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Novel variants in *LAMA3* and *COL7A1* and recurrent variant in *KRT5* underlying epidermolysis bullosa in five Chinese families

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Abstract **Epidermolysis bullosa (EB) is a group of clinically and genetically heterogeneous diseases characterized by trauma-induced mucocutaneous fragility and blister formation. Here, we investigated five Chinese families with EB, and eight variants including a novel nonsense variant (c.47G>A, p.W16*) in *LAMA3*, a known recurrent variant (c.74C>T, p.P25L) in *KRT5*, 2 novel (c.2531T>A, p.V844E; c.6811_6814del, p.R2271fs) and 4 known (c.6187C>T, p.R2063W; c.7097G>A, p.G2366D; c.8569G>T, p.E2857*; c.3625_3635del, p.S1209fs) variants in *COL7A1* were detected. Notably, this study identified a nonsense variant in *LAMA3* that causes EB within the Chinese population and revealed that this variant resulted in a reduction in *LAMA3* mRNA and protein expression levels by nonsense-mediated mRNA decay. Our study expands the mutation spectra of Chinese patients with EB.**

Keywords epidermolysis bullosa; *LAMA3*; *COL7A1*; *KRT5*; Chinese families

Introduction

Epidermolysis bullosa (EB) is a group of clinically and genetically heterogeneous diseases characterized by trauma-induced mucocutaneous fragility and blister formation. EB can be grouped into categories, including EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and kindler EB (KEB). The EBS is the most common type of EB and is characterized by fragility within the epidermis. The EBS with mottled pigmentation (EBS-MP; OMIM 131960) is a rare subtype of EBS characterized by intraepidermal blistering and progressive reticular hyperpigmentation and is caused by mutations in keratin 5 (*KRT5*). The JEB is characterized by blistering within the lamina lucida of the basement membrane zone (BMZ) and can be caused by mutations of genes, including type XVII collagen (*COL17A1*), laminin subunit α3 (*LAMA3*), laminin subunit β3 (*LAMB3*), laminin subunit γ2 (*LAMC2*), integrin α6β4 (*ITGA6* and *ITGB4*), and the integrin α3 subunit (*ITGA3*). The JEB can be broadly divided into Herlitz (OMIM 226700) and non-Herlitz (OMIM 226650) types on the basis of severity and survival years. The Herlitz JEB is severe and usually cannot survive past the first years of life [1,2]. The laryngo–onycho–cutaneous syndrome (LOCS; OMIM 245660) is a subtype of JEB that primarily affects the Punjabi Muslim population and is characterized by prominent skin and mucosal granulation tissue, leading to delayed wound healing, laryngeal obstruction, and blindness. The bi-allelic mutations located in the *LAMA3A*-specific exon, such as c.169C>T [3,4] and c.151dupG, have been reported to cause LOCS [5]. The DEB (OMIM 226600) is characterized by blisters formed beneath the BMZ and can be caused by mutations in the type VII collagen gene (*COL7A1*). The KEB (OMIM 173650), the rarest type of EB, is characterized by blistering on multiple levels within and beneath the BMZ and is caused by mutations in the fermitin family member 1 gene (*FERMT1*) [6].

EB is extremely rare and has a prevalence of less than 1 case per 2000 individuals [7], and differences about the

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prevalence of specific EB subtypes among different geographical areas have been revealed. However, the clinical features of EB are not yet fully understood among the Chinese population due to the limited reports of patients with EB [8,9].

Results

Five Chinese families with EB were investigated. Clinical information was collected, and written informed consent was obtained. Genetic testing was performed in accordance with the *Helsinki Declaration* and approved by the Peking Union Medical College Institutional Review Board. Variants were screened by whole-exome sequencing (WES) and confirmed by Sanger sequencing. Clinical manifestations of 7 patients with EB and 8 different pathogenic variants of three genes (i.e., *LAMA3, COL7A1,* and *KRT5*) are summarized in Table 1.

