RDEB (Christiano, McGrath, & Uitto, 1996; Kern, Kohlhase, Bruckner-Tuderman, & Has, 2006), as well

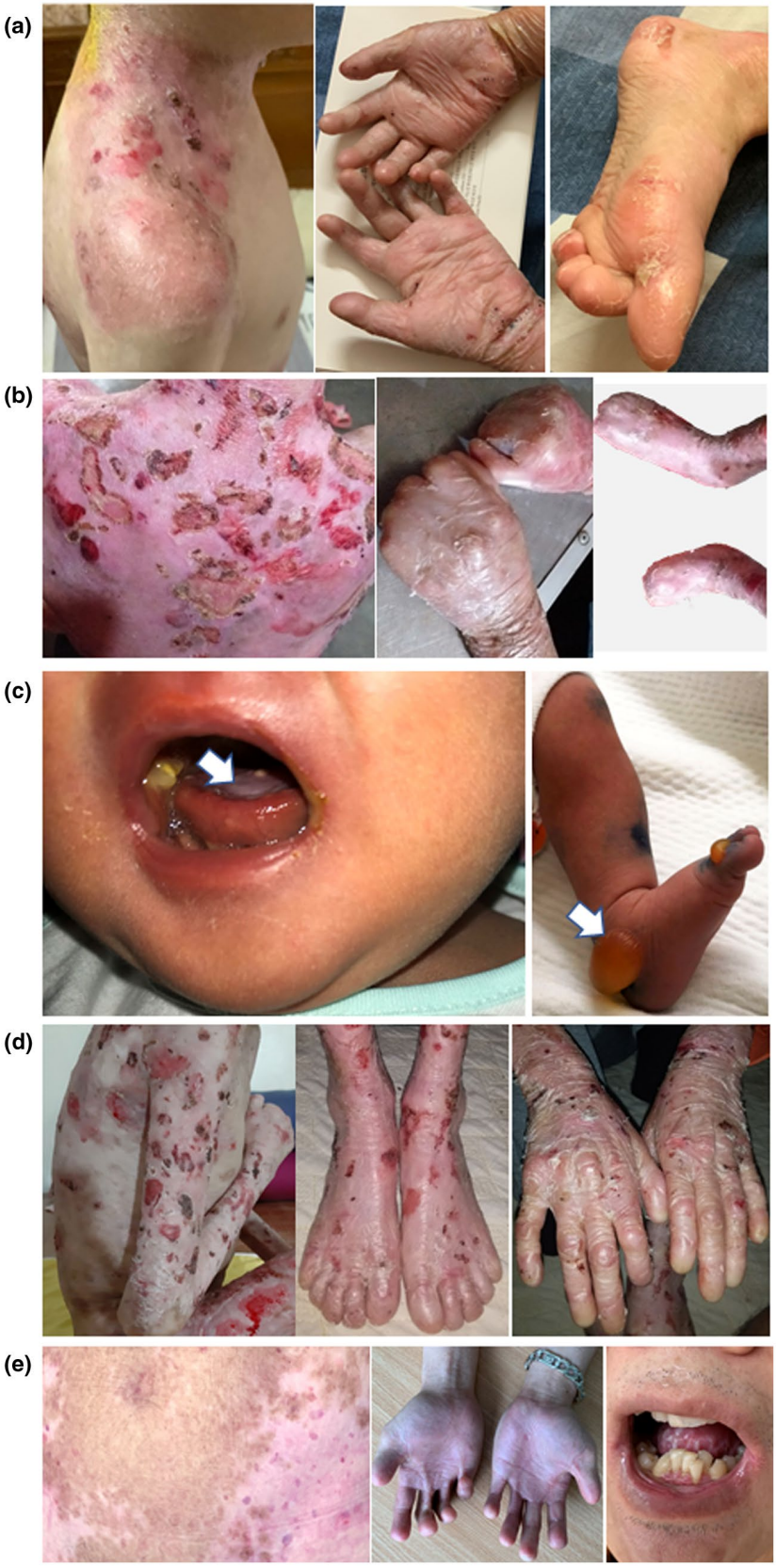


**FIGURE 1** Clinical features of 6 EB cases in Vietnam. (a) Moderately blistering, erosions, and scaring in shoulder hands and feet were observed in patient 1. (b) Patient 2 presented with generalized bullae, erythema, erosions, and scars in the back. Joint contractures of fingers and toes, pseudosyndactyly with mitten deformity in feet were also identified. (c) Blisters on the tongue and feet were found in patient 3. (d) Extensive severe lesions in the body, abnormal bending of joints, and absent nails on fingers and toes were presented in 2 probands (patients 4 and 5). (e) Hypopigmentation in groin, contractures of hands, reduction in the oral opening (microstomia) with ankyloglossia, and lesions in tongue were observed in patient 6

as in ClinVar with pathogenic classification. Confirmation by Sanger sequencing also showed that the affected child inherited c.6081delC from mother and c.2858_2859del from father (Figure 3b).

