

Genetic diagnosis of a rare COL7A1 variant causing dystrophic epidermolysis bullosa pruriginosa through whole-exome sequencing

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Abstract. Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare subtype of inherited DEB. In the present study, whole-exome sequencing was conducted on 12 individuals from the same affected family and a rare heterozygous variation was identified in the collagen type VII, $\alpha 1$ (COL7A1) gene, namely c.6859G>A (p.Gly2287Arg). Subsequently, this heterozygous variant was confirmed using Sanger sequencing of individual plasma cell-free DNA (cfDNA) and it was demonstrated for the first time, to the best of our knowledge, that COL7A1 exons can be amplified from plasma cfDNA. Within the large pedigree examined, 14 out of 18 individuals carried the variant, 3 carried the wild type, and one exceptional case, III-9, showed no disease symptoms despite carrying the disease variant. A general association between genotype and

phenotype was established. Of note, the mutation landscape indicated that this G2287R variant is primarily reported in Asian countries. *In silico* structure prediction suggested that the residue resulting from the mutation may affect collagen protein stability. In conclusion, the present study provides evidence for the involvement of the COL7A1 G2287R gene variant in the development of DEB-Pr and highlights the potential utility of cfDNA in genetic disease diagnosis.

Introduction

Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare clinical subtype of inherited DEB and there is currently no specific treatment (1). All DEB-Pr variants reported so far are caused by mutations in the collagen type VII (COL7) gene, whose encoded protein is a major component of the anchoring fibrils in the dermo-epidermal junction. In addition, DEB-Pr shows genetic heterogeneity, as autosomal dominant [dominant DEB (DDEB)] and recessive [recessive DEB (RDEB)] inheritance patterns have been previously reported and current studies summarized that dominant DEB tends to have heterozygous glycine substitutions and recessive DEB have nonsense, frameshift or splice site mutations on both COL7, $\alpha 1$ (COL7A1) alleles (2). To date, >60 variants have been reported to be associated with DEB-Pr and glycine substitutions are the most common variants (3). Although all forms of DEB result from COL7A1 mutations, there is phenotypic variability and both intra- and inter-family variability have been reported (4-8).

Compared to skin microscopy and classical Sanger sequencing, next-generation sequencing-based whole-exome sequencing (WES) is a more accurate and sensitive technique and has shown promise in improving the diagnosis of DEB, particularly in the differential diagnosis from other types of EB and other genetic diseases (9-13). The existence of cell-free

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DNA (cfDNA) in human blood was first reported in 1948 (14). CfDNA and cell-free fetal DNA (cffDNA) are always present in plasma and there has been growing interest in prenatal diagnostics, as well as cancer diagnosis and surveillance (15,16). However, there are limited studies using cfDNA for DEB-Pr diagnosis.

Molecular investigation is extremely important for the comprehensive diagnosis of EB subtypes, disease prognosis, genetic counseling, and patient management. In the present study, WES technology was applied in a Chinese family with a four-generation pedigree of DDEB, and a heterozygous variation was identified in the COL7A1 gene. Using cfDNA as a template, it was possible to confirm the disease-causing variant by Sanger sequencing. To further understand the prevalence of the variants identified to date and provide an overview of DEB-Pr-causing variants, we summarized all variants associated with DEB-Pr reported.

Patients and methods

Ethical approval. This research project was conducted after receiving approval from the Institutional Review Board (IRB) at the Dermatology Institute of Jiangxi Province, The Affiliated Dermatology Hospital of Nanchang University (Nanchang, China), and is in accordance with the Helsinki Declaration. All participants, including their parents or legal guardians, provided written informed consent. Use of the clinical picture of the proband was not permitted.

Patients. A total of 46 individuals from a DEB-Pr family were recruited for a comprehensive questionnaire and physical examination in this study at the Dermatology Institute of Jiangxi Province, The Affiliated Dermatology Hospital of Nanchang University between July 2020 and March 2021. Among them, 18 individuals donated their blood, 12 individuals agreed to the use of their samples for WES and all donors agreed to the use of their genomic material for COL7A1 PCR amplification. In addition, in order to have non-DEB controls, blood was also collected from 50 healthy controls who visited hospitals for annual physical examination and had a confirmed lack of DEB-associated genetic disease in the family history (median age 45.5 years; age range, 26-76 years; 32% women), and blood from one 32-year-old pregnant woman at week 37 who also presented as healthy and with a lack of DEB-related disease was collected in order to test whether cffDNA could be used for COL7A1 PCR amplification.

