

# Novel Pathogenic Mutations of FERMT1 in two Chinese Kindler Syndrome Families

**Min Li**

First Affiliated Hospital of Soochow University

**Weisheng Li**

First Affiliated Hospital of Soochow University

**Dan Zhu**

First Affiliated Hospital of Soochow University

**Likui Lu**

First Affiliated Hospital of Soochow University

**Jingliu Liu**

First Affiliated Hospital of Soochow University

**Yajun Shi**

First Affiliated Hospital of Soochow University

**Zhimiao Lin**

Peking University First Hospital

**Miao Sun** (✉ [miaosunsuda@163.com](mailto:miaosunsuda@163.com))

First Affiliated Hospital of Soochow University <https://orcid.org/0000-0002-6414-368X>

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## Research

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# Abstract

**Background:** Kindler syndrome (KNDLRS) is a very rare autosomal recessive disorder characterized by bullous poikiloderma with photosensitivity. Loss-of-function mutations in *FERMT1*, which located on chromosome 20p12.3, were responsible for KNDLRS. Numerous mutations in *FERMT1* have been reported to be associated with KNDLRS.

**Results:** The present study reported two Chinese KNDLRS families, and affected individuals from both families presented with poikiloderma, palmoplantar hyperkeratosis, and diffuse cigarette paper like atrophy on hands. Skin biopsy of the proband from family 2 showed atrophy of epidermis, hyperkeratosis, dilated blood vessels in upper dermis, and microbubbles at the dermis and epidermis junction. Medical Whole Exome Sequencing V4 combined with Sanger sequencing revealed mutations in *FERMT1* with affected individuals. Compound heterozygous nonsense mutations (c.193C>T, c.277C>T) were found with family 1, and a homozygous frameshift mutation (c.220delC) was observed in family 2. According to the clinical features and genetic analysis, KNDLRS was diagnosed in two Chinese families.

**Conclusions:** This study revealed two novel pathogenic mutations in *FERMT1* that caused KNDLRS and briefly summarized all pathogenic mutations in *FERMT1* that have been documented via the PubMed.

## Background

Kindler syndrome (KNDLRS; OMIM #173650) is a very rare autosomal recessive genodermatosis characterized by acral blistering, progressive poikiloderma, skin atrophy, abnormal photosensitivity, and gingival fragility [1]. It was first reported by Theresa Kindler in a 14-year-old girl with congenital blistering of her hands and feet. By linkage and homozygosity analysis, the pathogenic gene responsible for KNDLRS was mapped to chromosome 20p12.3, and homozygous as well as compound-heterozygous mutations of *FERMT1* were identified with the KNDLRS patients. Although light microscopy may be helpful, it is best to directly sequence *FERMT1* for unequivocal diagnosis. At present, about 91 mutations of *FERMT1* have been documented in the literature. Mutation types include missense, splicing, regulatory, small indels, gross deletions/insertions/duplications (HGMD) [2]. Most pathogenic mutations in *FERMT1* reported are predicted to lead to premature termination of translation, which results in the loss of the kindlin-1 protein and impairs its function. The clinical and genetic aspects of the disease have recently been reviewed, however no clear genotype-phenotype correlations are established [3, 4].

The *FERMT1* gene encodes kindlin-1, a member of the fermitin family (kindlins-1, -2, and -3), and contains a filopodin and ezrin/radixin/moesin (FERM) domain and a pleckstrin homology (PH) domain. In skin, kindlin-1 is localized within the epidermis and particularly in basal keratinocytes but not in epidermal melanocytes and dermal fibroblasts. How kindlins act is less well-defined, while disease-causing mutations show that kindlins are essential for integrin activation, adhesion, cell spreading and signaling [5]. Kiritsi et al has reported that the restoration of kindlin-1 led to structurally normal skin, while loss of kindlin-1 severely impaired keratinocyte proliferation [6]. Loss of kindlin-1 in mouse keratinocytes

recapitulates KNDLRS and produces enlarged and hyperactive stem cell compartments, which lead to hyperthickened epidermis, ectopic hair follicle development and increased skin tumor susceptibility [7].

