Identification of novel *KRT5* gene variants in two Chinese patients with sporadic form of epidermolysis bullosa simplex: A case report

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Abstract. Epidermolysis bullosa simplex (EBS), a rare genetic disorder characterized by fragile skin that is prone to blistering and tearing, is primarily caused by mutations in genes encoding keratin proteins, such as *KRT5* and *KRT14*. This study aimed to identify the pathogenic gene variants responsible for the sporadic form of EBS in two Chinese patients. Blood samples were collected from patients and their parents, and next-generation sequencing (NGS) was performed for variant screening. Two novel gene variants were identified within the *KRT5* gene: c.1399A>T (p.Ile467Phe) in patient 1 and c.1412G>A (p.Arg471His) in patient 2. These variants were absent in the unaffected parents and a control group of 100 healthy individuals. These two novel gene variants within the *KRT5* gene may be responsible for EBS, thus improving understanding of the genetic basis of EBS.

Introduction

Epidermolysis bullosa simplex (EBS), a mechanobullous genodermatosis characterized by skin fragility and blisters on the skin and mucous membranes, results from external trauma (1). EBS exhibits an incidence rate of 7.87/one million live births (2). The severity of EBS varies, ranging from mild blisters on the hands and feet to more generalized forms with extracutaneous involvement. Occasionally EBS can have a fatal outcome. The onset of EBS varies based on its subtype, typically manifesting at birth or during infancy (3). For accurate classification of EBS, it is imperative to consider the specific clinical features alongside molecular findings. A previous reclassification identified numerous clinical variants of EBS, including localized (previously referred to as Weber-Cockayne), intermediate (formerly known as generalized intermediate or Koebner) and severe EBS (previously termed as EBS Dowling-Meara) (4). The mildest, most common subtype is localized EBS, with a reported incidence of 3.67/one million live births (2). However, since a notable percentage of mild cases may remain undiagnosed, the actual incidence may be higher than that reported (2). One of the typical comorbidities associated with EBS is basal cell carcinoma, which primarily occurs in patients with the most severe form of EBS. This elevated risk of basal cell carcinoma in patients with most severe EBS is attributed to recurrent and chronic basal keratinocyte injury (5).

In EBS, gene aberrations have been implicated in seven genes, with 75% of the patients harboring mutations in genes encoding keratin 5 (*KRT5*) and *KRT14*, which are the primary cytoskeletal components of basal keratinocytes (6). The predominant genetic alterations are amino acid substitutions (missense variants). Inheritance typically follows an autosomal dominant pattern, with some exceptions. The clinical severity of EBS, in most cases, is associated with the specific loci of these genetic changes (7). Traditionally, diagnosis of EBS necessitated procedures such as skin biopsies, immunofluorescence microscopy and transmission electron microscopy. However, modern diagnostic strategies prioritize the identification of heterozygous pathogenic variants in *KRT5* or *KRT14* through molecular testing (8).

The present study describes two novel heterozygous *KRT5* gene variants from two patients with the sporadic form of EBS: c.1399A>T (p.Ile467Phe) and c.1412G>A (p.Arg471His). These individuals exhibited typical clinical features of intermediate and localized EBS, respectively.

Case report

Two patients consulted the Department of Dermatology in Suining Central Hospital (Suining, China) on August 2022. Patient 1 was a 43-year-old man with progressive trauma-induced blisters on the arms and legs since birth. Initially, the blisters appeared on legs at birth, followed by blister formation and skin erosion on arms and joints after a minor trauma. There were no signs of nail dystrophy, nail lysis or oral mucosal involvement. The patient presented with pruritus, although he had no apparent history or family history of similar disease (Fig. 1A). Patient 2 was a 31-year-old woman with erosions and blisters on the hands from 6 months of age. Typically, lesions healed without scarring or miliary formation. Clinical observation showed stable blistering on the palms and soles, with remission of