WES. The DNA was extracted by DNA isolation kit (cat. no. DC111; Vazyme Biotech Co., Ltd.) and DNA was measured by Nanodrop2000 (Thermo Fisher Scientific, Inc.). Genomic DNA (1 μ g) was sheared to 150-250 bp using a Covaris LE220 instrument with the following parameters: Duty cycle, 20%; intensity, 5; cycles per burst, 200; time, 90 sec; and shearing tubes, Crimp-Cap microtubes with AFA fibers (Covaris Inc.). The DNA fragments were then selected by VAHTSTM DNA Clean Beads (cat. no. N411; Vazyme Biotech Co., Ltd.). The library was constructed using the MGIEasy Exome FS Library Prep Set kit v2.0 (cat. no. 1000009658; MGI Tech Co., Ltd.) following the manufacturer's instructions. Briefly, the end-repair for DNA

fragments was performed by adding an 'A' nucleotide to the 3' end of each strand. Adapters were then ligated to both ends of the end-repaired/dA-tailed DNA fragments for amplification and sequencing. Size-selected DNA fragments were amplified by ligation-mediated PCR, purified and hybridized to the exome array for enrichment. Non-hybridized fragments were then washed out. Finally, the captured products were then circularized by MGIEasy circularization commercial kit (cat. no. 1000005259; MGI Tech Co., Ltd.). Rolling circle amplification was performed to produce DNA Nanoballs. The library was sequenced on the BGISEQ-500 sequencing platform (MGI Tech Co., Ltd.) and the overall quality control data is presented in Table SI. The raw sequence data reported in this paper were deposited in the National Institute of Biotechnology Information (NCBI) Sequence Read Archive database (accession no. SAMN36949954-SAMN36949963) and are accessible through <https://www.ncbi.nlm.nih.gov/sra/PRJNA1000907>.

Variant calling. The reads were mapped to the hg19/GRCh37 human reference genome by the BWA.v0.7.12 tool and Picard was used to mark the duplicates. A Genome Analysis Toolkit v 3.7 and Samtools (<http://www.htslib.org/>) were used to detect single nucleotide variants and short insertions/deletions (Indels). Short Indels in the repeat regions and within the 10 bp range from the start and end of the read were also excluded. The remaining variants were filtered from public databases comprising 1000 Genomes (<ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/release>) and gnomAD (The Genome Aggregation Database; <https://gnomad.broadinstitute.org/>).

Variant annotation and prediction. The variants were annotated with the ANNOVAR program (<https://annovar.openbioinformatics.org/en/latest/>). The *in-silico* analysis was performed by Sorting Intolerant From Tolerant (SIFT) (<https://sift.bii.a-star.edu.sg/>), Polymorphism Phenotyping v2 (PolyPhen2) (<http://genetics.bwh.harvard.edu/pph2/>), mutation assessor (<http://mutationassessor.org/r3/>), mutation taster (<https://www.mutationtaster.org/>), FATHMM (<http://fathmm.biocompute.org.uk/>) and CADD (<https://cadd.gs.washington.edu/score>) to anticipate the functional effect of missense and nonsense variants. Mega version 11 (11.0.13), Jalview Version 2 (2.11.2.7) and BioEdit software (v7.2) were used to align the sequences against the COL7A1 reference sequence (accession no. NC_000003).

ELISA. ELISAs for desmogelin (Dsg)1 (cat. no. 7880E), Dsg3 (cat. no. 7885E), BP180 (cat. no. 7695E), BP230 (cat. no. 7613E) and Type VII collagen (cat. no. 7845R2) were performed using commercial kits purchased from Medical and Biological laboratories, Co., Ltd., and the procedures were performed according to the manufacturer's instructions.

Immunoblotting. For immunoblotting, recombinant proteins including LM332 (cat. no. LN332-0502, Biolamina AB), LM111 (cat. no. LN111-0501, Biolamina AB) and integrin α 6 β 4 (cat. no. 5497-A6-050; BD Biosciences) were used for the antigen source (100 ng per reaction), and final blots were incubated with patients' sera at 1:5-1:800 dilution. The detailed method was performed as previously described (17-19).

Indirect immunofluorescence (IF). Indirect IF studies of normal human skin and 1M NaCl-split normal human skin were performed as previously described (19).

CfDNA and cffDNA extraction. Both cfDNA and cffDNA were extracted from plasma using a QIAamp DNA Blood Mini extraction kit (Qiagen GmbH) according to the manufacturer's instructions. The concentration of the DNA was measured by a Nanodrop 2000 (Thermo Fisher Scientific, Inc.) and the quality of the isolated DNA was checked by 1% agarose gel electrophoresis.