The diagnosis of KNDLRS is determined based on typical clinical manifestations, pathogenic variants in *FERMT1* or histologic findings on skin biopsy. Here, we reported two Chinese KNDLRS families based on typical clinical manifestations and two novel mutations (c.277C>T and c.220delC) in *FERMT1*. Pathogenic *FERMT1* mutations reported in KNDLRS between 1984 and 2020 was summarized.

## Results

### Pedigree of two families

Pedigree of two families were shown in Fig. 1A and 2A. Both pedigrees presented an autosomal recessive inheritance manner. All parents of the proband are healthy without any clinical phenotype. In addition, family 2 has a [consanguineous marriage](#) history.

### Patients with Kindler syndrome in two Chinese families

In family 1, physical examination (Fig. 1B. a-i) revealed an 11-year-old female presented with vulnerable gums, erosion on the gingivitis and skin blisters in the neck. In addition, hypogastric presence blisters and heterochromatism. Moreover, the skin of hands showed atrophy and blisters. The skin pigment of both lower limbs of patient is abnormal, and the skin is atrophic, spreading from the knee to the extremities.

The proband in family 2, a 30-year-old woman had a more severe phenotype (Fig. 2B. a-j). Both trunk and back of the skin showed diffuse blisters and heterochromatism. As for the limbs of patient, it showed that limb skin atrophy is obvious, accompanied by limb blisters, skin peeling with cracks. Skin biopsy showed atrophy of epidermis, hyperkeratosis, dilated blood vessels in upper dermis, and microbubbles at the dermis and epidermis junction (Fig 2B. k-m).

### Gene Variant Distributions in two Families

By using the Medical WES V4 and Sanger sequencing verification, we found c.193C>T and c.277C>T compound heterozygous mutations of *FERMT1* in proband III-1 from family 1 (Fig. 3C). A c.193C>T mutation inherited from the mother (Fig. 3A), and it had been reported as a pathogenic nonsense mutation previously. A c.277C>T mutation inherited from the father (Fig. 3B) was a novel nonsense mutation, and a bioinformatics analysis showed that it might be a pathogenic mutation.

As for proband IV-1 from family 2, a homozygous mutation (c.220delC (p.H74Tfs\*31) in the exon3 of *FERMT1* was found to be associated with disease phenotypes. Specifically speaking, a 1 bp deletion was in the exon3 of *FERMT1* (NM\_017671; c.220delC (p.H74Tfs\*31)) (Fig. 3F), which resulted in a frameshift and premature termination of *FERMT1*. It was confirmed that each of her parents was carrying a

heterozygous mutation (Fig. 3D-E). According to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines, the mutation was initially identified as a likely pathogenic.

## Three dimensional structural changes of proteins caused by mutations in two families

To further verify the effect of mutations on *FERMT1* coding protein, we use protein structure prediction software (SWISS-model) [8] to predict three-dimensional structure of mutated protein. The wild type (WT) three-dimensional structure of *FERMT1* (Fig. 4A) encoded protein generated by SWISS-model [8]. As for the compound heterozygous mutations of III-1 from family 1, we predicted two mutation sites, respectively. Two mutations c.193C > T (Fig. 4B) and c.277C > T (Fig. 4C) will lead to the generation of *FERMT1* variants (p.Q65X and p.Q93X), and eventually lead to the early termination of amino acids at positions 65 and 93, making the protein almost lose all functional structures. As shown in Fig. 4D, a 1 bp deletion of *FERMT1* (c.220delC (p.H74Tfs\*31)) will change 31 amino acids of the protein and make it lose almost all functional domains.