The combination of nonsense and another variant including missense variant involving an amino acid other than glycine and splicing variant in *COL7A1* gene was detected in patients 3, 4, and 5. In patient 3, compound heterozygous

**TABLE 1** *COL7A1* (NM_000094.4) variants were found in EB patients

| Patient | Variant change | | | | | Global population frequency | | In silico prediction | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cDNA | Amino acid | Zygosity | Type of mutation | Region | 1000 Genomes | gnomAD | SIFT | Polyphen2 | MutTaster | HSF | Alamut visual |
| 1 | **c.8279G>A** | **p.G2760E** | **hom** | **nonsyn** | **exon111** | – | – | **D** | **D** | **D** | – | – |
| | | p.E953fs*8 | het | FS del | exon22 | – | – | – | – | – | – | – |
| 2 | c.2858_2859del c.6081delC | p.P2029fs*177 | het | FS del | exon73 | – | – | – | – | – | – | – |
| 3 | c.6205C>T | p.R2069C | het | nonsyn | exon74 | – | $1.65 \times 10^{-5}$ | D | B | A | – | – |
| | c.8233C>T | p.R2745* | het | stopgain | exon111 | – | $3.99 \times 10^{-6}$ | – | – | A | – | – |
| 4, 5 brothers | **c.4518+2delT** | – | **het** | **splicing** | **exon43** | – | – | – | – | – | No significant impact on splicing signals | The consequence of this change is not predictable, but a skip of exon 43 is very likely |
| | c.5047C>T | p.R1683* | het | stopgain | exon54 | – | $8.48 \times 10^{-6}$ | – | – | A | – | – |
| 6 | **c.5821-2A>G** | – | **het** | **splicing** | **exon71** | – | – | – | – | **D** | Alteration of the WT Acceptor site, most probably affecting splicing | The consequence of this change is not predictable, but a skip of exon 71 is very likely |
| | c.6205C>T | p.R2069C | het | nonsyn | exon74 | – | $1.65 \times 10^{-5}$ | D | B | A | – | – |

The bold rows indicate novel variants

Abbreviations: A, disease causing automatic; B, benign; D, damaging/deleterious/disease-causing (in MutTaster); FS del, frameshift deletion. Bolds indicate the novel variants; het, heterozygous; hom, homozygous; nonsyn, nonsynonymous; wt, wild type.

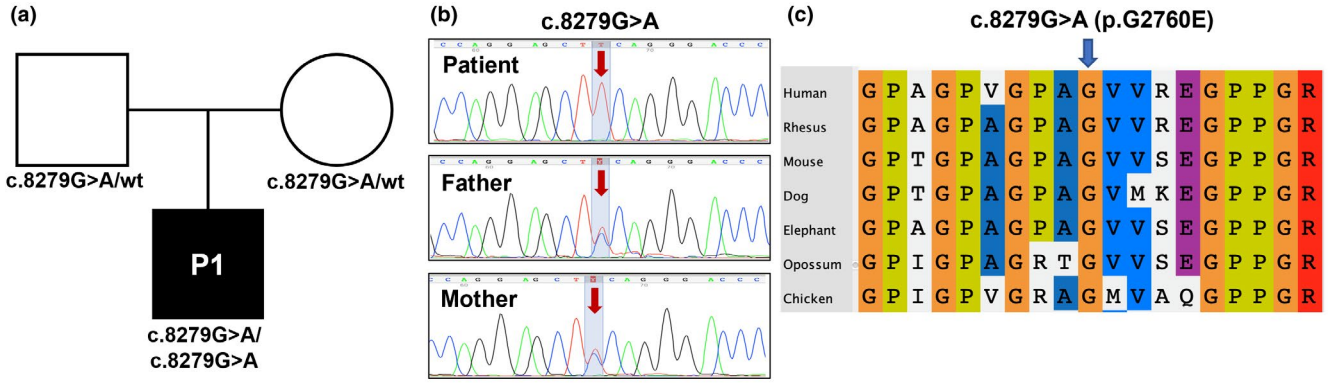


**FIGURE 2** (a) Pedigree chart of family 1 with an RDEB case. Black/white represent patient/not affected individuals. (b) Sanger sequencing showed a homozygous *COL7A1* (NM_000094.4) variant in affected individuals and carriers, partial sequence with mutated nucleotides marked with vertical lines and red arrows. (c) The amino acid substitutions at evolutionarily conserved residues were marked with a blue arrow