PCR and Sanger sequencing. The candidate variants were validated by direct Sanger sequencing. Primers for PCR and sequencing were provided by Tsingke Biological Technology. For PCR amplification, 10 ng of total genomic DNA was used as a template in 20 μ l of the reaction mixture using Vazyme Taq DNA polymerase as previously described (20). The primers used in this study and listed in Table SII. The thermocycling conditions were as follows: 95°C for 3 min, followed by 35 cycles of 95°C for 15 sec, 61°C for 15 sec and 72°C for 30 sec, and a final extension at 72°C for 3 min. The PCR products were checked by DNA electrophoresis and sent to Tsingke Biological Technology for sequencing. The COL7A1 consensus sequence generated from sanger sequencing results from 50 healthy controls, the reference sequence from NC_000003 and the sequences from affected individuals were also aligned, as shown in Fig. S1.

Software predication. Three-dimensional structure prediction of selected proteins was performed using I-TASSER (<https://zhanggroup.org/I-TASSER/>) (21). In details, the complete gene sequence of COL7A1 was retrieved from the NCBI GenBank (<https://www.ncbi.nlm.nih.gov/gene/1294>) under accession no. NC_000003. The wild-type protein of COL7A1 with an exonic region of 70 to 118 was used for 3D structure prediction. The C-score=-1.33, TM-score=0.55 \pm 0.15 and root-mean-square deviation (RMSD)=12.2 \pm 4.4 Å were selected, where C-score is a confidence score for estimating the quality of predicted models by I-TASSER and TM-score is a metric for assessing the topological similarity of protein structures; it is designed to solve two major problems in traditional metrics such as RMSD.

Literature review. A review of the literature was performed using PubMed and Google search with the search terms 'epidermolysis bullosa pruriginosa', 'DEB pruriginosa', 'EB pruriginosa' and 'COL7A1' covering the period of 1994 to November 2022, and the systematic reviews were limited to studies published in English.

Results

Case presentation. A 50-year-old female proband presented to the Dermatology Hospital of Jiangxi Province (Nanchang, China) with complaints of itching, skin nodules and blackish discoloration of the skin on both lower limbs for >32 years. Upon physical examination, depigmentation at the center with scarring was seen over the larger nodules and multiple lichenified papules to nodules were present over the lower limbs extending from the knee to the ankle joint. Routine

investigations, including chest X-ray, computed tomography, electrocardiogram, upper and lower gastrointestinal endoscopy, complete blood count, liver and renal function tests, as well as serum IgE, ferritin and thyroid function tests, all showed normal results. It was further confirmed that direct immunofluorescence using a biopsy of lesioned skin of the left leg showed no IgG, IgA, IgM and complement C3 deposition at the basement membrane zone or on keratinocyte cell surfaces. This patient's serum showed negative IgG, IgA and IgM results on indirect immunofluorescence using normal human skin and indirect immunofluorescence using salt-split skin (22,23). According to ELISAs using commercially available kits (Medical and Biological Laboratories, Co., Ltd.), IgG autoantibodies against Dsg1, Dsg3, BP180, BP230 and COL7 were all negative. Other diagnostic tests, including immunoblotting of normal human epidermal extract, dermal extract and recombinant proteins of laminin 332, laminin 111 and integrin α 6 β 4 (24-26), which are routinely used in our laboratory for the detection of known autoimmune bullous disease (AIBD) autoantibodies (18,27), showed negative results for this patient's serum. Based on all of these clinical and laboratory data, the diagnosis of AIBD was ruled out for this patient.

Identification of disease-causing variant by WES. The proband and her family under investigation comprised 46 individuals and a questionnaire revealed the absence of consanguineous marriage. Among the first three generations, 14 individuals had similar mild clinical disease phenotypes, as indicated in the pedigree chart (Fig. 1A). Based on the clinical examinations and questionnaires completed for the proband's family, the diagnosis of DEB-Pr was suspected.

To thoroughly investigate the potential genetic basis of DEB-Pr, WES of 12 individuals from the proband's family was performed, including the proband, affected individuals and unaffected individuals (Fig. 1B and C). Analysis of the WES data led to the identification of a G-to-A transition at the first base of codon 6,859 in exon 87 of the COL7A1 gene, resulting in the substitution of Gly (GGA) with Arg (AGA) (Fig. 1C). This variant (c.6859 G>A) in the COL7A1 gene has been reported in the DEB-Pr by Japanese studies (28-30). To predict the potential impact of the variant on the protein functions, different bioinformatic analysis tools were used and returned with predicted values, including the SIFT (value: 0.0), PolyPhen2 (value: 0.998), mutation assessor (value: 4.32), mutation taster (value: 0.999), FATHMM (value: -6.29) and CADD (value: 6.70). Overall, the variant was predicted to be 'deleterious' with high confidence, suggesting that COL7A1 mutation is the genetic basis of DEB-Pr.