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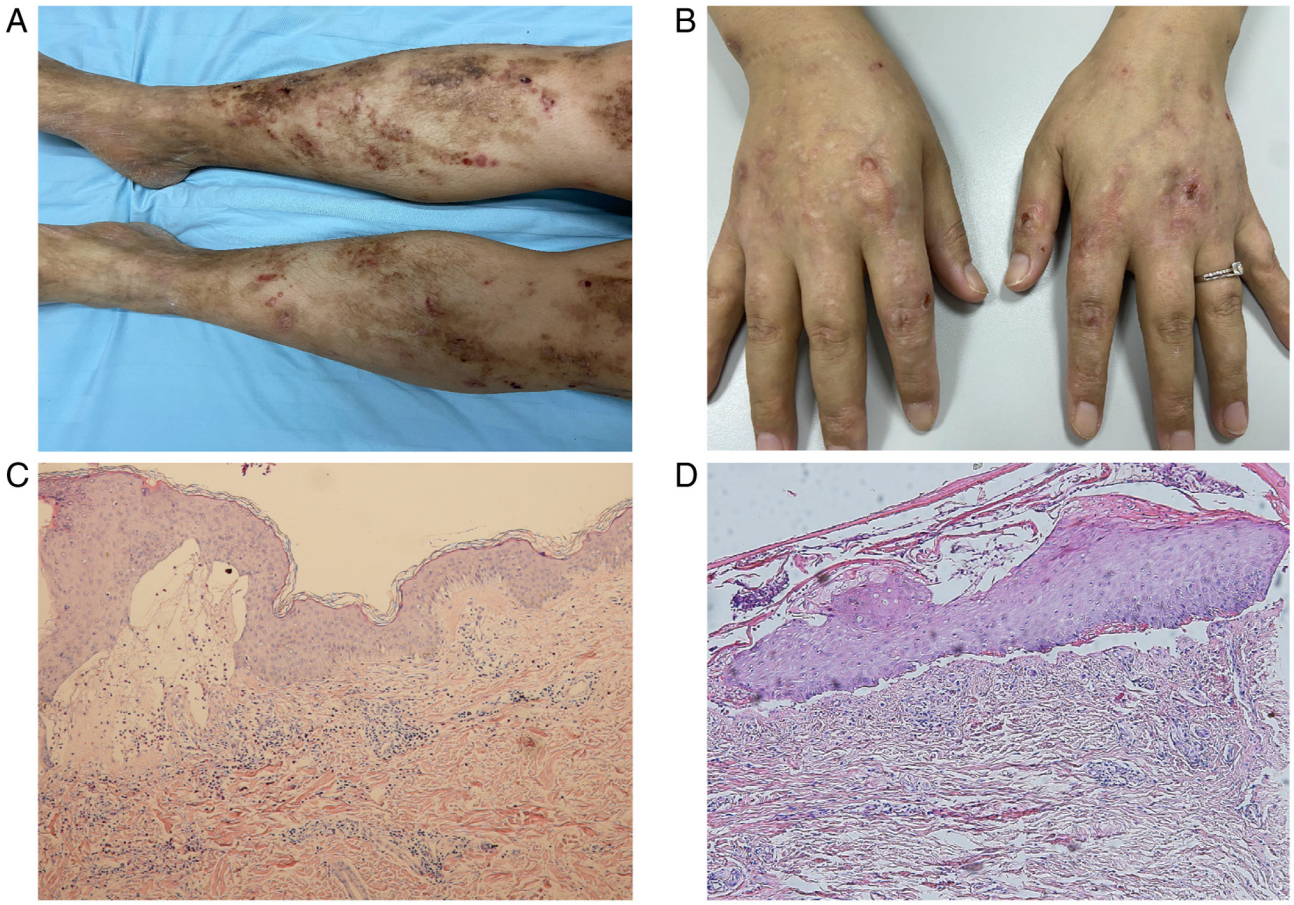

Figure 1. Representative images of the clinical features and histological examination of skin tissue of patients with epidermolysis bullosa simplex. (A) Image showing trauma-induced papules, scars and vesicles on the legs of patient 1. (B) Trauma-induced vesicles and skin erosion on the hands of patient 2. (C) Representative histological examination of the skin tissue from patient 1 showing subepidermal blisters surrounded by eosinophils and lymphocytes. (D) Representative histological examination of the skin tissue from patient 2 showing subepidermal blisters surrounded by neutrophils. Magnification, x100.