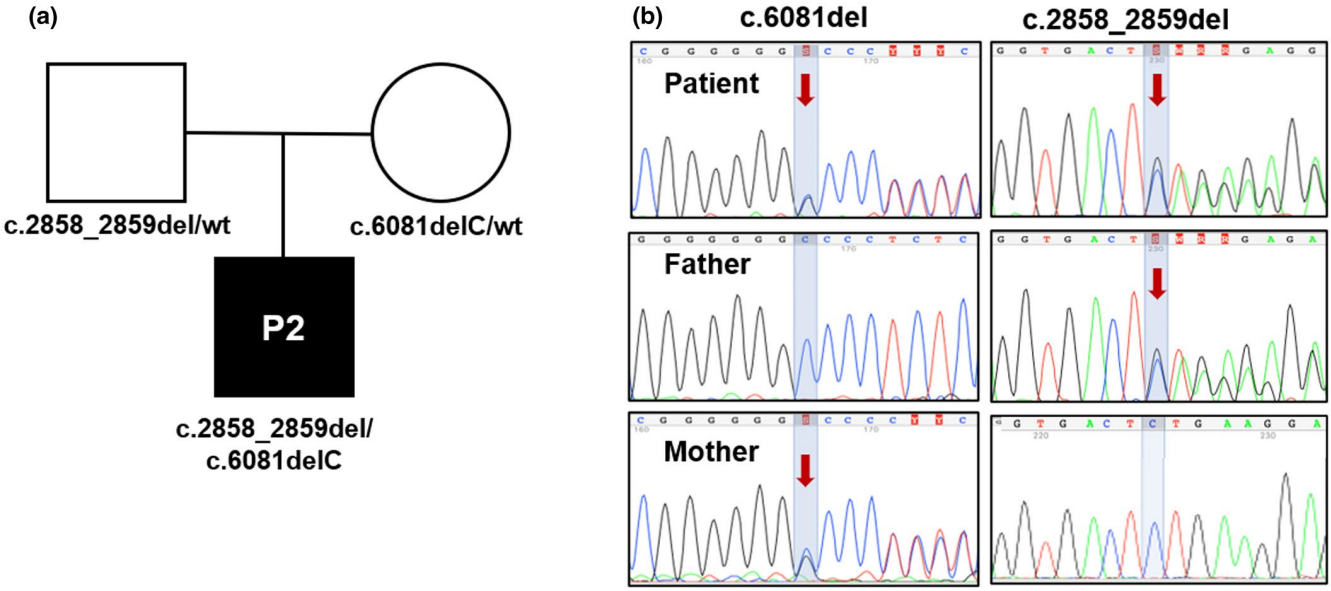


**FIGURE 3** (a) Pedigree chart of family 2 with an RDEB case. Black/white represent patient/not affected individuals. (b) Electropherogram of the patient with *COL7A1* (NM_000094.4) compound heterozygote, which was inherited from the parents. Mutated nucleotides were marked with vertical lines and red arrows

of one nonsynonymous SNV (c.6205C>T) and one stop-gain mutation (c.8233C>T) was identified, corresponding to rs121912855 and rs768202310 in dbSNPs, respectively. The c.6205C>T (p.R2069C) was described as pathogenic in ClinVar, involving individuals affected with DEB. The c.8233C>T apparently resulted in the expression of a truncated protein (p.R2745*). Verification by Sanger sequencing indicated that the mother and the father were heterozygous carriers for c.6205C>T and c.8233C>T, respectively. Coexistence of these genetic variants was also not detected in patient's parents (Figure 4b). In two brothers (patients 4 and 5), two heterozygous variants were identified: a novel deletion point (c.4518+2delT) in intron 43 and a single-base substitution (c.5047C>T) in exon 54 (Figure 5b). In in silico splice site prediction by Alamut Visual, the effect of skipping exon 43 very likely for c.4518+2delT was predicted. However, HSF Pro predicted no significant impact on splicing signals (Table 1). In contrast, RT-PCR result showed one amplicon of 125 bp in wild type and two amplicons in mutation with an additional shorter amplicon of 89 bp (Figure 5c), suggesting aberrant splicing, as revealed the same consequence to splice prediction by Alamut Visual. The lack of 36 bp corresponded to the skipping of exon 43, which could be constructed by splice site (c.4518+2delT), leading to the removed 12 amino acids in transcription. The replacement of a single base at the codon 5047 produced a premature stop codon (p.R1683*). The parent was heterozygous for

