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Whole exome sequencing in a sample of Peruvian patients diagnosed with epidermolysis bullosa

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Abstract

Background: Epidermolysis bullosa (EB) is a complex and heterogeneous dermatological disease. Four main types of EB have been described, each of them with distinct characteristics: EB simplex (EBS), dystrophic EB (DEB), junctional EB (JEB) and Kindler EB (KEB). Each main type varies in its manifestations, severity, and genetic abnormality.

Methods: We sought mutations in 19 genes known to cause EB and 10 genes associated with other dermatologic diseases in 35 Peruvian pediatric patients of a rich Amerindian genetic background. Whole exome sequencing and bioinformatics analysis was performed.

Results: Thirty-four of 35 families revealed an EB mutation. Dystrophic EB was the most frequently diagnosed type, with 19 (56%) patients, followed by EBS (35%), JEB (6%), and KEB (3%). We found 37 mutations in seven genes; 27 (73%) were missense mutations; 22 (59%) were novel mutations. Five cases changed their initial diagnosis of EBS. Four were reclassified as DEB and one as JEB. Inspection into other non-EB genes revealed a variant, c.7130C>A, in the gene *FLGR2*, which was present in 31 of the 34 patients (91%).

Conclusion: We were able to confirm and identify pathological mutations in 34 of 35 patients.

Keywords: dystrophic, epidermolysis bullosa, junctional, Kindler, sequencing, whole exome

Introduction

Epidermolysis bullosa (EB) is a heterogeneous group of rare congenital genetic disorders, characterized by skin fragility and blistering induced by minimal mechanical trauma or slight touch. Epidermolysis bullosa is classified using the "onion skin" hierarchical approach, in which four major types have been described: EB simplex (EBS), dystrophic EB (DEB), junctional EB (JEB) and Kindler EB (KEB). These are subsequently divided into several subtypes, depending upon severity, prognosis, and extra-cutaneous involvement [1,2]. Epidermolysis bullosa has a prevalence of 8.22 newborns per million births in the U.S. However, studies in other countries show a different prevalence, probably related to geographical and ethnic background [1,3]. In Latin America some reports point to an EB frequency similar to the described in the U.S. [4-7].

Epidermolysis bullosa is caused by mutations coding for structural proteins that give stability to the epidermis (keratin, cytoskeleton components) and the cutaneous basement membrane zone (hemidesmosomes, anchoring filaments). These structures link keratinocytes to the underlying basement membrane and the connective tissue.[8]. De novo mutations have been mainly reported, but inherited mutations can also be found.

Epidermolysis bullosa simplex is characterized by blistering at the epidermal level. It is the most frequent and least severe EB type in the global

population (70-80%), [1,9]. Currently four major subtypes and 17 minor subtypes exist. Some rarer forms of EBS are syndromic, associated with pyloric atresia, muscular dystrophy, and other traits [1]. Most patients have an autosomal dominant) mode of inheritance and have mutations in the *KRT5* and *KRT14* genes, but mutations in *EXPH5* and *TGM5* have been described too [1,8].

Dystrophic epidermolysis bullosa is reported as the second-most frequent type of EB and its phenotype can be mild, intermediate, or severe. Two major subtypes, autosomal dominant and autosomal recessive, have been described. Recessive EB (RDEB) is often more severe, affecting the whole body with blisters, whereas dominant DEB (DDEB) is less severe, affecting hands, feet, knees, and elbows [1]. Only *COL7A1* mutations have been reported in this type of EB.

Junctional Epidermolysis bullosa, in contrast, is mostly inherited in an autosomal recessive manner and more profoundly affects the skin. Since the lamina lucida is affected, both skin and mucosa may be compromised. Most patients have mutations in the *LAMB3* or *COL17A1* genes, but mutations involving *ITGB4*, *LAMA3*, or *LAMC2* have also been reported [1,10]. Lastly, KEB is the least frequent type and it is inherited in an autosomal recessive manner. Blistering occurs throughout the different layers of the skin and is associated with photosensitivity. All patients currently reported have mutations in the *FERMT1* gene [8].