Pathogenic variant validation using cfDNA. To confirm the results of the WES analysis and explore the potential use of cfDNA for DEB-Pr diagnosis, target DNA amplification and Sanger sequencing were next conducted. The dominant peaks observed in the cfDNA samples were ~166 and 300 bp, which aligns with the size range reported in previous studies (31,32) (Fig. 2A). The targeted region in exon 87 of COL7A1 was amplified and Sanger sequencing was performed in patients and 50 ethnically matched healthy controls (Fig. 2B-D). The results showed that the affected individuals carried AGA, whereas the unaffected individuals and healthy controls were GGA at this

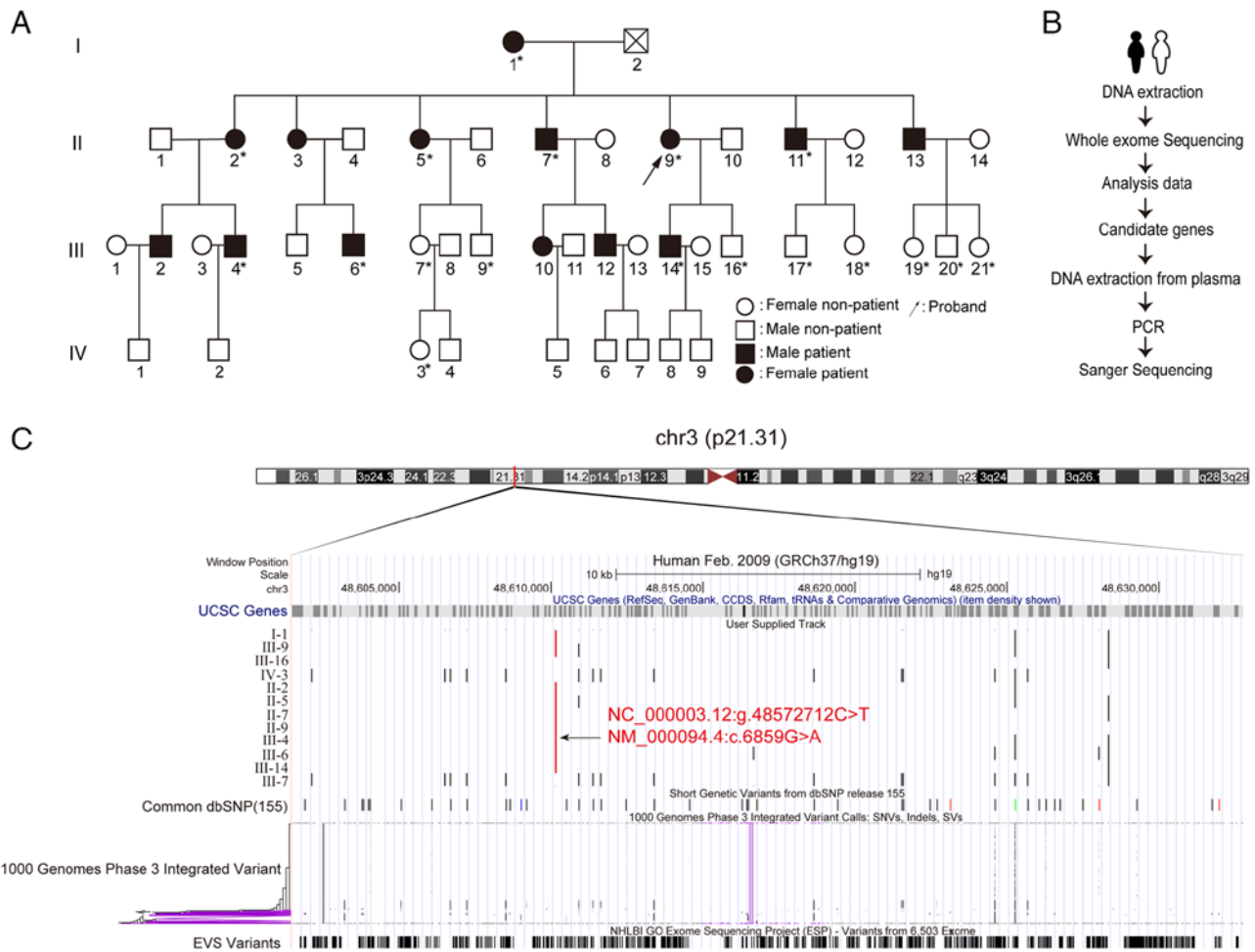


Figure 1. Molecular diagnosis of DEB-Pr. (A) Pedigree of the DEB-Pr family spanning 4 generations. The squares indicate male and circles female individuals; black filling denotes affected individuals; the slash indicates a deceased individual; the arrow points out the proband; asterisks indicate that the sample was used for sanger sequencing. (B) Overview of the experimental design. (C) Graphic view of COL7A1 generated by the UCSC genome browser. A total of 12 individual whole-exome sequencing results were aligned and compared with common dbSNP, 1000 genomes variant and the EVS variants datasets. The candidate Chr3:48610145 C>T position in the genome was marked in red. COL7A1, collagen type VII, $\alpha 1$; DEB-Pr, dystrophic epidermolysis bullosa pruriginosa; EVS, Exome Variant Server; UCSC, University of California Santa Cruz; SNV, single nucleotide variant; dbSNP, single nucleotide polymorphism database; Chr, chromosome; Indel, insertion and deletion; SV, structural variation.

position, further supporting the association between genotype and phenotype. Of note, one affected individual (III-9) carried heterozygous variants according to both WES (Fig. 1C) and Sanger sequencing (Fig. 2D). However, clinical examination indicated that this individual did not show any symptoms of DEB-Pr, which is consistent with other reports, suggesting other factors may also