Family 1

In Family 1, the proband is a 42-year-old male. He had severe fingernail onychodystrophy (Fig. 1A(a)) ; extensive mutilating erosions and scars involving extremities, elbows, and orofacial regions; and hypertrophic scars in the armpit (Fig. 1A(b)). Intraoral examination revealed severe enamel hypoplasia, which presented with discolored, pitted teeth, hypodontia, and excessive carious lesions in his oral cavity (Fig. 1A(c)). The brother of the proband was also affected (denoted as II-2 in Family 1, Fig. 1B(a)) and had eyelid scarring and symblepharon, severe scars in his extremities and neck, especially the lower limb (Fig. 1A(d–f)). WES revealed a novel homozygous nonsense variant in the exon 1 of *LAMA3* (NM_000227.4: c.47G>A; NP_000218.3: p.W16*) in the proband and his affected brother, and the mother of the proband was found to be heterozygous for the nonsense variant (Fig. 1B(b)). Informed consent was obtained first, and the RNA/protein from the skin tissue of the proband and brother of the proband in Family 1 was collected and extracted to elucidate the effect of this nonsense variant on *LAMA3* expression level. Real-time quantitative PCR results showed that the *LAMA3A* (NM_000227.4) mRNA levels of the proband and brother of the proband in Family 1 were reduced to approximately 23% and 28%, respectively (Fig. 1B(c)). The Western blotting results also revealed that the protein levels of *LAMA3A* (NP_000218.3) in the proband and his brother were reduced remarkably compared with those of the normal individual (Fig. 1B(d)).

Families 2–4

In Family 2, the proband was an 11-year-old boy (denoted as Family 2-II-1 in Fig. 2A(a)). He suffered from generalized blistering since birth, and the skin of extremities was most severely affected. He presented with serious fingernail dystrophy. Examination revealed extensive blistering, wounds, and scarring in his neck, backs, hands, and elbows (Fig. 2B(a–c)). In Family 3, the proband was a 54-year-old man (denoted as Family 3-II-1 in Fig. 2A(b)). He was referred to the clinic with a chief complaint of nonhealing skin ulceration on the feet. He suffered from predominantly acral blistering since birth, scarring, erythema, ulceration, crust on his hands, feet, and elbows (Fig. 2B(d–f)). He had a history of esophageal strictures. The histopathological examination of the skin biopsy specimen showed the absence of squamous cell carcinoma. In Family 4, the proband was a 5-year-old girl (denoted as Family 4-II-1 in Fig. 2A(c)). Since birth, she suffered from intermittent blistering and excoriated lesions on her neck, abdomen, and extremities after minor trauma and healed with hypertrophic scars. Severe hemorrhagic blisters were present on her neck, and she suffered from dysphagia, nail dystrophy, and partial nail loss (Fig. 2B(g–k)). Novel compound variants in *COL7A1*, including a novel missense variant (NM_000094: c.2531T>A; NP_000085.1: p.V844E) in exon 19 and a frameshift deletion (c.6811_6814del, p.R2271fs) in exon 86 of *COL7A1*, were detected in Family 2. These novel variants were not present in population databases (ExAC no frequency). *In silico* analysis performed using SIFT, PolyPhen-2, Mutation Taster, M-CAP, and CADD predicted them as pathogenic variants. The proband was compound heterozygous for both variants and inherited the variant c.2531T>A from his mother and the variant c.6811_6814del from his father. Four known variants in *COL7A1* were detected in Families 3 and 4 (Fig. 2A). These variants included compound variants of a nonsense variant (c.8569G>T, p.E2857*), an 11 bp deletion (c.3625_3635del, p.S1209fs) in the proband of Family 3, and compound variants of a missense variant (c.6187C>T, p.R2063W) in exon 74, which was inherited maternally, and a *de novo* missense variant (c.7097G>A, p.G2366D) in exon 92 of *COL7A1* in proband of Family 4. The locations of variants identified in our study in the domains of COL7A1 protein are shown in Fig. 2C.