Diagnosis is mainly achieved by clinical examination, skin biopsy for immunochemistry with fluorescent antibodies, and tests like transmission electron microscopy. Genetic analysis should be performed to get an accurate diagnosis and proper classification to clarify prognosis and facilitate genetic counseling [8]. Worldwide, several studies describing mutations in EB patients have been published. In Latin America, a few studies from Chile, Brazil, and Mexico have found autochthonous mutations, some recurrent; in some cases, founder mutations seem to arise from European populations [4,5,7,11,12].

In this study we sought to find the mutational spectrum of the different EB phenotypes in a sample of Peruvian patients referred to a main pediatric center in Peru, using whole exome sequencing and filtering with a selection of 29 genes associated to EB and other EB-like skin diseases.

Methods

We performed a cross-sectional study involving pediatric patients representing 35 independent families from the dermatology department at the Instituto Nacional de Salud del Niño (INSN Lima, Perú, the national reference center for pediatric diseases). Molecular genetic examinations were offered to these patients to establish accurate diagnosis, prognosis, and treatment recommendations [1,8]. We recruited patients by convenience, between June 2018 and May 2019, with known clinical and/or laboratory diagnosis of EB, using Has et al. criteria [1,2]. Data from patients (age, sex, family history) and laboratory tests (immunofluorescence, and transmission electron microscopy) were obtained from the clinical history of each patient in most cases.

DNA extraction, whole exome sequencing and bioinformatics analysis

We collected blood samples in 3ml EDTA tubes. DNA was extracted using the salting-out procedure with some modifications [13]. Samples were sent to Macrogen (Korea) for library preparation and whole exome sequencing using Paired End Sequencing Sure selectXT (Illumina Inc.). We received the raw data (fastq files) and proceeded to the bioinformatics analysis using an in-house protocol (Obispo et al. in preparation). Variants were analyzed after selecting 29 genes; 19 were related to EB and 10 were related to other dermatological diseases similar to EB (see **Table 1**). Reads were aligned to the GRCh37/hg19 human reference genome with BWA and variant calling and annotation was performed using VarScan, Variant Effect Predictor (VEP), and SnpEff/Sift tools. Extracted variants were eliminated if it had a frequency higher than 0.1% in control databases (ExAC, gnomAD, and 1000 Genomes Project, and ClinVar database). The selected variants were evaluated with in silico predictor for potential deleterious effects on protein functioning and

Table 1. *Selected genes used to perform variant search in patients of this study.*

Main epidermolysis bullosa type	Gene	Chromosomic location (hg19)	
Epidermolysis bullosa simplex	*DSP*	7541808	7586946
	JUP	39942964	39942964
	PKP1	201252580	201302121
	TGM5	43559055	43559055
	KRT5	52908359	52914328
	KRT14	39738531	39743147
	EXPH5	108376158	108464492
	KLHL24	183353398	183402307
	PLEC	144989321	145050913
	ITGA6	173291954	173371181
	ITGB4	73717516	73753899
	DST or BPAG1	56322785	56819426
Junctional epidermolysis bullosa	*LAMA3*	21269562	21535030
	LAMB3	209788215	209825820
	LAMC2	183147952	183214262
	COL17A1	105791046	105845638
	ITGA3	48133340	48167849
Dystrophic epidermolysis bullosa	*COL7A1*	48601506	48632593
Kindler syndrome	*FERMT1*	6055492	6104191
Other skin diseases	*CD151*	832952	838840
	CDSN	31082865	31088252
	CHST8	34112861	34264414
	CSTA	122044011	122060816
	DSG1	28898052	28937394
	FLG2	152321213	152332482
	KRT1	53068520	53074191
	KRT10	38974369	38978863
	PKP1	201252580	201302121
	SERPINB8	61637263	61656608

pathogenicity (i.e., SIFT, PolyPhen-2, MutationTaster2, Human Splice Finder).

