Correspondence

DOI: 10.5582/irdr.2025.01033

Clinical and genetic study of a family with epidermolysis bullosa simplex caused by a novel *KRT5* gene mutation c.987C>G (p.Asn329Lys)

Ruohan Zhang[1,§], Xu Chen[1,§], Zhenying Wang[1,*],Chao Xu[2], Shenhao Li[2]

[1] Department of Dermatology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan, Shandong, China;
[2] Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan, Shandong, China.

SUMMARY: This study investigated the association between the novel *KRT5* gene mutation c.987C>G (p.Asn329Lys) and the clinical phenotype of epidermolysis bullosa simplex (EBS), to provide a basis for the molecular diagnosis and genetic counseling of EBS. Clinical data were collected from a 20-year-old female patient. Whole-exome sequencing was performed on the proband, 5 affected family members, and 2 healthy family members, with mutations verified by Sanger sequencing. Functional prediction was conducted using SIFT, PolyPhen-2, and MutationTaster, while conservation analysis was performed using ConSurf and NCBI CDD databases. The pathogenicity of the mutation was evaluated according to the 2015 ACMG guidelines. Results showed that the proband and all affected family members carried the heterozygous *KRT5* gene mutation c.987C>G (p.Asn329Lys), while healthy members did not, consistent with autosomal dominant co-segregation. This mutation was not recorded in databases such as gnomAD, indicating it is a novel mutation. Functional prediction showed SIFT score 0.00 (damaging), PolyPhen-2 score 1.000 (PROBABLY DAMAGING), and MutationTaster classification as "Deleterious". Conservation analysis confirmed that the 329th amino acid is located in the highly conserved Filament domain (ConSurf score 0.92, CDD E-value = 1.04e-158). The ACMG classification determined it as "Pathogenic". Affected family members exhibited a mild phenotype characterized by "friction-induced blisters, seasonal dependence, and scarless healing". The *KRT5* gene mutation c.987C>G (p.Asn329Lys) is a novel pathogenic mutation for EBS. Its unique phenotype enriches the genotype-phenotype spectrum of EBS and has important reference value for clinical practice.

Keywords: epidermolysis bullosa simplex, *KRT5* gene, novel mutation

1. Introduction

Epidermolysis bullosa (EB) is a group of rare hereditary skin fragility disorders characterized by increased fragility of the skin and mucous membranes. The core pathogenesis involves mutations in genes encoding key proteins of the epidermal-dermal junction structure, leading to decreased resistance of the skin to mechanical damage, with clinical manifestations including recurrent blisters, erosions, and wound healing abnormalities (*1*). EB is caused by mutations in genes encoding keratins, desmosomes, hemidesmosomes, or other intraepidermal or dermal-epidermal adhesion filaments, which are characterized by poor cell adhesion, lack of tissue repair or barrier function, resulting in varying degrees of blister and ulcer formation (*2,3*). Based on the ultrastructural changes of the skin and the level of blister formation (from top to bottom), EB is classified into four main types: epidermolysis bullosa simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler EB (KEB). Among them, EBS is the most common, accounting for approximately 70% of all EB cases (*4*). Epidermolysis bullosa is clinically and genetically heterogeneous, with inheritance patterns of autosomal dominant (AD) or autosomal recessive (AR) (*5*). EBS exhibits significant genetic heterogeneity, and identified pathogenic genes include *KRT14*, *KRT5*, *etc*. Among them, the *KRT5* gene encodes type II cytokeratin 5, a key structural protein in epidermal basal cells, which forms heterodimers with type I cytokeratin 14, assembles into an intermediate filament network, and anchors to the basement membrane through hemidesmosomes, providing mechanical stability to the epidermis (*6,7*).

KRT5 gene mutations are one of the main causes of EBS, most of which are dominant-negative missense mutations inherited in an autosomal dominant manner. The location of *KRT5* mutations is closely related to the severity of clinical phenotypes (*7*). Currently reported

pathogenic mutations in *KRT5* are mostly concentrated in the highly conserved rod domain, but discovery of new mutations still helps to further clarify the genotype-phenotype relationship of EBS. Significant progress has been made in clinical and genetic research on EB in recent years, and application of molecular diagnostic techniques has laid the foundation for accurate typing and genetic counseling. This study identified a novel mutation c.987C>G (p.Asn329Lys) in the *KRT5* gene through clinical and genetic analysis of a family with typical EBS phenotypes, and clarified its pathogenicity and clinical significance by combining functional prediction, conservation analysis, and family co-segregation verification.