Family 5

In Family 5 (denoted as the pedigree in Fig. 3A), the proband (II-1) and her mother (I-2) suffered from blistering since birth, and the tendency to blister decreased with age. The additional mottled and reticulate macular pigmentation increased especially at the previous blistering sites. The variant c.74C>T (p.P25L) in *KRT5* (NM_000424.4) was detected in the proband and her mother, and the variant was inherited maternally in the family (Fig. 3B).

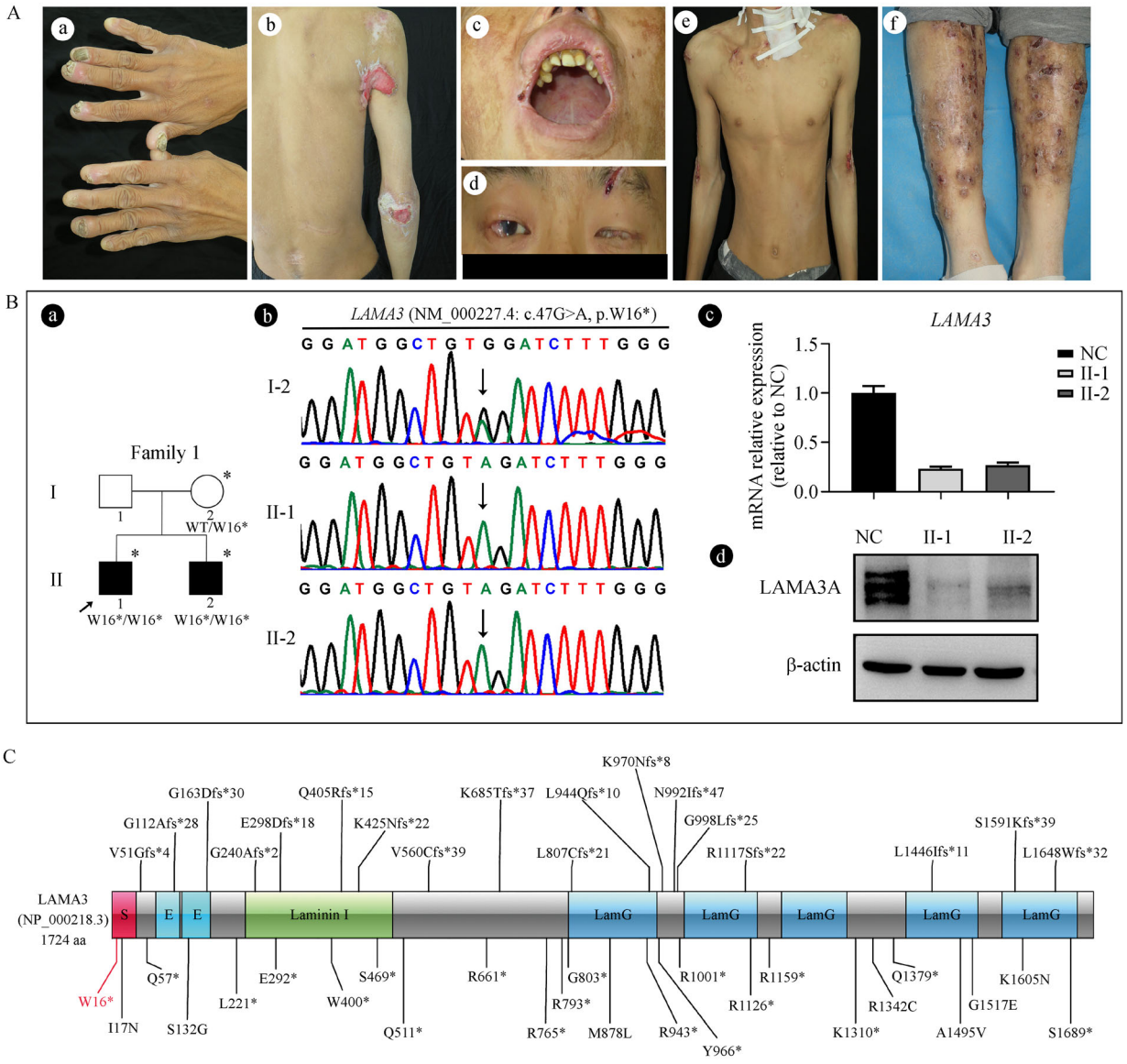

Fig. 1 Clinical features and molecular characterization of Family 1 with nonsense variant c.47G>A [p.W16*] in *LAMA3*. (A) Clinical manifestations of the affected proband (II-1) and proband's brother (II-2) in Family 1. (a–c) Clinical manifestations of proband (II-1): (a) Severe fingernail onychodystrophy, (b) blisters and scars on extremities and elbows and hypertrophic scars in the armpit, and (c) severe enamel hypoplasia (discoloration, pitted teeth, and hypodontia), excessive carious lesions in oral cavity, and erosions in orofacial regions. (d–f) Clinical manifestations of proband's brother (II-2): (d) Eyelid scarring and symblepharon in his eye margin, (e) scars on the extremities and neck, (f) severe scars widely distributed in his lower limb. (B) Molecular characterization of the nonsense variant c.47G>A [p.W16*] in *LAMA3*. (a) Pedigree with indicated genotypes of studied individuals (WT, wild type) in Family 1. Males, females, and affected individuals are represented by squares, circles, and shading, respectively, and arrow indicates the proband. Individuals who underwent genetic testing are marked with an asterisk (*). (b) Sequence chromatogram depicting the nonsense variant c.47G>A [p.W16*] of *LAMA3* in the pedigree, including mother of the proband (I-2), proband (II-1), and brother of the proband (II-2). (c) Real-time quantitative PCR results showing that the *LAMA3A* mRNA levels of the proband (II-1) and brother of the proband (II-2) in Family 1 were reduced to 23% and 28%, respectively, compared with the normal control (NC) individual. Each bar represents the mean ± standard deviation ($n = 4$). (d) Western blotting results revealing that the protein levels of *LAMA3A* transcript (170 kDa) in the proband (II-1) and brother of the proband (II-2) were reduced significantly compared with that of the normal control (NC) individual. β-actin (42 kDa) was used as a loading control. (C) Schematic of LAMA3 with identified variants to date. The nonsense variant c.47G>A [p.W16*] identified in our study was highlighted in red. (S, laminin N-terminal; E, laminin EGF-like.)