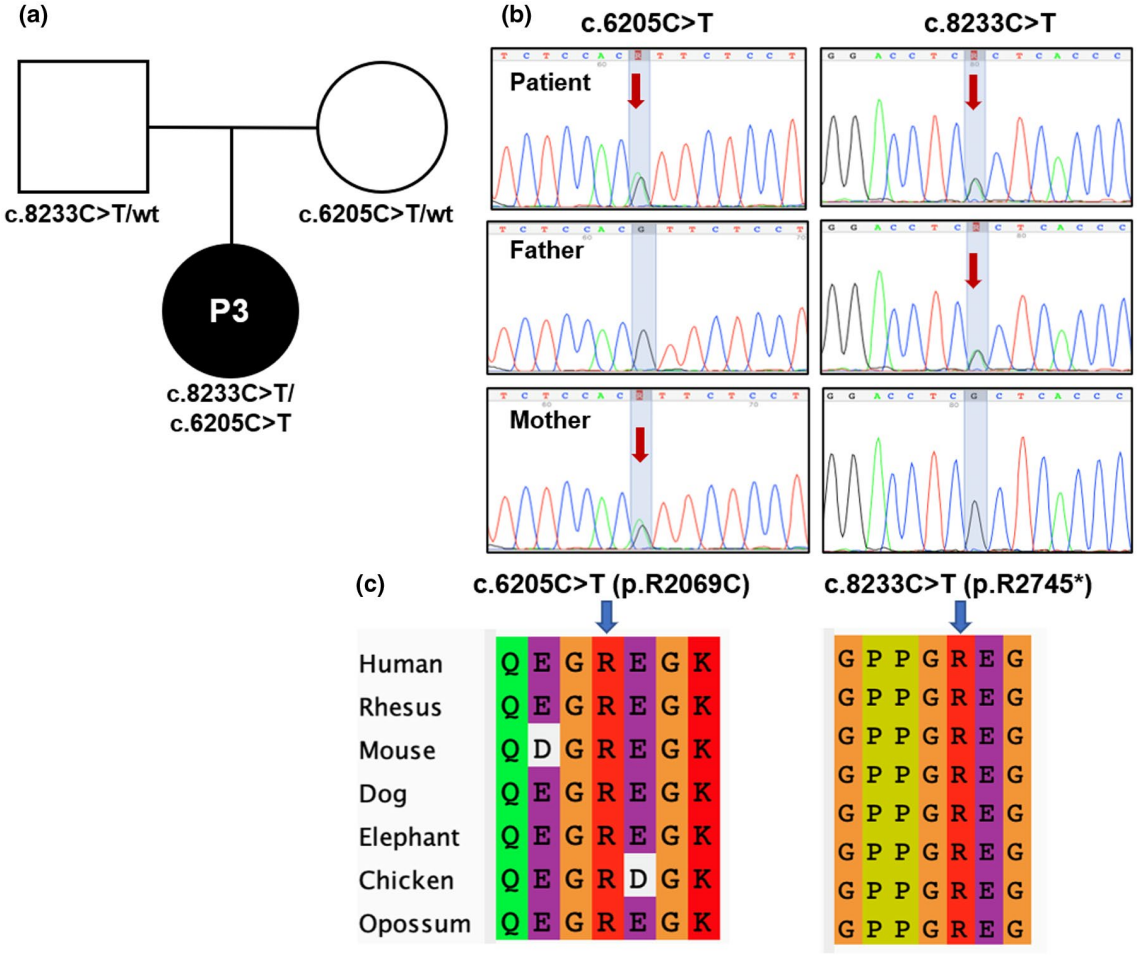


**FIGURE 4** (a) Pedigree chart of family 3 with an RDEB case. Black/white represent patient/not affected individuals. (b) Compound heterozygous of *COL7A1* (NM_000094.4) was detected in patient 3 and father/mother carried once. Mutated nucleotides were marked with vertical lines and red arrows. (c) The amino acid substitutions at evolutionarily conserved residues were marked with a blue arrow

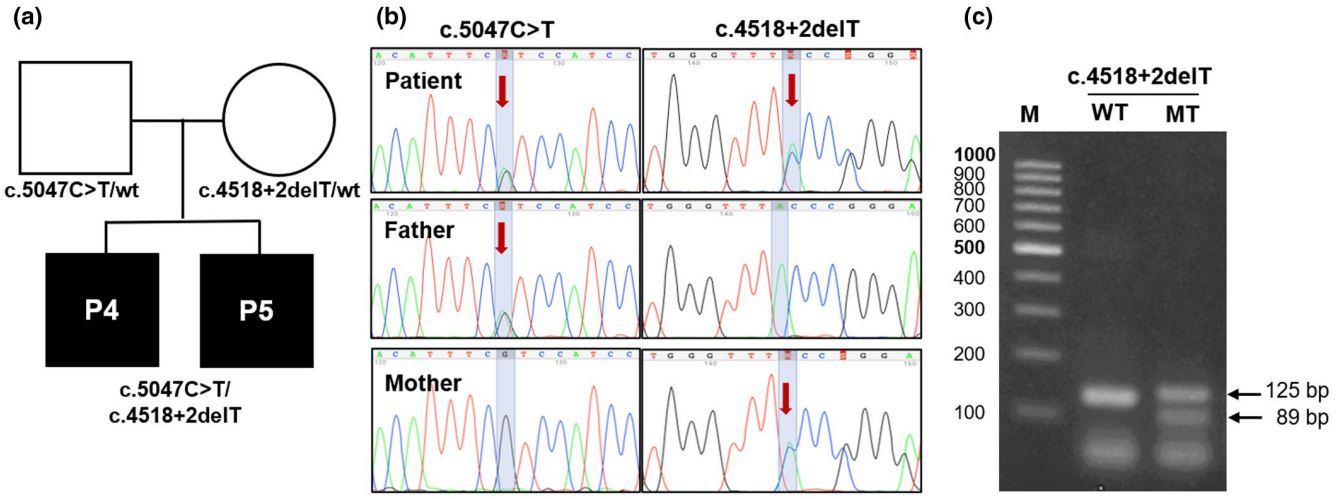


**FIGURE 5** (a) Pedigree chart of family 4 with two RDEB cases. Black/white represent patient/not affected individuals. (b) Sequencing analysis indicated that two siblings carried a compound heterozygous *COL7A1* (NM_000094.4) variant and inherited from mother and father. Mutated nucleotides were marked with vertical lines and red arrows. (c) Splice defect of c.4518+2delT. Agarose gel electrophoresis of RT-PCR products, the longer band with 125 bp in length presented the wild-type (WT) transcript, the shorter band with 89 bp in length presented the mutated transcript (MT), the bottom bands are primer dimer