Ethical considerations

We received recommendations and ethical approval from the "Comité de Ética de la Universidad de San Martín de Porres" (IRB IORB00003251 OHRP/FDA). Since all patients were under the age of 18 we received a signed informed consent from one or both parents at the time of the interview. A session for each patient was performed to clarify doubts about the procedure and to explain the results obtained.

Results

A total of 35 patients, ranging from newborn to 15 years of age, with clinical diagnosis of EB were analyzed using whole exome sequencing. We analyzed 19 EB causal genes and 10 genes related to other skin diseases. All patients were diagnosed in their first year of life following clinical exam, immunofluorescence microscopy, and transmission electron microscopy studies. We were able to confirm the clinical diagnosis in 34 out of 35 patients: 19 DEB (56%), 12 EBS (35%), two JEB (6%), and one (3%) case of KEB. In five events (14%), the results of the molecular analysis helped to change the diagnosis, all from presumptive cases of EBS to four events of DEB and one of JEB. In the studied group, we identified 16 dominant EB and 18 recessive EB cases. These were caused by 37 mutations involving 7 genes, of them, 22 were novel (59%). Of the initial 35 patients, EB29 remained without a molecular diagnosis. In **Figure 1**, the position of the mutations found in the most frequently affected genes (*COL7A1, KRT5, KRT14*) are depicted. Finally, we found that in 31 of 34 (91%) patients there was the variant c.7130C>A p.Ser2377Ter (rs12568784) in gene *FLG2*. (**Table 2**, **Table 3**)

Dystrophic epidermolysis bullosa

We found 19 patients with DEB, six had a single *COL7A1* mutation corresponding to DDEB. The other 13 patients showed at least two mutations in *COL7A1* suggesting a recessive form of RDEB. One of these

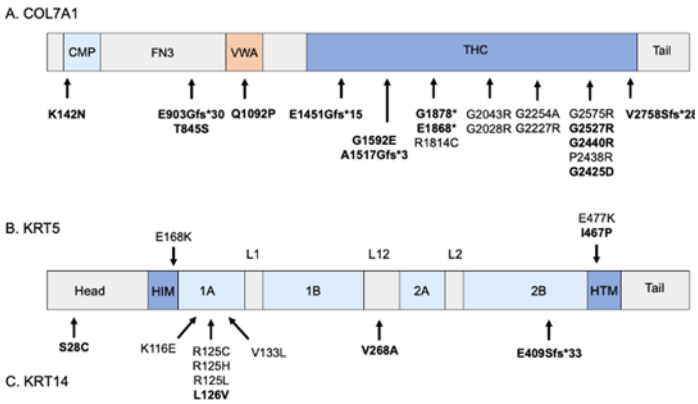

Figure 1. *Localization of mutations in **A)** COL7A1, **B)** KRT5 (upper 3), and **C)** KRT14 (lower 9). Mutations in bold are novel.*

CMP, cartilage matrix protein; Fn3, fibronectin III-like domain; HIM, helix initiation motif; HTM, helix termination motif; THC, triple helix collagenous domains; VWA, von Willebrand factor A domain.

cases (EB01) had three mutations (**Table 3**). In RDED, we identified nine individuals that were homozygous and four that were compound heterozygous.

We found 20 mutations in *COL7A1*, 16 (80%) corresponding to the "*triple helix collagenous domains*" (THC) region, nine (60%) of these were in a glycine residue. The fibronectin III-like domain (Fn3) was affected in two (10%) mutations and both the cartilage matrix protein (CMP) region, and the Von Willebrand factor A domain (VWA) were affected with one mutation, respectively (**Figure 1A**). Six truncating mutations (fs/stop codon) were found: four in the THC region and two in the Fn3 domain. Of the 20 mutations, we identified 14 that were novel. Finally, two cases of recurrent mutations, both present in a recessive form of DEB, were found. One was c.4550_4554delCCAAG (p.Ala1517GlyfsTer3) in three homozygote (EB25, EB27 and EB30) patients and one compound heterozygote (EB26) patient. The other mutation was c.5632G>T (p.Gly1878Ter), found in two cases; one was homozygous (EB05) and one was compound heterozygous (EB01).