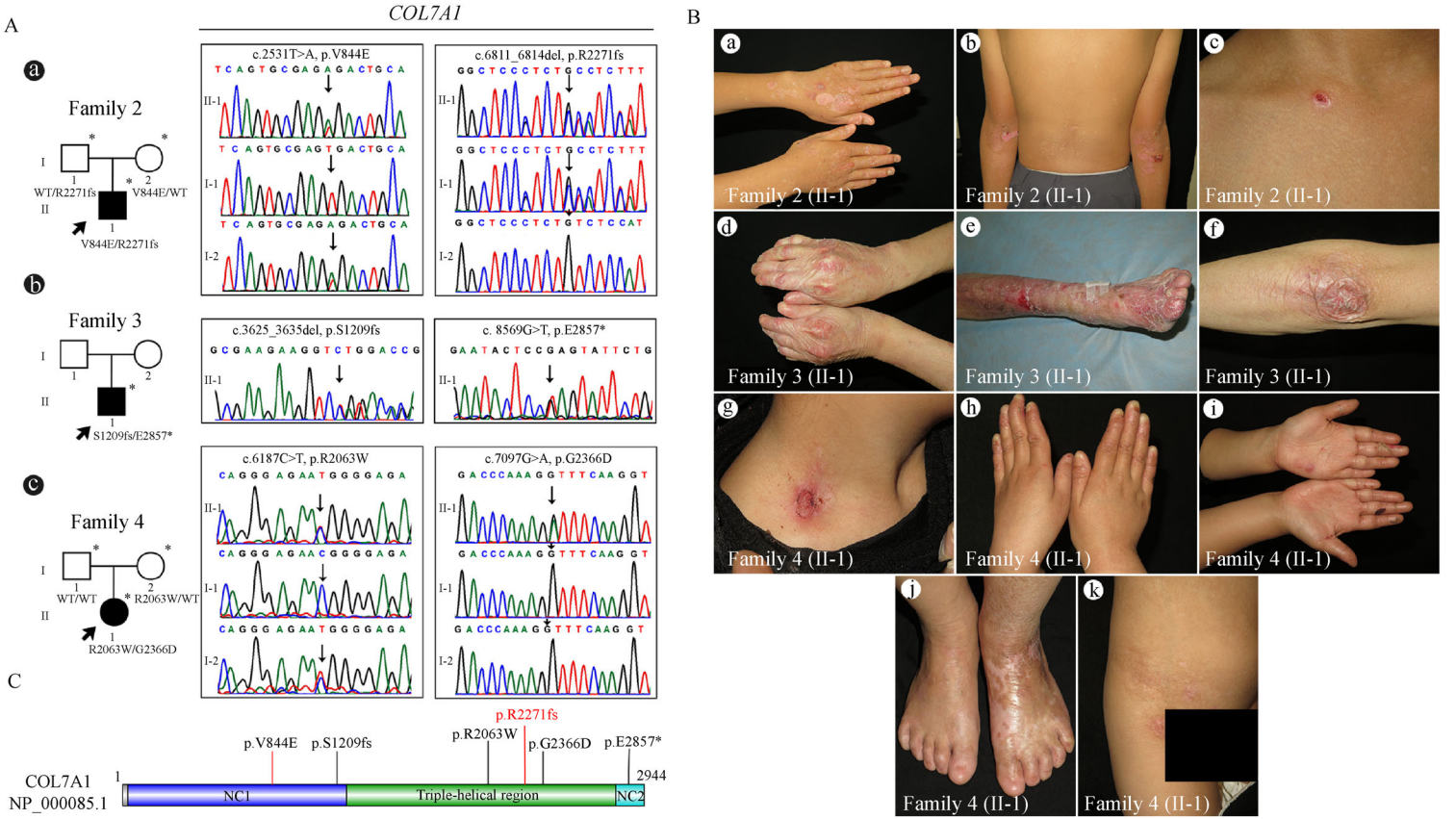

Fig. 2 Clinical features and molecular characterization of the Families 2, 3, and 4 with compound variants in *COL7A1*. (A) Pedigree with indicated genotypes of studied individuals (WT, wild type) in Families 2, 3, and 4. Males, females, and affected individuals are represented by squares, circles, and shading, respectively, and arrow indicates the proband. Individuals who underwent genetic testing are marked with an asterisk (*). Sequence chromatogram depicting the compound variants of the c.6811_6814del [p.R2271fs] and c.2531T>A [p.V844E] of *COL7A1* in Family 2, compound variants of the c.3625_3635del [p.S1209fs] and c.8569G>T [p.E2857*] of *COL7A1* in Family 3, and compound variants of the c.6187C>T [p.R2063W] and c.7097G>A [p.G2366D] of *COL7A1* in Family 4. (B) Clinical manifestations of affected probands of Families 2, 3, and 4: (a–c) Blistering on hands, elbows, and hemorrhagic blisters on neck of proband (II-1) in Family 2; (d–f) blistering, scarring, erythema, ulceration, and crust on hands, feet, and elbows of proband (II-1) of Family 3; and (g–k) severe hemorrhagic blisters in neck, blistering, and excoriated lesions on abdomen, hands, and feet of proband (II-1) in Family 4. (C) Overview of locations of variants identified in our study in the type VII collagen protein with the noncollagenous-1 (NC1), triple-helix (THD), and noncollagenous-2 domains (NC2).