2. Research design and data collection

The proband was a 20-year-old female who had recurrent blisters all over her body since birth, especially in friction-prone areas such as hands, feet, and extensor surfaces of joints (Figure 1). The blisters showed obvious seasonal dependence — frequent attacks in summer (when the temperature was above 30°C, 3–5 blisters per week) and significant reduction in winter (1-2 blisters per month). Skin lesions could heal spontaneously without scarring or pigmentation. Family survey showed that there were 5 affected individuals in 3 generations of the family (maternal grandmother, mother, younger brother, uncle, and cousin), all showing similar phenotypes.

Laboratory examination results were as follows: Physical examination revealed multiple blisters and broken surfaces on both feet, and scattered blisters on both lower limbs, without scarring or pigmentation. Direct immunofluorescence (DIF) showed negative IgG, C3, IgM, and IgA in the intercellular space of the epidermis and basement membrane, ruling out autoimmune bullous diseases. Skin biopsy showed hyperkeratosis, extensive subepidermal clefts and blisters, and sparse mononuclear cell infiltration in the superficial dermis. Transmission electron microscopy showed intraepidermal basal layer blisters and clefts, basement membrane and hemidesmosomes at the dermal edge, and a large number of tonofilaments arranged as homogenized masses, consistent with the ultrastructural characteristics of EBS. (Figure 2).

To clarify the pathogenic genotype, peripheral blood samples were collected from the proband, 5 affected family members, and 2 healthy members (father and aunt) (Figure 3). Genomic DNA was extracted for whole-exome capture and sequencing. Candidate variants were verified by Sanger sequencing in family members. Functional prediction was performed using SIFT, PolyPhen-2, and MutationTaster tools, while conservation analysis was conducted using ConSurf platform (parameters: HMMER E-value = 0.0001, MAFFT alignment) and the NCBI CDD database. Pathogenicity of the variants was evaluated according to the 2015 ACMG guidelines.

3. Key research findings

Whole-exome sequencing results showed that the proband had a heterozygous missense mutation c.987C>G (p.Asn329Lys) in the *KRT5* gene, which was located in the 1A exon, resulting in the substitution of

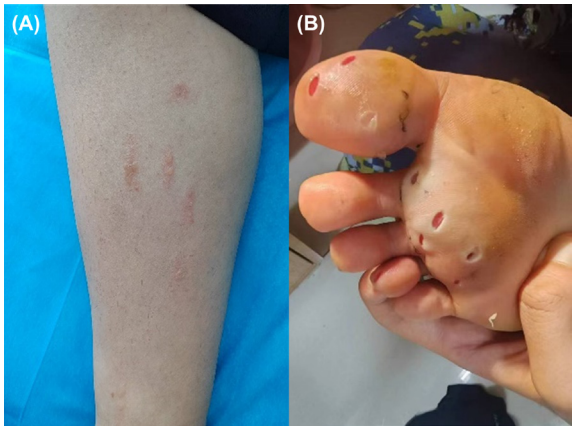

Figure 1. The patient's clinical manifestations. (A) Small blisters can be seen on the skin of the lower extremities after friction; **(B)** There are numerous blisters on both feet, and some of them are ruptured.