symptoms in autumn and winter. Patient 2 had no family history of blister disease (Fig. 1B). Skin lesions measuring 1.2x1.0x0.6 cm from patient 1 and 1.0x1.0x0.8 cm from patient 2 were excised for histopathological examination. The tissues underwent fixation in 4% neutral formalin at room temperature for 48 h, followed by dehydration with alcohol and xylene. Subsequently, they were embedded in paraffin at 62˚C and cooled. Serial sections of a 4-μm thickness were prepared and stained with hematoxylin for 5 min and eosin for 2 min at room temperature. Imaging was performed using light microscopy (Olympus BX51; Olympus Corporation). The histology report of patient 1 showed subepidermal blisters surrounded by eosinophils and lymph (Fig. 1C) while that of patient 2 showed subepidermal blisters surrounded by neutrophils (Fig. 1D). Informed consent was obtained from both patients and their parents, and ethics approval was granted by the Ethics Committee of the Suining Central Hospital. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

DNA was isolated from a 200-μl blood sample from each patient using the Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH,). Genomic DNA fragments were generated using a Covaris Ultrasonicator (Covaris, Inc.) and the DNA library was prepared. Exon capturing was performed using Streptavidin-Coated Magnetic Beads by NimbleGen (Roche NimbleGen, Inc.). The library quality was assessed by linear PCR amplification. Subsequently, next-generation sequencing (NGS), performed at Shanghai Anbailong Biotechnology, was conducted on an Illumina HiSeq X Ten platform (Illumina, Inc.) with a sequencing depth >200x and Q30>90% (9). The raw sequencing data was converted to FASTQ format and the reads were aligned with the human genome reference sequence (hg19) using Burrows-Wheeler Alignment to detect the genetic variants (9). All the identified variants were assessed by consulting various databases, such as the NCBI database of Single Nucleotide Polymorphisms (http://www.ncbi.nlm.nih.gov/SNP/), Online Mendelian Inheritance in Man (http://www.omim.org/), The Human Gene Molecular Biology Reports Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and NCBI ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

Sanger sequencing was performed for verification, targeting the potential pathogenic variants identified by NGS. *In silico* analysis tools, including PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (https://www.mutationtaster.org/), were employed to predict the impact of amino acid substitutions on KRT5 protein structure and function. From January 2021 to September 2021, a total of 100 unrelated healthy Chinese Han individuals (52% male,