have an important role in disease progression, such as metabolic, genetic, epigenetic or environmental factors (33,34), and follow-up of this individual may be recommended. To further determine whether the cfDNA may be used as a template to amplify other regions, pairs of primers for 12 out of 118 exons were randomly selected. The results indicated that all amplicons may be amplified with target sizes, implying that cfDNA may be used for genetic testing (Fig. 2E). To further extend its potential for prenatal diagnosis, cfDNA was subsequently isolated from a pregnant woman (Fig. 2F) and amplification of the COL7A1 gene were performed. Successful amplification of the COL7A1 gene from cfDNA suggested that cfDNA may be used as a non-invasive means of detecting the COL7A1 variants in suspected hereditary epidermolysis bullosa (Fig. 2G).

Structural prediction of the variant in COL7A1 gene causing DEB-Pr. Multi-alignments analysis indicated high evolutionary conservation of p2287 glycine and the Gly-X-Y motif across several vertebrates, including human, mouse, pig, chicken, dog, frog, zebrafish and lizard, suggesting critical roles of the amino acid in maintaining normal protein function (Fig. 3A). Next, the possible molecular mechanism of the variant on the COL7A1 gene causing DEB-Pr was investigated using the structure prediction tool I-TASSER, which is considered the finest tool for predicting the tertiary structure of a protein (<https://seq2fun.dcm.med.umich.edu/I-TASSER/>) (21). Analysis of the predicted 3D protein structures did not reveal any difference in the hydrogen bond interactions between wild-type and mutant residues. However, the residue size and charge differences may impact the protein folding and thus overall protein stability (Fig. 3B). To gain an overview of the COL7A1 mutations causing DEB-Pr, a total of 65 unique mutations in COL7A1 gene were summarized, of which 37 were specifically reported in DEB-Pr cases (Fig. 3C). This summary demonstrated that the major cause of DEB-Pr involves glycine substitutions (86.5%), although deletions or