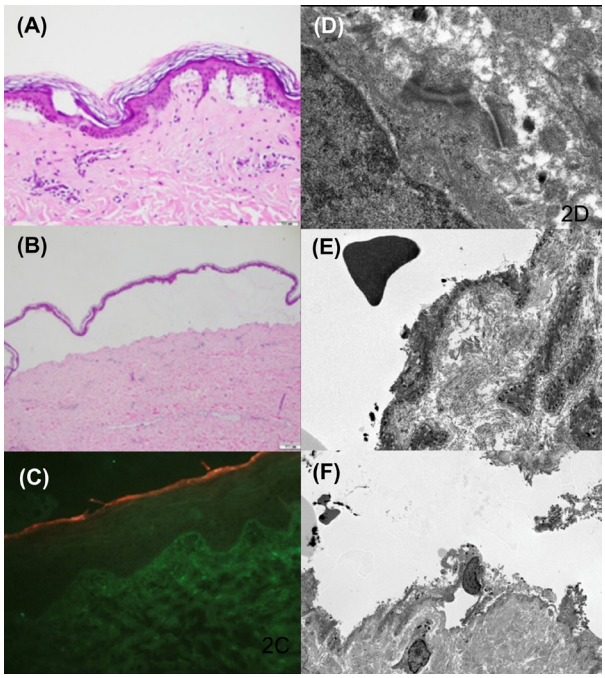

Figure 2. The patient's skin biopsy pathology, immunopathology and transmission electron micrographs. (A) Extensive cleavage can be observed beneath the epidermis. There is a sparse infiltration of mononuclear cells in the superficial layer of the dermis, and no obvious eosinophils are found. **(B)** Bulla formation can be seen beneath the epidermis. **(C)** By direct immunofluorescence, IgG, C3, IgM and IgA are negative among epidermal cells and in the basement membrane. **(D)** The basement membrane and complete desmosomes are visible at the dermal margin (DPx15.0k) **(E)** A large number of tonofilaments are arranged in a homogenized mass (DP3.0k). **(F)** Blisters and clefts are seen within the basal layer of the epidermis (DPx1.0k).

asparagine (N) with lysine (K) at the 329th amino acid (Figure 4). Sanger sequencing verification showed that all 5 affected family members carried this heterozygous mutation, while 2 healthy members (father and aunt) did not, completely consistent with the autosomal dominant co-segregation pattern (segregation ratio 1:1, $\chi^2 = 0.02$, $p = 0.89$). No record of this mutation was found in large-scale population databases such as gnomAD or in published literature, suggesting that it is the first reported novel mutation internationally.

Bioinformatics and conservation analysis further supported the pathogenicity of this mutation: In terms of functional prediction, SIFT tool scored 0.00 (determined to damage protein function), PolyPhen-2 scored 1.000 (determined as "PROBABLY DAMAGING"), and MutationTaster determined it as "Deleterious". The consistent results of the three tools strongly suggested that the mutation is harmful. Conservation analysis showed that the 329th amino acid had a conservation score of 0.92 (close to the full score of 1, belonging to a highly conserved site) calculated by the ConSurf platform. NCBI CDD database analysis confirmed that this site is located in the highly conserved Filament domain (167–480 amino acids) of the KRT5 protein, with an E-value of 1.04e–158 (approaching 0), indicating that it is subject to strong functional constraints during evolution and is the core functional region for keratin heterodimer assembly and intermediate filament network formation.

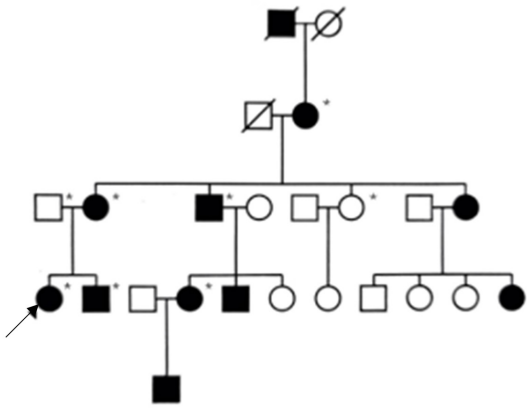

Figure 3. Patient's pedigree.

According to the 2015 ACMG guidelines for variant pathogenicity classification, this mutation met multiple pathogenicity criteria: It belonged to strong evidence (PS1) because the mutation was located in a known pathogenic functional domain (Filament domain) with no record of benign variants in this region; It belonged to moderate evidence (PM2) as it was not recorded in large-scale population databases and was an extremely rare variant; It belonged to supporting evidence (PP3) as multiple bioinformatics tools consistently predicted it to be harmful; It belonged to supporting evidence (PP4) as the patient's phenotype was completely consistent with EBS and family co-segregation was verified. Based on the above evidence, the mutation was clearly determined to be "Pathogenic" (Table 1).