Epidermolysis bullosa simplex

We found 12 patients with EBS, involving the gene *KRT14* and *KRT5* (seven and two cases, respectively) in dominant inheritance and one in a recessive fashion (EB36). Six *KRT14* mutations were in a commonly known hotspot, region 1A of the helix initiation motif. Another five mutations were within the critical region of amino acids 116-129 associated with the generalized severe EBS type. Lastly, one had a mutation in c.397G>T (p.Val133Leu) related to the localized EBS type (**Figure 1B**, **C**).

One mutation, c.1225delG (p.Glu409SerfaTer33), was a predicted truncation caused by a frameshift in codon 409 of the region 2B (EB16). The recessive event of *KRT14* showed a compound heterozygosity with a mutation c.373C>T (p.Arg125Cys) from the region 1A described previously, accompanied with a novel c.83C>G mutation at the head region.[14]. We also identified a case (EB12) of EBS associated with continuous palmoplantar hyperkeratosis, acanthosis, and basal vascularization. Gene analysis revealed a probable concurrence of EBS with hyperkeratosis with a heterozygotic mutation in two genes: c.502G>A(p.Glu168Lys) in *KTR5* and a novel mutation, c.359G>A (p.Gly120Asp in *KRT10*.

Another patient (EB33), presented as a syndromic case. This patient was born prematurely at 35 weeks due to fetal distress, to healthy non-consanguineous parents from a province (Huacho) 150 Km north of Lima, Peru's capital. The newborn had a dysmorphic face and aplasia cutis of both arms and legs, among other features. The molecular analysis found a homozygous novel change, c.1035G>A (p.Trp345Ter) in exon 11 of the *PLEC* gene. Finally, we did not identify EB mutations in patient EB29, born with dystrophic aplasia cutis, with localized EBS of hands and feet.

Junctional epidermolysis bullosa

We identified two cases of JEB. One case (EB04) was a homozygous novel mutation in *COL17A1*, c.2706dupC (p.Phe903LeufsTer62). Her parents were consanguineous and came from the south Andean region of Peru. The other JEB case, was compound heterozygous for gene *LAMB3* with a previously reported mutation c.628G>A (p.Glu210Lys) in the domain IV of *LAMB3* and a novel mutation c.2011delC (p.Leu671TrpfsTer36) that truncates the protein in domain II.

Kindler epidermolysis bullosa

We found a case of KEB (EB02) in siblings of a consanguineous couple, the homozygous mutation (c.676delC) in the *FERMT1* gene causing a reported frameshift and protein truncation (p.Gln226SerfsTer26).[15].

Distribution of *FLGR2* in epidermolysis bullosa patients

We found a variant of the gene *FLGR2*, c.7130C>A p.Ser2377Ter present in 31 of our 34 EB patients (91%); 15 were homozygous and 16 were heterozygous. This variant (rs12568784) has a minor allele frequency of 0.26 in Latin Americans of European and Native American origin (the closer proxy population at NCBI). In our EB cohort the frequency of this SNP was 0.68.

Discussion

We report the molecular analysis of 35 unrelated Peruvian families affected with EB. We registered 37

different mutations in 34 patients involving 7 of the genes known to cause epidermolysis bullosa (*COL7A1, KRT14, KRT5, COL17A1, PLEC, LAMB3, FERMT1)*. We found 22 (59%) novel mutations. We could not identify a causal mutation associated with EB in one case (EB29). It is possible that our technology was not able to detect, for example, an intronic mutation. The high proportion of novel mutations probably reflects the poorly studied genetic background in the Peruvian population with an average of 70% Amerindian ancestry depending on the geographic origin in the country. A particularly high proportion of indigenous ancestry is found, that is not present in most countries of Latin America [16].