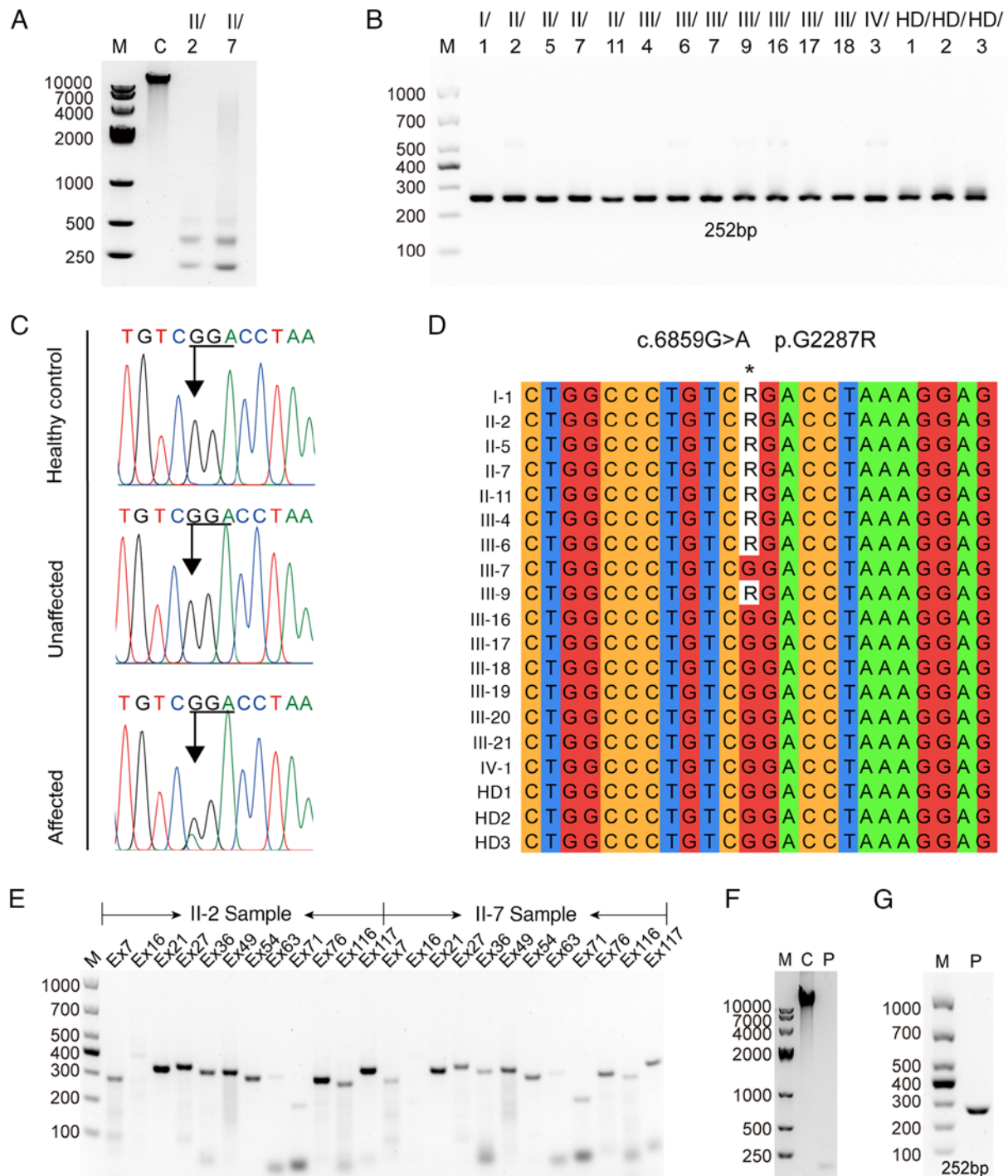


Figure 2. Molecular basis of dystrophic epidermolysis bullosa pruriginosa confirmed by Sanger sequencing. (A) cfDNA was extracted and the size distribution was checked by 1% DNA gel electrophoresis. (B) The target region-carrying variant was amplified from 13 members mentioned in Figs. 1A and 3 representative healthy controls. The PCR product was visualized by 1% DNA gel electrophoresis. (C) Representative data of direct nucleotide sequencing indicating a heterozygous, single nucleotide substitution in exon 87, c.6859G>A. (D) Sequence alignment of tested family members and healthy controls. R represents a heterozygous G/A of the variant. (E) PCR amplification of randomly selected exons in the COL7A1 gene using two individual-derived cfDNA. (F) Visualization of cfDNA extracted from a plasma sample. (G) COL7A1 exon 87 amplification using cfDNA. M, DNA marker; C, control DNA extracted from Jurkat T cells; P, DNA extracted from plasma derived from a pregnant subject; COL7A1, collagen type VII, $\alpha 1$; Ex, exon; HD, healthy donor; cfDNA, cell-free fetal DNA; cfDNA, cell-free DNA.

splice-site mutations may appear in certain cases, consistent with previous findings (2,6). Notably, all variants are located within the triple helix of COL7A1 in DDEB. By contrast, the mutations include nonsense, splice site, exon skipping and non-glycine missense mutations within the triple helix or non-collagenous NC-2 domain in RDEB.

Discussion

DEB-Pr represents a rare variant within the broad spectrum of DEB and was first reported by McGrath *et al* (1) in 1994. It has been established that mutations in the COL7A1 gene, which encodes the COL7 protein, underlie all subtypes of DEB. WES