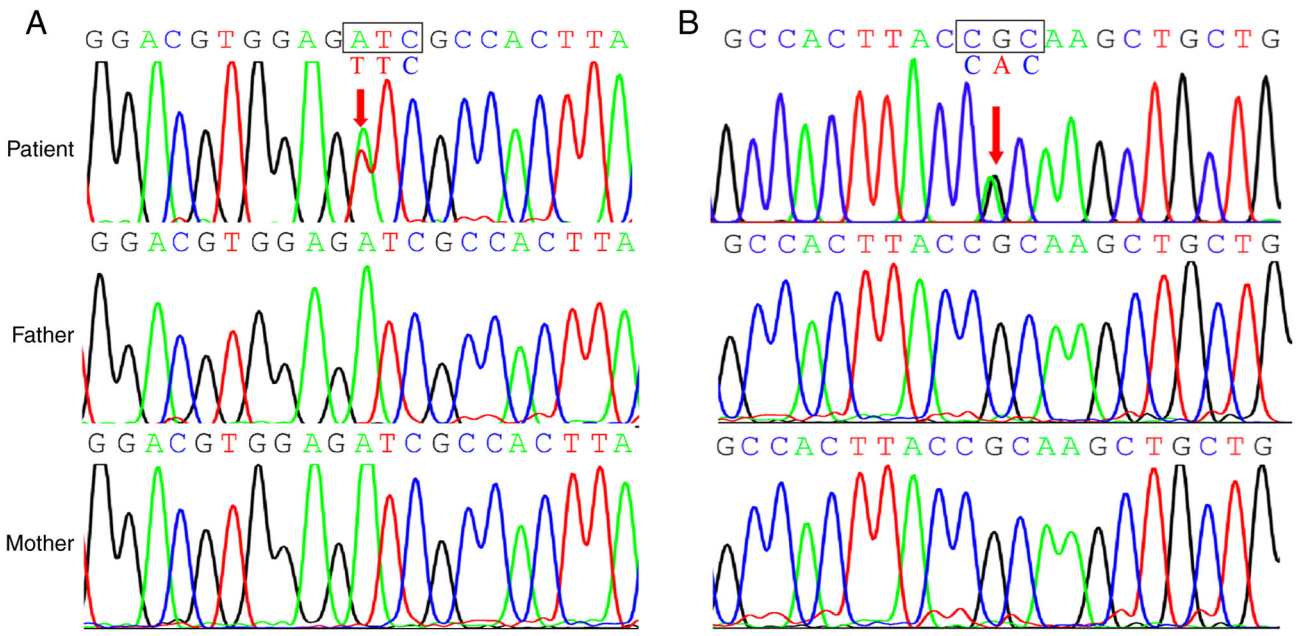

Figure 2. *KRT5* mutation analysis. (A) Sequencing analysis of patient 1 and his parents; arrow indicates mutation site with a missense mutation c.1399A>T (p.Ile467Phe) in exon 7 of the *KRT5* gene. (B) Sequencing analysis of patient 2 and her parents; arrow indicates the mutation site with a missense mutation c.1412G>A (p.Arg471His) in exon 7 of the *KRT5* gene. KRT, keratin.

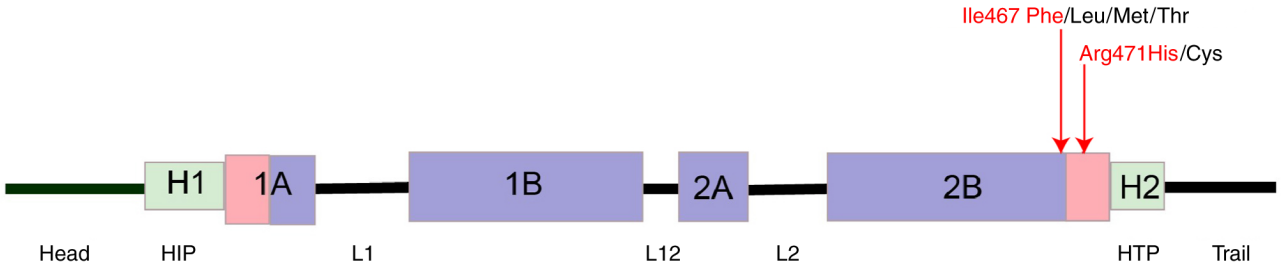

Figure 3. Domain information of *KRT5* gene. The mutations identified in the present study are highlighted in red. HIP, helix initiation peptide; HTP, helix termination peptide.

48% female; aged 18-30 years) consisted of the control group in Suining Central Hospital.

NGS analysis performed on the DNA isolated from the blood sample of patient 1 revealed a novel *KRT5* gene variant, c.1399A>T (p.Ile467Phe; Fig. 2A) within the 2B-domain of exon 7. This gene variant resulted in an amino acid substitution from isoleucine to phenylalanine at position 467 in the KRT5 protein. Notably, this variant was absent in both the unaffected parents and a control group of 100 healthy individuals. Patient 1 was diagnosed with intermediate EBS.

Similarly, patient 2, who was diagnosed with localized EBS, harbored a novel point mutation, c.1412G>A (p.Arg471His), in exon 7 of *KRT5* gene. This mutation was also within the 2B-domain (Fig. 2B) and resulted in amino acid substitution from arginine to histidine at position 471 in the KRT5 protein. PolyPhen2 prediction of pathogenicity revealed that both p.Ile467Phe and p.Arg471His variants were harmful. MutationTaster predicted that these two variants were 'disease-causing'. In addition, these variants were considered novel since they were not found in the ExAC (http://exac.broadinstitute.org/dbsnp), ESP (https://esp.gs.washington.edu/drupal/), 1000G (http://www.1000genomes.org/) and HGMD (http://www.hgmd.org) databases. In line with the American College of Medical Genetics and Genomics guidelines (10), both variants were classified as 'likely pathogenic', further supporting their potential role in the development of EBS and confirming the diagnosis.

For the two patients, the treatment approach primarily targeted symptom relief to enhance functionality and improve quality of life. Given the significant role of inflammation in EBS and its exacerbating effects on phenotypes, antibiotics and corticosteroids were administered to patient 1 while botulinum toxin was administered to patient 2 to decrease blistering in the affected surface area.