Epidermolysis bullosa simplex is frequently described as the most prevalent type but in our sample the majority of cases belonged to DEB instead. A previous study from Torres et al. (2017), depicted clinical and epidemiological data from a group of 93 Peruvian patients registered at the INSN from 1993 to 2015 [17]. They already identified DEB as the predominant form of EB in Peru (DEB 44.1% versus EBS 41.9%) and we corroborate this previous observation. A similar high frequency of DEB (66.7%) has been reported in neighboring Brazil [7].

We found 6 DDEB and 13 RDEB caused by 20 distinct mutations in *COL7A1*, 14 of them novel. Of interest is the presence of two novel and recurrent mutations in RDEB. The first mutation is c.4550_4554delCCAAG (p.Ala1517GlyfsTer3) with a frequency less than 0.001% for the Americas and in no other continent (NCBI gnome Exome America). Thus, it probably is an autochthonous mutation. This mutation was present in three severe DEB homozygous cases and in one compound heterozygous case with c.7274G>A (p.Gly2425Asp) exhibiting intermediate DEB. The second recurrent mutation is the novel c.5632G>T (p.Gly1878Ter) also associated with severe RDEB either in homozygous or heterozygous. A study of mutations in 64 DEB patients in the neighboring Andean country of Chile, pointed to a frequency of 70% of recurrent mutations c.6527dupC (42%) and c.7708delG (28%) in *COL7A1*. The c.6527dupC mutation is a frequent finding in DEB patients from Andalusia, Spain, suggesting a founder effect from European migration [4,18,19]. Owing to the geographical vicinity we expected to find both recurrent *COL7A1* mutations, but they were not present in our sample.

Regarding EBS, most pathogenic mutations occur predominantly in *KRT5* and *KRT14* with roughly similar proportions [20,21]. In our sample, more patients (8/12 patients) had *KRT14* mutations, compared with three patients who bore a *KRT5* mutation and a single case with *PLEC* mutation. This proportion differs from what is described elsewhere, such as Brazil and Korea, and probably relates to the low number of samples in our study [7,22].

The Helix initiation motif is known to be frequently affected in EBS caused by *KRT14* mutations spanning residues 116-129/130-133 in region IA, which dysregulates keratin filament assembly [23]. Six of the nine patients with mutations in *KRT14* exhibited mutations in this region, including the novel mutation c.376C>G (p.Leu126Val) in patient EB06. The other mutations have been reported in Brazil, Poland, China, and Korea [9,21,24]. We are not able at this moment to elucidate if there is a common ancestry of Peruvian patients with the same mutations or if the finding of recurrent mutations at this hotspot is coincidental. We have two cases of autosomal dominant EBS with mutations in *KRT5* (EB22 and EB38), both corresponding to the protein region two B; one (EB22), was previously reported in a case of generalized severe EBS and the other is a novel mutation [14].

We studied a patient with EBS and hyperkeratosis (EB12), bearing mutations in the *KRT5* gene at Gly 168 and a mutation c.359G>A (p.Gly120Asp) in the *KRT10* gene associated with hyperkeratosis. Regarding *KRT5*, substitution of the highly conserved residue among different species, the Glu168 site results in the most severe type of EBS (generalized, severe). The Glu168Asp variant found here has been reported in a German patient causing intermediate EBS, suggesting that other factors related to the protein structure, epigenetic changes, or ethnic background may result in distinct phenotypes [25].

We report the case of a newborn with EBS that presented with aplasia cutis of the arms and legs,

dysmorphic facies, and premature death within a few days of birth. Although an autopsy was not permitted by the parents, it is evident that abnormalities were not limited to the skin. As expected, this EBS newborn was a probable case of syndromic presentation and it was corroborated by a homozygous novel mutation c.1035G>A (p. Trp345Ter) in the *PLEC* gene. This gene codes for the plectin protein which acts as a cytoskeleton link protein on several tissues like muscle, skin, and nerves. The mutation reported is situated in the globular plakin-like domain, where EB with muscular dystrophy has been reported [26].