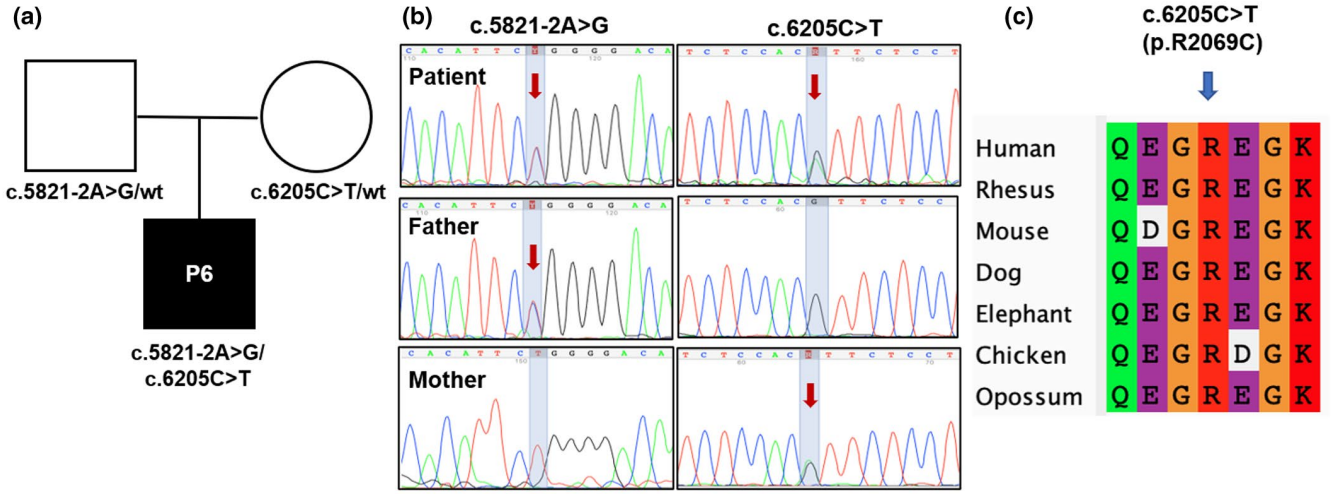


**FIGURE 6** (a) Pedigree chart of family 5 with an RDEB case. Black/white represent patient/not affected individuals. (b) Sanger sequencing confirmation indicated a compound heterozygous of *COL7A1* (NM_000094.4) in patient 6, and each variant was carried from the parents. Mutated nucleotides were marked with vertical lines and red arrows. (c) The amino acid substitutions at evolutionarily conserved residues were marked with a blue arrow

c.4518+2delT and c.5047C>T, corresponding to the maternal and paternal variants, respectively (Figure 5b).

The conjunction of a splicing variant (c.5821-2A>G) and a missense variant (c.6205C>T) was found in patient 6. The c.5821-2A>G was a novel substitution variant in intron 71. This variant was predicted in "the skipping exon 71 very likely" and "alteration of the WT acceptor site, most probably affecting splicing as a consequence of substitution in intron 71" by Alamut Visual and HSF Pro, respectively (Table 1). The c.6205C>T was a pathogenic variant and was also found in patient 3. Sequencing analysis indicated that both mother and father were heterozygous carriers for c.C6025T and c.5821-2A>G, respectively (Figure 6b).

## 4 | DISCUSSION

The mutation in the *COL7A1* gene causes the dystrophic epidermolysis bullosa (DEB), which is inherited in an autosomal dominant (DDEB) or an autosomal recessive (RDEB) manner. The diagnosis of DEB base on the clinical manifestations and the variant causing in *COL7A1*. Up to now, more than 800 genetic variants of *COL7A1* are related to DEB (http://www.col7a1-database.info) (Wertheim-Tysarowska et al., 2012). Among these, almost all mutations are glycine substitutions located in triple helix domain (THD; Leverkus et al., 2011). The clinical phenotypes of DDEB are comparatively milder than that of RDEB. In DDEB, the blistering is often limited to hands, feet, knees, and blows. It is relatively benign but nonetheless heals with scaring. RDEB is a severe inherited skin disorder characterized by extensive blisters, atrophic scarring, milia, minor dystrophic, or absent nails (Laimer, Prodinger, & Bauer, 2015). Although almost all mutations in DDEB were found in exons 73–75, mutations including insertions, deletions, single-base substitution, and splice junction alteration in RDEB occur in both alleles that result in either null allele or out-of-frame mutations (Christiano, Amano, Eichenfield, Burgeson, & Uitto, 1997; Fine et al., 2014; Van den Akker et al., 2011). In our study, a homozygous and four compound heterozygous variants in *COL7A1* were found in six EB cases, and the heterozygous carriers of each variant did not show any symptoms, leading to the prediction of recessive in inheritance trait.