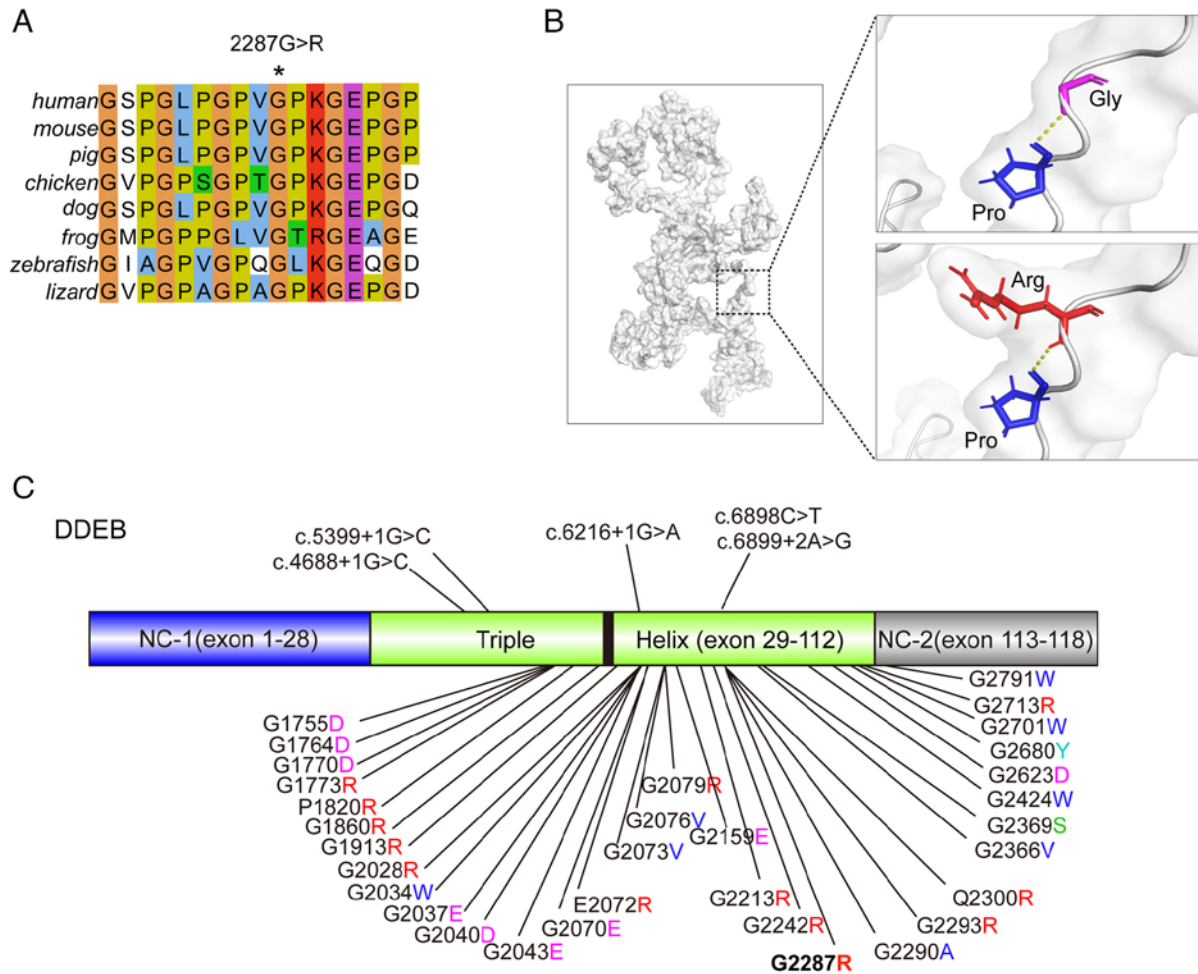


Figure 3. *In silico* prediction and summary of COL7A1 variants in DEB-Pr. (A) Sequence alignment of high evolutionary conservation of p2287. (B) The structure of COL7A1 was predicted by I-TASSER and a detailed differential structure analysis of the variant was displayed by Pymol. The hydrogen bond is indicated with a dashed line. The top panel indicates wild-type COL7A1 and the bottom panel indicates the mutant COL7A1. (C) Summary of current variants in dominant DEB-Pr. COL7A1, collagen type VII, α 1; NC, non-collagenous domain; DDEB, dominant dystrophic epidermolysis bullosa.

provides a promising tool for disease diagnosis. In the present study, on the basis of clinical and genetic examinations, a large family was diagnosed with DEB-Pr using WES, and this was further confirmed by Sanger sequencing. In this family, DEB-Pr was caused by the COL7A1 variant at position 6859 with G to A substitution, and an amino acid level change of Gly to Arg. In addition, it was also demonstrated that plasma cfDNA can be used as a source for diagnosis.

In the present study, it was observed that G2287R resulted in mild disease manifestations in the proband and related family members, which is consistent with previous studies in both Japanese and Chinese patients (28,29,35). These findings support the notion that specific glycine substitutions in the collagenous domain of COL7A1 relate to mild diseases. Previous studies have indicated that the onset of DEB-Pr varies from infancy to late adulthood. Typically, clinical manifestations begin to appear during the first two decades of life. However, in certain patients, the onset may be delayed to adulthood, with the eldest case reported being that of a 71-year-old male (29,36). Of note, in the present study, an individual (III-9) in their teens who carried the disease-causing variants but did not exhibit any disease manifestations was identified. Conversely, it was confirmed that III-19, 20 and 21 carried the

wild-type allele, despite their father being affected by DEB-Pr. The underlying causes of the phenotypic disparity observed in DEB-Pr remain elusive. It has been proposed that additional factors, such as genetic, epigenetic, metabolic, immunological or environmental factors, may also contribute to the DEB-Pr phenotype (33,37,38). Further research is needed to elucidate the mechanism underlying this phenotypic disparity.