Two patients (EB07 and EB04) were classified as JEB. Patient EB04, with consanguineous parents, was found to be homozygous for a novel mutation in *COL17A1*. The other patient EB07, was heterozygous for two mutations in gene *LAMB3*, one previously reported missense and one novel frameshift. A study in Chile, found three splice and a 77 insertion as recurrent mutations in a sample of 23 JEB patients on *LAMB3*, suggesting that nearly 93% of alleles for affected patients would present these mutations. Another study, from Brazil, found a similar low proportion of JEB cases. In those cases, two novel frameshift mutations in *LAMB3* and *COL17A1* were reported [7].

We have only one familial case with two siblings, from consanguineous parents, diagnosed with KEB (EB02). Both showed a homozygous mutation in the *FERMT1* gene, previously reported by Chmel et al. in 2015 [15]. This mutation has been reported as heterozygous in a patient with German origin [15]. At the same position, an insertion (c.676insC) or duplication (c.676dup) has been reported in patients from several countries including Pakistan, Brazil, Albania, and Australia, suggesting that the nucleotide position 676 at *FERMT1* is a recurrent hotspot for mutation [27].

Incidentally, we found that in 31 (91%) cases the mutation c.7130C>A p.Ser2377Ter (rs12568784) in the gene *FLG2* was present. This gene codes for filaggrin2 which is located in the stratum corneum and binds to keratin intermediate filaments. In its absence, epithelial barrier defects are formed and an incomplete accumulation of keratin can occur [28]. This mutation allows a 50% more likelihood to develop atopic dermatitis [29]. We do not understand at this moment this bias towards a high prevalence of this particular mutation in our sample or its role in the EB phenotype. The frequency for this particular mutation fluctuates between 0.12 in Nordic countries to 0.50 in Siberians and east Asians (NCBI and 1000 genomes data). A reference for Latin American of European and Native American descent shows a proportion of 0.266. In our 35 patients, we estimated an allelic frequency of 0.66.

Conclusion

Our study presents cases of EB using the molecular approach. Since the INSN is the national public reference center for EB, we were able to assess patients from different Peruvian regions. This work allows us to further understand the disease in our country and to identify common mutations that could be targeted through therapies based in genetics. We also were able to improve therapies according to their genetic profile. For example, a patient (EB01) with a stop codon in *COL7A1,* was successfully treated with intravenous gentamicin during a hospitalization, a therapeutic option known to be beneficial in stop mutations in JEB and RDEB.

Our approach of studying 29 genes allowed the identification of 34 out of 35 EB cases (efficiency of 97%). Further, in five out of 34 cases we were able to refine the diagnosis, changing from presumptive EBS to four cases of DEB and one of JEB. Although this work involves a relatively small cohort of patients, it is the first report of its type in our country. Further studies should be performed to understand the recurrent mutations found and to understand the role of *FLG2* in the EB phenotype and clinical progression.

Potential conflicts of interest

The authors declare no conflicts of interest.

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Table 2. *Candidate mutations in patients with epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JEB) and Kindler epidermolysis bullosa (KEB).*