Compound heterozygous PTC variant (c.2858_2859del and c.6081delC) was identified in patient 2 with severe phenotype (Figures 1b and 3). Widespread blistering, extensive scarring, flexion contractures of limbs, and pseudosyndactyly with mitten deformity were observed in clinical findings. Genotype to phenotype correlation indicates that PTC mutations in both alleles of *COL7A1* known as the main mutation of RDEB generalize severe (RDEB-GS) cases, causing in nonsense-mediated decay or truncated polypeptides, resulting in significant reduction or complete absence of type VII collagen (COLVII) or anchoring fibrils (AFs) (Christiano et al., 1997; Fine et al., 2014; Van den Akker et al., 2011). In few cases, RDEB-GS could be caused by compound heterozygosity of two missense mutations or the conjunction of a PTC and a missense mutation (Christiano, McGrath, Tan, & Uitto, 1996). In cases of two brothers (patients 4 and 5), the severe phenotype consists of extensive blisters, erosions, scarring in the body, pseudosyndactyly, and contractures

of the hands and feet, with absent nails on fingers and toes (Figure 1d). The combination of a PTC (p.R1683*) and a splice site variant (c.4518+2delT) was shown in Figure 5. The milder RDEB was often caused by the conjunction of one PTC mutation and one missense mutation (Shimizu, McGrath, Christiano, Nishikawa, & Uitto, 1996). Patient 3 presented with a mild phenotype characterized by the limited blistering on hands, feet, and tongue (Figure 1c), who carried compound heterozygosity, including a PTC variant (p.R2745*) and a missense variant (p.R2069C; Figure 4). Other *COL7A1* mutations that did not cause PTCs codon usually produce less severe disease. This could be explained in patients 1 and 6, who were characterized with moderately blistering, erosions, scarring in body (patient 1, Figure 1a), hypopigmentation in the lower back and groin area, limited oral opening, ankyloglossia, and tongue lesion (patient 6, Figure 1e). In detail, the homozygous glycine substitution (p.G2760E, Figure 2) and compound heterozygous variant (c.5821-2A>G, c.6205C>T, Figure 6) were detected in patients 1 and 6, respectively. Due to the genotypes to phenotypes observed from the studied patients, PTC mutations resulted in the most serious cases, it was the same aspect of previously published studies.

Three novel *COL7A1* variants were revealed from WES data of RDEB patients. The novel homozygous glycine substitution (p.G2760E) in collagenous encoding domain would lead to the instability and affect critical amino acids in the triple helix structure. The recessive glycine substitution lead to impairment of COLVII secretion as well as AFs assemblage or a conjunction thereof (Uitto, 2011). The other single base substitution (c.8278G>A), that resulted in the glycine substitution at the same codon 2760 and produced a missense mutation (p.G2760R) was previously reported (Yan et al., 2018). The two novel splicing variants residing in a donor splice site (c.4518+2delT) and an acceptor splice site (c.5281-2A>G) of *COL7A1*. These exon/intron boundary sequences are highly conserved, which consist of GT and AG motifs at 5′ and 3′ end of the intron, respectively. Splice site affecting +1 and +2 residues at 5′ donor site and −1 and −2 residues at the 3′ acceptor had been known as the most classical variants. The mutation in canonical sequence can alter the interaction between pre-mRNA and protein, which responsible for the intron removal (Abramowicz & Gos, 2018). Functional splicing prediction by Alamut Visual indicated that the consequence of exon skipping in both variants. Nevertheless, the variant c.5281-2A>G in intron 70 was predicted by HSF as probably affect splice site signal. Despite of HSF prediction resulted in none splicing defect of c.4518+2delT, this variant was demonstrated in RNA level of two siblings (patients 4 and 5) with complete in-frame skipping of exon 43 (12 amino acids) within THC domain of COLVII. Our research finding suggested the complete inactivation of *COL7A1* and loss of COLVII function. Since the RNA of patient 6 was not available, functional analysis by another assay should be considered to further investigate the c.5281-2A>G impact.

## 5 | CONCLUSION

In conclusion, this is the first report on mutational screening of DEB patients in Vietnam, providing an important genetic variant spectrum for Vietnamese EB. To sum up, our study had identified 3 novel mutations (p.G2760E, c.4518+2delT, and c.5281-2A>G) and 5 very rare variants (p.E953*8, p.R1683*, p.P2029*177, p.R2069C, and p.R2745*). In addition, pre-mRNA splicing confirmed that the c.4518+2delT led to an in-frame deletion of exon 43. The results of the molecular tests would be valuable for appropriate genetic counseling and providing a theoretical prognosis for the progression of the disease.