Discussion

KRT5 gene, located on chromosome 12qx, comprises nine exons spanning ~6.1 kilobases (11). The rod domain of this keratin protein, which is encoded by the *KRT5* gene, comprises four α-helical regions (1A, 1B, 2A and 2B) interspersed with three short linker sequences (L1, L12 and L2) at conserved regions (12). At the C-terminus of the 2B domain is a distinct sequence motif, TYRKLLEGE, which exhibits

near-perfect conservation across all intermediate filament proteins (13).

A previous study revealed a genotype-phenotype correlation exists in patients with EBS (14). Disease severity is associated with the physicochemical properties of the substituted amino acids and their location within the protein structure (15). Specifically, substitutions of highly conserved amino acids in the helix initiation or termination motifs disrupt heterodimerization of keratin 5 and 14 polypeptides resulting in severe EBS (4). Conversely, substitutions in other regions tend to result in milder clinical phenotypes of EBS (16).

The present study identified two novel variants in the *KRT5* gene: c.1399A>T (p.I467F) in patient 1 resulting in the amino acid substitution p.Ile467Phe and c.1412G>A (p.R471H) in patient 2 resulting in the amino acid substitution p.Arg471His. These two *KRT5* variants have not been reported in the ExAC, ESP, 1000G and HGMD databases. The *in silico* pathogenicity predictions for these variants using Polyphen-2 revealed that they were harmful. Furthermore, pathogenicity prediction using MutationTaster revealed that these two variants were pathogenic.

The p.I467F variant in patient 1 resulted in intermediate EBS while p.R471H variant in patient 2 led to localized EBS. Notably, these variants were situated within the 2B domain of the *KRT5* gene (Fig. 3), a well-established hotspot for variants (17). Similar substitutions, namely, p.I467L, p.I467M, and p.I467T, have been previously identified as pathogenic variants resulting in EBS-localized, EBS-generalized and EBS Dowling-Meara, respectively (15). Additionally, another similar substitution, p.R471C, was previously reported as a pathogenic variant resulting in EBS-generalized (15). The various clinical phenotypes associated with these substitutions may be attributed to their occurrence within the TYRKLLEGE motif of the KRT5 protein, which may cause the surface-exposed residues to be affected. This motif exhibits low tolerance for side chain modifications such that even minor alterations in side chain chemistry can lead to significant differences in EBS severity (13). A comprehensive analysis of phenotypical changes introduced by amino acid substitutions may reveal the genotype-phenotype association in EBS (18).

Early stage molecular genetic analysis is essential in patients suspected of having EBS as the results obtained can contribute to personalized treatment, thereby resulting in improved prognosis. A multidisciplinary approach, with recent advancements in medicine focusing on wound care, pain management, pruritus relief and nutritional support, is pivotal for the management of EBS (6). Restoration of skin integrity has become the primary goal of targeted therapy, including protein-, cell- and gene-based interventions for EBS (19). Topical calcipotriol and diacerein are potential drugs to enhance the healing of skin lesions in patients with EBS (20).

The present *KRT5* gene variants contribute to the expanding mutation spectrum of EBS. The present study identified two novel *de novo* heterozygous missense variants of *KRT5*, c.1399A>T (p.Ile467Phe) and c.1412G>A (p.Arg471His), within the 2B domain of *KRT5*. These findings not only broaden understanding of the underlying pathophysiology of EBS but also hold potential significance for genetic diagnosis, counseling and therapy. Furthermore, they also provide valuable insights into the phenotype-genotype association observed in EBS.

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Availability of data and materials

The data generated in the present study may be found in the NCBI database under accession number (PRJNA1054300) or at the following URL: http://www.ncbi.nlm.nih.gov/bioproject/1054300.

Authors' contributions

LL and CY designed the study and confirm the authenticity of all the raw data. LL conducted the genetic and bioinformatics analysis. HL and QL collected the clinical data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Suining Central Hospital (Suining, China; approval no. LLSNCH20200042), and written informed consent was obtained from all subjects.

Patient consent for publication

The patients provided consent for publication of their data and images.

Competing interests

The authors declare that they have no competing interests.

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