Diagnosis	Patient	Identification (rs)	Inheritance	Gene	Mutation	Domain (Protein)	SIFT[a]	Polyphen-2[b]	Ref
EBS	EB06	-	AD	*KRT14*	c.376C>G (p.Leu126Val)	Region 1A (116-129) HIM	Deleterious(0)	Probably damaging(0.997)	This study
EBS	EB08	rs58330629	AD	*KRT14*	c.374G>A (p.Arg125His)	Region 1A (116-129) HIM	Deleterious(0.02)	Possibly damaging(0.813)	[22]
EBS	EB16	-	AD	*KRT14*	c.1225delG (p.Glu409SerfsTer33)	Region 2B	-	-	This study
EBS	EB19	rs61027685	AD	*KRT14*	c.397G>T (p.Val133Leu)	Region 1A	Deleterious(0)	Probably damaging(0.996)	[30]
EBS	EB20	rs58330629	AD	*KRT14*	c.374G>T (p.Arg125Leu)	Region 1A	Deleterious(0)	Possibly damaging(0.584)	[31]
EBS	EB32	rs60338701	AD	*KRT14*	c.346A>G (p.Lys116Glu)	Region 1A	Deleterious(0)	Probably damaging(0.997)	[20]
EBS	EB34	-	AD	*KRT14*	c.803T>C (p.Val268Ala)	Region L12	Deleterious(0)	Probably damaging(0.990)	This study
EBS	EB36	rs60399023	AR (het)	*KRT14*	c.373C>T (p.Arg125Cys)	Region 1A (116-129) HIM	Deleterious(0.03)	Possibly_damaging(0.813)	[14]
		-	ar	*KRT14*	c.83C>G (p.Ser28Cys)	Head	Deleterious(0.03)	Probably_damaging(0.98)	This study
EBS	EB12	rs58619430	AD	*KRT5*	c.502G>A (p.Glu168Lys)	Head	Deleterious(0)	Probably damaging(0.997)	[32]
			AD	*KRT10*	c.359G>A (p.Gly120Asp)	-	Deleterious low confidence(0.05)	Probably damaging(0.964)	This study
EBS	EB22	rs59190510	AD	*KRT5*	c.1429G>A (p.Glu477Lys)	Region2B	Deleterious(0.01)	Probably damaging(0.954)	[33]
EBS	EB38	rs58288198	AD	*KRT5*	c.1399A>T (p.Ile467Phe)	Region2B	Deleterious(0)	Probably damaging(0.999)	This study
EBS*	EB33	-	AR (homo)	*PLEC*	c.1035G>A (p.Trp345Ter)	-	-	-	This study
JEB	EB04	-	AR (homo)	*COL17A1*	c.2706dupC (p.Phe903LeufsTer62)	-	-	-	This study
JEB	EB07	rs121912482	AR (het)	*LAMB3*	c.628G>A (p.Glu210Lys)	-	Tolerated(0.47)	Benign(0.1)	[34]
		rs1057516822	ar	*LAMB3*	c.2011delC (p.Leu671TrpfsTer36)	-	-	-	This study
KEB	EB02	rs748240859	AR (homo)	*FERMT1*	c.676delC (p.Gln226SerfsTer26)	-	-	-	[15]

*Syndromic; AD, autosomal dominant; AR, autosomal recessive; ar, autosomal recessive, lesser impact variant; EBS, epidermolysis bullosa simplex; het, heterozygous; HIM, helix initiation motif; homo, homozygous; JEB, junctional epidermolysis bullosa; KEB, Kindler epidermolysis bullosa; Ref, reference.

[a]Sorting intolerant from tolerant (SIFT), scores allow to predict if an aminoacid substitution could lead to a functional effect. Ranges from deleterious (0) to tolerated (1).

[b]Polymorphism Phenotyping v2 (PolyPhen-2), scores allow to predict if an aminoacid substitution can produce a structural or functional change in the protein. Ranges from benign (0) to probably damaging (1).

Table 3. *Candidate mutations in patients with dystrophic epidermolysis bullosa (DEB).*