Clinical phenotype analysis showed that all affected family members exhibited mild EBS characteristics. The impact of the disease on quality of life was mainly

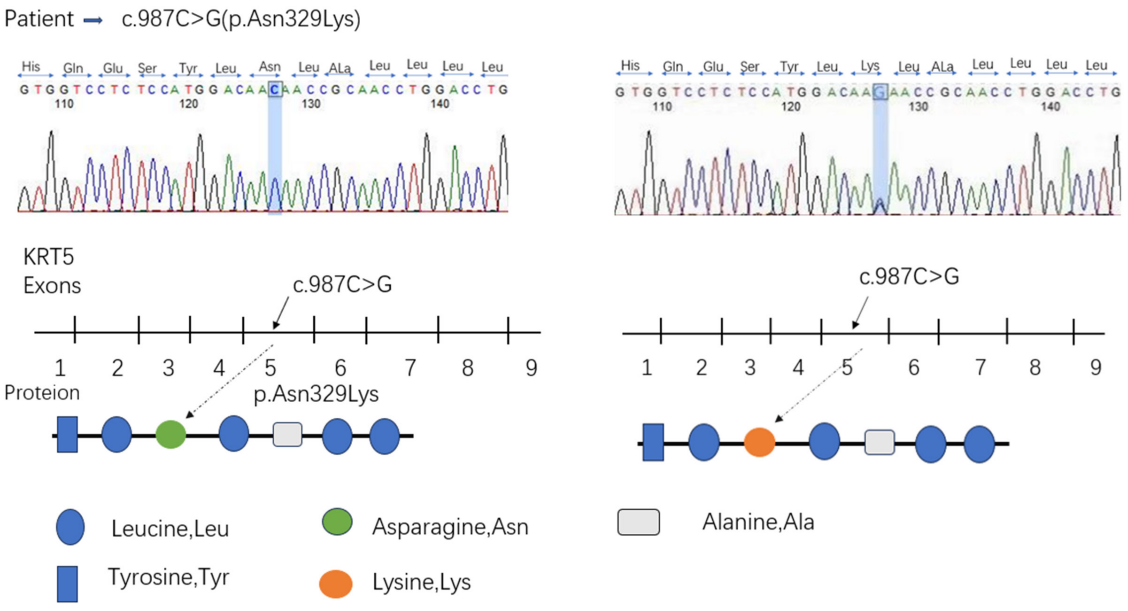

Figure 4. A missense mutation, specifically c.987C>G (p.Asn329Lys), was identified in the coding region of the keratin 5 (*KRT5*) gene in all patients. This mutation results in the substitution of polar uncharged asparagine with positively charged lysine at amino acid position 329 of keratin 5, thereby disrupting the native charge balance and hydrogen bond network. Such alterations impair keratin filament assembly and may lead to protein misfolding, consequently weakening the mechanical strength and barrier function of the skin and ultimately contributing to disease pathogenesis.

Table 1. ACMG evidence codes applied to evaluate KRT5 mutation pathogenicity in an epidermolysis bullosa simplex family

Evidence Type	Code	Criteria	Application to our mutation
Strong (P)	PS1	Located in a known pathogenic domain with no benign variants reported	Met: Mutation in *KRT5* Filament domain (core functional region with no benign variants)
Moderate (PM)	PM2	Absent/extremely rare in large population databases (*e.g.*, gnomAD)	Met: Not recorded in gnomAD or other databases (extremely rare)
Supporting (PP)	PP3	Consistent prediction of harmfulness by multiple bioinfor-matic tools	Met: SIFT (0.00), PolyPhen-2 (1.000), MutationTaster all predicted damage
Supporting (PP)	PP4	Phenotype matches the disease; variant co-segregates with phenotype in family	Met: Typical EBS phenotype; 5 affected family members carried the mu-tation, healthy members did not

reflected in social avoidance (such as refusing to participate in activities involving skin exposure like swimming due to skin appearance) and work restrictions (such as inability to engage in heavy physical labor), with no life-threatening complications.

4. Discussion

In this study, a novel mutation c.987C>G (p.Asn329Lys) in the *KRT5* gene was identified in an EBS family. Its pathogenicity was confirmed through multi-dimensional evidence, and a unique genotype-phenotype association was revealed, which holds significant implications for the clinical practice of EBS.