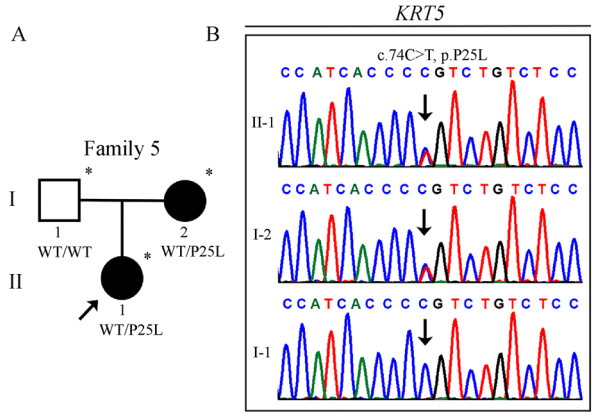

Fig. 3 Molecular characterization of Family 5 with missense variant in *KRT5*. (A) Pedigree with indicated genotypes of studied individuals (WT, wild type) in Family 5. Males, females, and affected individuals are represented by squares, circles, and shading, respectively, and arrow indicates the proband. Individuals who underwent genetic testing are marked with an asterisk (*). (B) Sequence chromatogram depicting the variant c.74C>T [p. P25L] in *KRT5* in the proband (II-1) and mother (I-2) of the proband.

Discussion

Laminin-332 genes, including *LAMA3*, *LAMB3,* and *LAMC2,* code for heterotrimeric noncollagenous glycoproteins consisting of α, β, and γ subunits that build anchoring filaments in the lamina lucida. To date, 52 *LAMA3* (NM_000227.4) mutations of EB (i.e., 16 nonsense mutations, 7 missense mutations, 9 splice site mutations, 19 frameshift mutations, and 1 gross deletion) have been identified (Fig. 1C). *LAMA3*, which is located on 18q11.2, contains 76 exons and 3 major alternative transcripts. These transcripts encode three laminin α3 polypeptides, i.e., α3a, α3b1, and α3b2. Two major transcripts, including *LAMA3A* and *LAMA3B*, encode laminin a3a and a3b1. *LAMA3A* is expressed from a promoter within intron 38, and its protein product is encoded by exons 39 to 76 (5175 bp open reading frame, encoding 1724 amino acids). *LAMA3B* is long and consists of exons 1 to 38 and the common 3′ exons 40 to 76, and exon 39 is skipped (10 002 bp open reading frame, encoding 3333 amino acids) [10]. Here, we identified a novel homozygous nonsense variant p.W16* located in the exon specific to the laminin α3a transcript (designated exon 1 of *LAMA3A*, NM_000227.4). Clinically, our case harboring this nonsense mutation most closely resembles LOCS particularly the symblepharon, leading to visual impairment, fingernail dystrophy, and laryngeal obstruction in our patients. Moreover, we reveal that the *LAMA3* homozygous nonsense variant remarkably reduces the *LAMA3A* mRNA and protein levels. Thus, the reduction in the *LAMA3A* may affect its function to the assembly competent and ability of making anchoring filaments extracellularly in the cutaneous BMZ, whereas about 30% of *LAMA3A* mRNA with the variant may escape from nonsense-mediated mRNA decay. Considering that the majority of *LAMA3* mutations reported in H-JEB patients are premature termination codon mutations located on *LAMA3A* and *LAMA3B* transcripts, the loss of laminin a3a and a3b expression is hypothesized to cause H-JEB and that the loss of a3a alone causes LOCS [5], which can explain the mild phenotype of patients with this

Table 1 Phenotype and molecular characterization of probands with epidermolysis bullosa from five Chinese families