### ACKNOWLEDGMENT

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### AUTHOR CONTRIBUTIONS

Conceptualization: NDT, NVH; Funding acquisition: NDT; Data curation, Formal analysis, and Investigation: MTHT, NHH, NTTH, NDB, LTLA, VTH, TVK, TTS; Methodology; Roles/Writing—original draft: MTHT, NHH, NDT, NVH; Writing—review and editing: MTHT, NHH, NDT, NVH.

### DATA AVAILABILITY STATEMENT

The data used to support the findings of this study may be requested from the corresponding author.

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### REFERENCES

Abramowicz, A., & Gos, M. (2018). Splicing mutations in human genetic disorders: Examples, detection, and confirmation. *Journal of Applied Genetics*, *59*(3), 253–268. https://doi.org/10.1007/s13353-018-0444-7

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., Kondrashov, A. S., & Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. *Nature Methods*, *7*(4), 248–249. https://doi.org/10.1038/nmeth0410-248

Christiano, A. M., Amano, S., Eichenfield, L. F., Burgeson, R. E., & Uitto, J. (1997). Premature termination codon mutations in the type VII collagen gene in recessive dystrophic epidermolysis bullosa result in nonsense-mediated mRNA decay and absence of functional protein. *Journal of Investigative Dermatology*, *109*(3), 390–394. https://doi.org/10.1111/1523-1747.ep12336276

Christiano, A. M., McGrath, J. A., Tan, K. C., & Uitto, J. (1996). Glycine substitutions in the triple-helical region of type VII collagen result in a spectrum of dystrophic epidermolysis bullosa phenotypes and patterns of inheritance. *American Journal of Human Genetics*, *58*(4), 671–681.

Christiano, A. M., McGrath, J. A., & Uitto, J. (1996). Influence of the second COL7A1 mutation in determining the phenotypic severity of recessive dystrophic epidermolysis bullosa. *Journal of Investigative Dermatology*, *106*(4), 766–770. https://doi.org/10.1111/1523-1747.ep12345814

Fine, J.-D. (2010). Inherited epidermolysis bullosa. *Orphanet Journal of Rare Diseases*, *5*(1), 12. https://doi.org/10.1186/1750-1172-5-12

Fine, J.-D., Bruckner-Tuderman, L., Eady, R. A. J., Bauer, E. A., Bauer, J. W., Has, C., Heagerty, A., Hintner, H., Hovnanian, A., Jonkman, M. F., Leigh, I., Marinkovich, M. P., Martinez, A. E., McGrath, J. A., Mellerio, J. E., Moss, C., Murrell, D. F., Shimizu, H., Uitto, J., Woodley, D., & Zambruno, G. (2014). Inherited epidermolysis bullosa: Updated recommendations on diagnosis and classification. *Journal of the American Academy of Dermatology*, *70*(6), 1103–1126. https://doi.org/10.1016/j.jaad.2014.01.903

Gong, L., Liu, C. C., Li, Y. H., & Xu, X. G. (2019). Whole exome sequencing identified two point mutations of COL7A1 and FLG in a Chinese family with dystrophic epidermolysis bullous pruriginosa and ichthyosis vulgaris. *The Journal of Dermatology*, *46*(2), 158–160. https://doi.org/10.1111/1346-8138.14731

Hu, J., & Ng, P. C. (2013). SIFT Indel: Predictions for the functional effects of amino acid insertions/deletions in proteins. *PloS One*, *8*(10), e77940. https://doi.org/10.1371/journal.pone.0077940

Kern, J. S., Kohlhase, J., Bruckner-Tuderman, L., & Has, C. (2006). Expanding the COL7A1 mutation database: Novel and recurrent mutations and unusual genotype–phenotype constellations in 41 patients with dystrophic epidermolysis bullosa. *Journal of Investigative Dermatology*, *126*(5), 1006–1012. https://doi.org/10.1038/sj.jid.5700219

Laimer, M., Prodinger, C., & Bauer, J. W. (2015). Hereditary epidermolysis bullosa. *Journal der Deutschen Dermatologischen Gesellschaft*, *13*(11), 1125–1133. https://doi.org/10.1111/ddg.12774

Leverkus, M., Ambach, A., Hoefeld-Fegeler, M., Kohlhase, J., Schmidt, E., Schumann, H., Has, C., & Gollnick, H. (2011). Late-onset inversa recessive dystrophic epidermolysis bullosa caused by glycine substitutions in collagen type VII. *British Journal of Dermatology*, *164*(5), 1104–1106. https://doi.org/10.1111/j.1365-2133.2011.10230.x