In terms of the pathogenic mechanism of the mutation, the Filament domain of the KRT5 protein contains four α-helical regions (1A, 1B, 2A, 2B). These regions assemble with KRT14 protein through hydrophobic interactions to form a stable intermediate filament network, which is crucial for maintaining the mechanical strength of the epidermis (*7*). The c.987C>G mutation identified in this study is located in the 1A helical region, causing the substitution of asparagine (a neutral amino acid) with lysine (a positively charged amino acid) at position 329. This change may affect protein function through two mechanisms: first, it disrupts the hydrophobic core structure of the α-helix, interfering with the formation of KRT5-KRT14 heterodimers; second, it alters the local charge distribution, undermining the stability of the intermediate filament network and reducing the resistance of basal cells to mechanical stress. This is consistent with the observation of "disordered arrangement of tonofilaments into homogenized masses" under transmission electron microscopy. Additionally, the 329th amino acid is highly conserved (all asparagine) across 12 species, further confirming its key role in maintaining keratin function and reinforcing the pathogenicity of the mutation.

Regarding genotype-phenotype correlation, the mild phenotypic characteristics caused by this mutation are closely related to its location and functional impact. Compared with classic mutations in the initial region of the 1A helix (such as p.Arg125Cys, which causes severe phenotypes with annual blister counts > 100), this mutation is located in the middle of the helix, exerting weaker interference on heterodimer assembly, thus resulting in a milder phenotype. This provides a new perspective for explaining the heterogeneity of EBS mucosal phenotypes.

In terms of clinical significance, the discovery of this novel mutation enriches the mutation spectrum of EBS, and its unique clinical phenotype offers important insights for clinical diagnosis and genetic counseling. In the early 1990s, prenatal diagnosis was performed using electron microscopy and/or fetal skin biopsy with IFM after 17 weeks of gestation (*8*). The main drawbacks of this technique include the possibility of sampling errors, the risk of miscarriage, and the emotional distress associated with terminating an affected fetus at an advanced stage. Prenatal diagnosis can also be accomplished by examining chorionic villus cells (usually around 10 weeks) or amniocentesis (usually around 16 weeks) (*9*). The accuracy of predicting postnatal EB diagnosis is over 98% (*10*). Many studies have attempted to isolate fetal 10lls from maternal blood. The successful implementation of this technology would enable prenatal diagnosis of EB from maternal blood samples as early as 6–7 weeks (*11*).

At the diagnostic level, for patients presenting with "season-dependent blisters, scarless healing, and mucosal involvement", priority should be given to detecting variations in the 1A helical region of the *KRT5* gene to improve the efficiency of molecular diagnosis. At the genetic counseling level, it is necessary to clearly inform family members about the autosomal dominant inheritance pattern of this mutation (50% risk of inheritance in offspring) and combine it with prenatal diagnostic technologies (such as chorionic villus sampling at 10 weeks of gestation) to provide a basis for family planning decisions. If the fetus carries the mutation, measures such as avoiding friction and high-temperature environments and wearing soft clothing after birth can reduce blister formation and significantly improve quality of life. For families

wishing to completely avoid genetic risks, prenatal testing and appropriate genetic counseling are integral to the management of EB patients and families at risk (*12*). Preimplantation genetic diagnosis (PGD) is a feasible option, and based on the clear mutation site in this family, the accuracy of PGD can be guaranteed.

The current treatment options for EB are mainly symptomatic, aiming to prevent mechanical damage, provide wound care, treat infectious complications, and address the external manifestations of the disease. To date, there is no cure for EB (*13*). In addition to basic symptomatic treatments, there are several emerging therapeutic approaches, such as gene therapy, cell therapy, protein replacement therapy, antisense oligonucleotides, and PCT interpretation (*14*). Emerging gene therapy research holds promise for the future. Current therapeutic research on EB includes the use of gene-corrected patient-specific iPS cells, gene editing technologies, and polymer-mediated DNA delivery systems (*15-17*). For epidermolysis bullosa (EB), the goal of gene therapy is to restore the function of skin structural proteins and enhance the mechanical strength of the skin. This can be achieved through various approaches, including gene addition, gene replacement, and gene editing technologies. The selection of gene vectors includes viral vectors, retroviral vectors, adeno-associated viral vectors (AAV), non-viral vectors, liposomes, and polymer vectors (*18*).