Diagnosis	Patient	Identification (rs)	Inheritance	Gene	Mutation	Domain (Protein)	SIFT[a]	Polyphen-2[b]	Reference
DDEB	EB11	rs121912836	AD	*COL7A1*	c.6127G>A (p.Gly2043Arg)	THC	Deleterious(0)	Probably damaging(1)	[35]
DDEB	EB13	-	AD	*COL7A1*	c.8272delG (p.Val2758SerfsTer28)	Fn3	-	-	This study
DDEB	EB15	-	AD	*COL7A1*	c.6679G>A (p.Gly2227Arg)	THC	Deleterious(0)	Probably damaging(0.999)	[36]
DDEB	EB17	rs762162799	AD	*COL7A1*	c.6082G>A (p.Gly2028Arg)	THC	Deleterious(0)	Probably damaging(1)	[37]
DDEB	EB28	rs185142403	AD	*COL7A1*	c.7313C>G (p.Pro2438Arg)	THC	Tolerated(0.12)	Possibly_damaging(0.588)	[4]
DDEB	EB35	-	AD	*COL7A1*	c.7318G>A (p.Gly2440Arg)	THC	Deleterious(0)	Probably_damaging(1)	This study
RDEB	EB05	-	AR (homo)	*COL7A1*	c.5632G>T (p.Gly1878Ter)	THC	-	-	This study
RDEB	EB09	-	AR (homo)	*COL7A1*	c.6761G>C (p.Gly2254Ala)	THC	Deleterious(0)	Probably damaging(0.998)	This study
RDEB	EB18	rs561709623	AR (homo)	*COL7A1*	c.3275A>C (p.Gln1092Pro)	VWE	Deleterious(0)	Probably damaging(0.996)	This study
RDEB	EB21	-	AR (homo)	*COL7A1*	c.2708delA (p.Glu903GlyfsTer30)	Fn3	-	-	This study
RDEB	EB23	-	AR (homo)	*COL7A1*	c.4350_4351delTG (p.Glu1451GlyfsTer15)	THC	-	-	This study
RDEB	EB24	-	AR (homo)	*COL7A1*	c.426G>T (p.Lys142Asn)	CMP	Deleterious(0.02)	Probably damaging(0.979)	This study
RDEB	EB25	rs756897026	AR (homo)	*COL7A1*	c.4550_4554delCCAAG (p.Ala1517GlyfsTer3)	THC	-	-	This study
RDEB	EB27	rs756897026	AR (homo)	*COL7A1*	c.4550_4554delCCAAG (p.Ala1517GlyfsTer3)	THC	-	-	This study
RDEB	EB30	rs756897026	AR (homo)	*COL7A1*	c.4550_4554delCCAAG (p.Ala1517GlyfsTer3)	THC	-	-	This study
RDEB	EB10	-	AR (het)	*COL7A1*	c.5602G>T (p.Glu1868Ter)	THC	-	-	This study
		rs778035441	ar	*COL7A1*	c.5440C>T (p.Arg1814Cys)	THC	Deleterious(0)	Probably damaging(0.998)	[38]
DDEB	EB26	rs756897026	AR (het)	*COL7A1*	c.4550_4554delCCAAG (p.Ala1517GlyfsTer3)	THC	-	-	This study
		rs1458877386	ar	*COL7A1*	c.7274G>A (p.Gly2425Asp)	THC	Deleterious(0)	Probably_damaging(0.991)	This study
DDEB	EB31	-	AR (het)	*COL7A1*	c.4775G>A (p.Gly1592Glu)	THC	Deleterious(0)	Probably damaging(0.999)	This study
		-	ar	*COL7A1*	c.2534C>G (p.Thr845Ser)	Fn3	Tolerated(0.24)	Probably damaging(0.978)	This study
RDEB	EB01	rs760891216	AR (het)	*COL7A1*	c.7723G>A (p.Gly2575Arg)	THC	Deleterious(0)	Probably damaging(1)	[39]
		-	ar	*COL7A1*	c.7579G>A (p.Gly2527Arg)	THC	Deleterious(0)	Probably damaging(1)	This study
		-	ar	*COL7A1*	c.5632G>T (p.Gly1878Ter)	THC	-	-	This study

AD, autosomal dominant; AR, autosomal recessive; ar, autosomal recessive, lesser impact variant; CMP, cartilage matrix protein; DDEB, dominant dystrophic epidermolysis bullosa; DEB, dystrophic epidermolysis bullosa; Fn3, fibronectin III-like domain; het, heterozygous; homo ; homozygous; RDEB, recessive dystrophic epidermolysis bullosa; THC, triple helix collagenous domains; VWE, von Willdebrand factor A domain.

[a]Sorting intolerant from tolerant (SIFT), scores allow to predict if an aminoacid substitution could lead to a functional effect. Ranges from deleterious (0) to tolerated (1).
[b]Polymorphism Phenotyping v2 (PolyPhen-2), scores allow to predict if an aminoacid substitution can produce a structural or functional change in the protein. Ranges from benign (0) to probably damaging (1).