cfDNA analysis has emerged as a game-changing tool in genetic disease diagnosis, offering significant advantages over traditional diagnostic methods. Its non-invasive nature, early detection capabilities and high accuracy have propelled the field of precision medicine forward, improving patient outcomes and quality of life (32,39). However, it is important to note that the level of cfDNA in peripheral blood is relatively low. Typically, the content of cfDNA reaches 4% after 10-12 weeks of pregnancy and various factors, such as body weight and gestational age, may influence the content of cfDNA, which may limit its application and accuracy (39-44).

Using I-TASSER, the finest structure prediction tool, the molecular basis of the influence of variants on functional changes was analyzed. The collagen molecule requires a glycine residue to maintain its specific triple helical conformation (45,46). In the present cases, arginine residues were

substituted for a glycine residue. In general, the characteristics of the amino acids have an important impact on the conformation of a protein. Although there is no difference in hydrogen bond interaction in the predicted model, arginine is a basic amino acid and has a more bulky side chain compared to glycine and it may alter protein stability as well as structural conformation. Therefore, different glycine substitutions may result in different characteristics of COL7 and may thus explain the differences in the clinical features. Further structure analysis will need to confirm this hypothesis and guide drug development.

The identification of variations in the COL7A1 gene has a critical role in diagnosing DEB and its specific subtypes. Genetic testing is utilized to detect mutations in the gene, allowing clinicians to confirm the presence of the disease and provide appropriate genetic counseling to affected families. Certain studies suggest that specific mutations in the COL7A1 gene may be associated with varying disease severities in individuals with DEB. Understanding this genotype-phenotype correlation may assist in predicting disease progression and guiding treatment strategies (1,3). For families with a history of DEB or known COL7A1 gene mutations, prenatal testing may be conducted to determine if a fetus carries the genetic variation. This information enables parents and healthcare providers to make informed decisions about the pregnancy and plan appropriate care after birth (47,48). Research focused on the COL7A1 gene and its variants has opened up new possibilities for gene therapy and other targeted treatments for DEB. Scientists are actively exploring strategies to correct or replace the defective gene as potential therapeutic options (8). Studying the COL7A1 gene and its protein product, type VII collagen, provides valuable insight into the molecular mechanisms underlying DEB. This knowledge is crucial for the development of drugs aimed at alleviating symptoms, promoting wound healing and improving the quality of life for affected individuals.

The present study's main strength lies in the extensive collection of individuals from the same family, allowing us to confirm the association between the genotype COL7A1 G2287R and the clinical phenotype. In addition, the potential application of cfDNA in genetic diagnosis was successfully demonstrated. Furthermore, identifying the specific variants responsible for the disease will markedly benefit disease management.

However, it is essential to acknowledge the limitations of the present study. First, the sample size of cffDNA was relatively small, prompting the need for further research with a larger sample to fully explore the application of cffDNA for prenatal diagnosis. Secondly, the COL7A1 protein structure is not available yet. We predicted and interpreted the impact of residue changes on protein structure and the consequences of the protein structure changes, which may affect downstream molecular mechanisms or the function of collagen as a cellular component. While the exact molecular mechanisms remain elusive. Further investigation is warranted to gain a more comprehensive understanding. Finally, we encountered an exceptional case where an individual carried the variant but did not exhibit any clinical manifestations. The reason behind this observation remains elusive and requires further investigation.

In conclusion, in the present study, the rare heterozygous variant c.6859G>A (p.Gly2287Arg) in the COL7A1 gene was identified through WES together with Sanger sequencing using individual plasma cfDNA in patients with DEB-Pr. Overall, the present study provides compelling evidence supporting the involvement of the COL7A1 G2287R gene variant in the development of DEB-Pr. Furthermore, the present findings underscore the potential of utilizing cfDNA in genetic disease diagnosis, offering new avenues for improved diagnostics and personalized disease management in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

XS and FH conceived and designed the study. YY, YG, WW, HQ, KZ, YM, MZ and DZ performed data analysis and conducted experiments. XS and XL wrote and edited the manuscript. FH and XL organized and provided clinical samples. All authors have read and approved the final manuscript. YY and XS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the IRB of the Dermatology Hospital of Jiangxi Province (Nanchang, China).

Patient consent for publication

All patients or their legal guardians provided written informed consent for genetic testing and publication of their results.

Competing interests

The authors declare that they have no competing interests.

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