Gene editing technology CRISPR/Cas9: The CRISPR/Cas9 technology identifies specific DNA sequences through guide RNA (gRNA), and the Cas9 enzyme cleaves the DNA double strand to achieve precise gene editing. In EB research, CRISPR/Cas9 has been used to repair mutations in the *COL7A1* gene and restore its normal function (*19*). ALENs and ZFNs: These technologies achieve precise DNA cleavage and repair by specifically recognizing DNA sequences. TALENs and ZFNs have high specificity in gene editing, but their large molecular weight limits their application in viral vectors (*20*). In a 2006 study by Mavilio *et al*., the skin structure of patients was successfully repaired by introducing the *LAMB3* gene into the patients' epidermal stem cells (*21*). In studies on dystrophic EB, AAV vectors have been used to deliver the *COL7A1* gene into patients, and some patients have shown improved skin healing (*22*). However, this form of gene therapy also presents complex issues and risks, involving the technical development of gene vectors, carcinogenic potential, future risk of malignant tumors, and the duration of therapeutic effects. It is worth noting that there is currently no approved gene therapy for EB. The mutation in this study is located in the highly conserved Filament domain, and its clear functional localization provides a potential target for targeted intervention. For example, local gene repair strategies may alleviate symptoms by restoring the stability of keratin intermediate filaments.

The limitation of this study is the lack of *in vitro* functional experiments to directly verify the impact of the mutation on protein structure. In the future, mutant expression vectors can be constructed to observe the interaction with KRT14 and the assembly of intermediate filaments, thereby further clarifying the pathogenic mechanism.

In conclusion, the *KRT5* gene mutation c.987C>G (p.Asn329Lys) is a novel pathogenic mutation for EBS. The related research provides new evidence for understanding the genetic heterogeneity of EBS and has direct guiding value for clinical diagnosis and genetic counseling.

Acknowledgements

We sincerely thank all patients and researchers for their contributions to this study.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Kotalevskaya YY, Stepanov VA. Molecular genetic basis of epidermolysis bullosa. Vavilovskii Zhurnal Genet Selektsii. 2023; 27:18-27.
2. Torres-Iberico R, Condori-Fernández Y, Apagüeño-Ruiz C, Andia-Ticona M, Pomar-Morante R. Kindler syndrome: A multidisciplinary management approach. Actas Dermosifiliogr (Engl Ed). 2020; 111:775-780.
3. Uitto J, Bruckner-Tuderman L, McGrath JA, Riedl R, Robinson C. EB2017-Progress in epidermolysis bullosa research toward treatment and cure. J Invest Dermatol. 2018; 138:1010-1016.
4. Has C, Liu L, Bolling MC, Charlesworth AV, El Hachem M, Escámez MJ, Fuentes I, Büchel S, Hiremagalore R, Pohla-Gubo G, van den Akker PC, Wertheim-Tysarowska K, Zambruno G. Clinical practice guidelines for laboratory diagnosis of epidermolysis bullosa. Br J Dermatol. 2020; 182:574-592.
5. Youssefian L, Vahidnezhad H, Uitto J. Kindler syndrome. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2025.
6. Rossi S, Castiglia D, Pisaneschi E, Diociaiuti A, Stracuzzi A, Cesario C, Mariani R, Floriddia G, Zambruno G, Boldrini R, Abeni D, Novelli A, Alaggio R, El Hachem M. Immunofluorescence mapping, electron microscopy and genetics in the diagnosis and sub-classification of inherited epidermolysis bullosa: A single-centre retrospective comparative study of 87 cases with long-term follow-up. J Eur Acad Dermatol Venereol. 2021; 35:1007-1016.
7. Sprecher E. Epidermolysis bullosa simplex. Dermatol Clin. 2010; 28:23-32.
8. Holbrook KA, Smith LT, Elias S. Prenatal diagnosis of genetic skin disease using fetal skin biopsy samples. Arch Dermatol. 1993; 129:1437-1454.
9. McGrath JA, Dunnill MG, Christiano AM, Lake BD,