Family	Phenotype	Gene	Inheritance
1	Scars on elbow and orofacial regions, dental enamel defects, nail defects, and hypertrophic scars in the armpit	*LAMA3*	AR
2	Nail dystrophy and loss with granulation tissue of nail beds and scarring and blistering on hands, neck, back hands, and elbow regions	*COL7A1*	AR AR
3	Scarring, erythema, ulceration, crust on hands, feet, and elbows; blistering on the joint of hands and feet; esophageal strictures	*COL7A1*	AR AR
4	Blistering and excoriated lesions on neck, abdomen, extremities and healed with hypertrophic scars; severe hemorrhagic blisters in neck; dysphagia; nail dystrophy; and partial nail loss	*COL7A1*	AR AR
5	Blistering from birth especially on friction sites, additional mottled or reticulate macular pigmentation typically of the previous blistering skin	*KRT5*	AD

Family	Variant type	Exon	cDNA change	Amino acid change	Reference
1	Homozygous	1	c.47G>A	p.W16*	Present study
2	Heterozygous Heterozygous	86 19	c.6811_6814del c.2531T>A	p.R2271fs p.V844E	Present study Present study
3	Heterozygous Heterozygous	27 116	c.3625_3635del c. 8569G>T	p.S1209fs p.E2857*	[20] [21,22]
4	Heterozygous Heterozygous	74 92	c.6187C>T c.7097G>A	p.R2063W p.G2366D	[18,19] [12]
5	Homozygous	1	c.74C>T	p.P25L	[11–17]

homozygous nonsense variant (c.47G>A, p.W16*) that only affects the *LAMA3A* transcript.

COL7A1 is a large gene located on chromosome 3p21.31 that comprises 118 exons coding for a pro-a1 (VII) procollagen polypeptide consisting of 2944 amino acids. Each pro-a1 (VII) polypeptide chain contains a central triple helical collagenous domain flanked by a large amino-terminal (NC-1) and a small carboxyl-terminal (NC-2) noncollagenous domains. We reported six variants (2 novel and 4 reported variants) in *COL7A1* from three patients. The novel missense variant is located at the NC1 domain and may affect its biological function to interact with BMZ components at one end and at the other end with type IV collagen in "anchoring plaques." The novel frameshift variant (c.6811_6814del, p.R2271fs) may interrupt the Gly-X-Y repeats within the crucial collagenous domain of COL7A1 protein.

EBS-MP is a rare subtype of EBS mostly caused by the missense variant of p.P25L in *KRT5*. Several reports about p.P25L in *KRT5* concerning multiple cases with European and Japanese origins are available [11–17]. Here, we report two patients with EBS-MP from a Chinese pedigree. A heterozygous p.P25L variant in the first exon of *KRT5* is identified in the proband and the proband's mother. To our knowledge, this study is the second report of a Chinese family harboring the recurrent variant. Our report extends the limited number of EBS-MP cases and provides further evidence that the *KRT5* variant is also responsible for this rare phenotype within the Chinese population.

Patients with EB show wide interfamilial clinical variability. However, the clinical features of EB are not yet fully understood among the Chinese population due to the rare reports of patients with EB. Here, clinical manifestations of 7 patients with EB and 8 different pathogenic variants of three genes (i.e., *LAMA3*, *COL7A1*, and *KRT5*) are summarized in Table 1 [11–22]. The genotype–phenotype relationship of known variants identified in our patients based on previous reports is summarized in Table S1 [19–23]. In this study, we characterize the genotypes and phenotypes in five Chinese families with EB. This study reported the homozygous nonsense variant in *LAMA3* that causes EB within the Chinese population and investigated its molecular characteristics, which will benefit our understanding of genotype–phenotype correlations.

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Compliance with ethics guidelines

Rongrong Wang, Liwei Sun, Xiaerbati Habulieti, Jiawei Liu, Kexin Guo, Xueting Yang, Donglai Ma, and Xue Zhang declare that they have no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the *Helsinki Declaration* of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-021-0878-x and is accessible for authorized users.

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