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Mahajan, R., Vellarikkal, S. K., Handa, S., Verma, A., Jayarajan, R., Kumar, A., De, D., Kaur, J., Panigrahi, I., Vineeth, V. S., & Sivasubbu, S. (2018). Utility of whole-exome sequencing in detecting novel compound heterozygous mutations in COL7A1 among families with severe recessive dystrophic epidermolysis bullosa in India – implications on diagnosis, prognosis and prenatal testing. *Journal of the European Academy of Dermatology and Venereology*, *32*(12), e433–e435. https://doi.org/10.1111/jdv.14909

Schwarz, J. M., Cooper, D. N., Schuelke, M., & Seelow, D. (2014). MutationTaster2: Mutation prediction for the deep-sequencing age. *Nature Methods*, *11*(4), 361–362. https://doi.org/10.1038/nmeth.2890

Shimizu, H., McGrath, J. A., Christiano, A. M., Nishikawa, T., & Uitto, J. (1996). Molecular basis of recessive dystrophic epidermolysis bullosa: Genotype/phenotype correlation in a case of moderate clinical severity. *Journal of Investigative Dermatology*, *106*(1), 119–124. https://doi.org/10.1111/1523-1747.ep12329600

Takeichi, T., Liu, L., Fong, K., Ozoemena, L., McMillan, J. R., Salam, A., Campbell, P., Akiyama, M., Mellerio, Je, McLean, Whi, Simpson, Ma, & McGrath, J. A. (2015). Whole-exome sequencing improves mutation detection in a diagnostic epidermolysis bullosa laboratory. *British Journal of Dermatology*, *172*(1), 94–100. https://doi.org/10.1111/bjd.13190

Trujillano, D., Bertoli-Avella, A. M., Kandaswamy, K. K., Weiss, M. E., Köster, J., Marais, A., Paknia, O., Schröder, R., Garcia-Aznar, J. M., Werber, M., Brandau, O., Calvo del Castillo, M., Baldi, C., Wessel, K., Kishore, S., Nahavandi, N., Eyaid, W., Al Rifai, M. T., Al-Rumayyan, A., … Abou Jamra, R. (2017). Clinical exome sequencing: Results from 2819 samples reflecting 1000 families. *European Journal of Human Genetics*, *25*(2), 176–182. https://doi.org/10.1038/ejhg.2016.146.

Uitto, J. (2011). Glycine substitution mutations in the COL7A1 gene: Implications for inheritance of dystrophic epidermolysis bullosa-dominant vs. Recessive. *Acta Dermato-Venereologica*, *91*(3), 259–261. https://doi.org/10.2340/00015555-1131

Vahidnezhad, H., Youssefian, L., Saeidian, A. H., & Uitto, J. (2019). Phenotypic spectrum of epidermolysis bullosa: The paradigm of syndromic versus non-syndromic skin fragility disorders. *Journal of Investigative Dermatology*, *139*(3), 522–527. https://doi.org/10.1016/j.jid.2018.10.017

Van den Akker, P. C., Jonkman, M. F., Rengaw, T., Bruckner-Tuderman, L., Has, C., Bauer, J. W., Klausegger, A., Zambruno, G., Castiglia, D., Mellerio, J. E., McGrath, J. A., van Essen, A. J., Hofstra, R. M. W., & Swertz, M. A. (2011). The international dystrophic epidermolysis bullosa patient registry: An online database of dystrophic epidermolysis bullosa patients and their COL7A1 mutations. *Human Mutation*, *32*(10), 1100–1107. https://doi.org/10.1002/humu.21551

Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, *38*(16), e164. https://doi.org/10.1093/nar/gkq603

Wertheim-Tysarowska, K., Sobczyńska-Tomaszewska, A., Kowalewski, C., Skroński, M., Swięćkowski, G., Kutkowska-Kaźmierczak, A., Woźniakal, K., Bal, J. (2012). The COL7A1 mutation database. *Human Mutation*, *33*(2), 327–331. https://doi.org/10.1002/humu.21651.

Yan, Y., Meng, Z., Hao, S., Wang, F., Jin, X., Sun, D., Gao, H., Ma, X. (2018). Five novel COL7A1 gene mutations in three Chinese patients with recessive dystrophic epidermolysis bullosa. *Annals of Clinical & Laboratory Science*, *48*(1), 100–105.

Yenamandra, V. K., Vellarikkal, S. K., Chowdhury, M. R., Jayarajan, R., Verma, A., Scaria, V., Sivasubbu, S., Ray, S., Dinda, A., Kabra, M., Sharma, V., & Sethuraman, G. (2018). Genotype-phenotype correlations of dystrophic epidermolysis bullosa in India: Experience from a tertiary care centre. *Acta Dermato Venereologica*, *98*(9), 873–879. https://doi.org/10.2340/00015555-2929

Yenamandra, V. K., Vellarikkal, S. K., Kumar, M., Chowdhury, M. R., Jayarajan, R., Verma, A., Scaria, V., Sivasubbu, S., Ray, S. B., Dinda, A. K., Kabra, M., Kaur, P., Sharma, V. K., & Sethuraman, G. (2017). 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. *Journal of Dermatological Science*, *86*(1), 30–36. https://doi.org/10.1016/j.jdermsci.2016.12.020

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