Atherton DJ, Rodeck CH, Pope FM, Eady RA, Uitto J. First trimester DNA-based exclusion of recessive dystrophic epidermolysis bullosa from chorionic villus sampling. Br J Dermatol. 1996; 134:734-739.
10. Pfendner EG, Nakano A, Pulkkinen L, Christiano AM, Uitto J. Prenatal diagnosis for epidermolysis bullosa: A study of 144 consecutive pregnancies at risk. Prenat Diagn. 2003; 23:447-456.
11. Jou HJ, Lo PH, Ling PY. Recent advances of microfluidic platform for cell based non-invasive prenatal diagnosis. Int J Mol Sci. 2023; 24:991.
12. Gonzalez ME. Evaluation and treatment of the newborn with epidermolysis bullosa. Semin Perinatol. 2013; 37:32-39.
13. Pânzaru MC, Caba L, Florea L, Braha EE, Gorduza EV. Epidermolysis bullosa-a different genetic approach in correlation with genetic heterogeneity. Diagnostics (Basel). 2022; 12:1325.
14. Bruckner-Tuderman L. Newer treatment modalities in epidermolysis bullosa. Indian Dermatol Online J. 2019; 10:244-250.
15. Kogut I, McCarthy SM, Pavlova M, Astling DP, Chen X, Jakimenko A, Jones KL, Getahun A, Cambier JC, Pasmooij AMG, Jonkman MF, Roop DR, Bilousova G. High-efficiency RNA-based reprogramming of human primary fibroblasts. Nat Commun. 2018; 9:745.
16. Kocher T, Peking P, Klausegger A, Murauer EM, Hofbauer JP, Wally V, Lettner T, Hainzl S, Ablinger M, Bauer JW, Reichelt J, Koller U. Cut and paste: Efficient homology-directed repair of a dominant negative KRT14 mutation via CRISPR/Cas9 nickases. Mol Ther. 2017; 25:2585-2598.
17. Mencía Á, Chamorro C, Bonafont J, Duarte B, Holguin A, Illera N, Llames SG, Escámez MJ, Hausser I, Del Río M, Larcher F, Murillas R. Deletion of a pathogenic mutation-containing exon of COL7A1 allows clonal gene editing correction of RDEB patient epidermal stem cells. Mol Ther Nucleic Acids. 2018; 11:68-78.
18. Shinkuma S. Advances in gene therapy and their application to skin diseases: A review. J Dermatol Sci. 2021; 103:2-9.
19. De Rosa L, Carulli S, Cocchiarella F, Quaglino D, Enzo E, Franchini E, Giannetti A, De Santis G, Recchia A, Pellegrini G, De Luca M. Long-term stability and safety of transgenic cultured epidermal stem cells in gene therapy of junctional epidermolysis bullosa. Stem Cell Reports. 2013; 2:1-8.
20. Hirsch T, Rothoeft T, Teig N, *et al*. Regeneration of the entire human epidermis using transgenic stem cells. Nature. 2017; 551:327-332.
21. Mavilio F, Pellegrini G, Ferrari S, Di Nunzio F, Di Iorio E, Recchia A, Maruggi G, Ferrari G, Provasi E, Bonini C, Capurro S, Conti A, Magnoni C, Giannetti A, De Luca M. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nat Med. 2006; 12:1397-1402.
22. Siprashvili Z, Nguyen NT, Gorell ES, Loutit K, Khuu P, Furukawa LK, Lorenz HP, Leung TH, Keene DR, Rieger KE, Khavari P, Lane AT, Tang JY, Marinkovich MP. Safety and wound outcomes following genetically corrected autologous epidermal grafts in patients with recessive dystrophic epidermolysis bullosa. JAMA. 2016; 316:1808-1817.

Received June 1, 2025; Revised August 13, 2025; Accepted October 29, 2025.

[§]These authors contributed equally to this work.

**Address correspondence to:*
Zhenying Wang, Department of Dermatology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 324 Jing Wu Road, Ji'nan 250021,Shandong, China.
E-mail: zywang@email.sdfmu.edu.cn

Released online in J-STAGE as advance publication November 21, 2025.