## Review of 91 *FERMT1* mutations reported in KNDLRS.

Literatures from 1984 to 2020 as well as the results gained in current study revealed about 91 different mutations in *FERMT1*. The mutations included missense, frameshift, nonsense, and splice mutations. The locations of *FERMT1* mutations spread from exons 1 to 15, introns (1, 7 to 11, 13 to 14), and regulatory regions (Fig. 5). The mutation c.193C > T reported previously was in BLUE, while the other two novel mutations identified in this study were highlighted in GREEN. The results demonstrated that most mutations produced a premature termination of *FERMT1* and may lead to production of nonfunctional proteins or even absent kindlin-1 protein.

## Discussion

KNDLRS is caused by *FERMT1* mutations on chromosome 20p12.3 [9]. *FERMT1* contains 15 exons and encodes the 677-amino acid protein kindlin-1, which plays an important role in keratinocyte migration, adhesion, and proliferation [1]. The *FERMT1* gene product belongs to a family of focal adhesion proteins (kindlin-1, -2, -3) that bind several beta integrin cytoplasmic domains. Ussar S and his collaborators found that deleting kindlin-1 in mice gives rise to skin atrophy [10]. Kindlin-1 play a critical role in integrin activation and that lack of this protein leads to pathological changes beyond focal adhesions, with disruption of several hemidesmosomal components and reduced expression of keratinocyte stem cell markers [11]. Has et al had reported a novel nonsense mutation E304X in the exon7 of *FERMT1*, which was predicted to cause loss of the FERM and PH domains and consequently to impair the function of the protein or, more likely, to lead to nonsense-mediated mRNA decay and complete loss of kindlin-1 protein expression [12]. Has C et al reviewed that there was an association of *FERMT1* missense and in-frame

deletion mutations with milder disease phenotypes, and later onset of complications. It has been reported that a c.1729del (p. Ser577AlafsX14) mutation results in a premature stop codon that deletes most of the FERM 3 domain of kindlin-1 or, more likely, triggers mRNA degradation via nonsense-mediated decay mechanisms [13]. All these loss-of-function *FERMT1* mutations demonstrate the importance of kindlin-1 in maintaining epithelial integrity, although the mechanism linking this mutant protein to photosensitivity and poikiloderma remains to be determined. The protein N-terminal was very important for its function. Harburger et al reported that the in-frame deletion of isoleucine 623 affects the FERM 3 subdomain close to the binding site for  $\beta 1$  integrin [14]. Except for nonsense mutation and small deletion mutation, a novel genomic deletion (about 3.9 kb) was identified in Italian KNDLRS patients, and this deletion resulted in the loss of exons 10 and 11 of *FERMT1* and leading to a truncated kindlin-1 [15].

To date, 91 different pathogenic *FERMT1* mutations have been reported (**Fig.5**) These mutations were scattered throughout *FERMT1*, including all the exons, introns (1, 7 to 11, 12 to 13), and regulatory regions, without obvious hotspots identified. Most of the mutations were nonsense mutations or frameshift variants, which will result in loss of function and may lead to absent kindlin-1 protein or the production of dysfunctional proteins [16]. As a recessive disorder, most of these mutations in *FERMT1* were reported to be homogenous in KNDLRS patients and few were compound heterogeneous mutations. To date, excluding two milder phenotype related mutations Has et al reported in 2011, no additional homozygous *FERMT1* missense and in-frame deletion mutations had been identified, therefore it still needs more evidence to clarify the genotype-phenotype correlations with KNDLRS.

Here we reported two patients from different KNDLRS families. With the detailed medical history consultation and clinical examination, they are preliminarily diagnosed as KNDLRS. Validated by the Medical WES V4 and Sanger sequencing, we found the pathogenic variants in *FERMT1*. The patient III-1 has the compound heterozygous variant, and the patient IV-1 who is from a **consanguineous marriage** family has the homozygous variant. It was known that the majority of KNDLRS patients are homozygous for their mutations, and are predominantly offspring of consanguineous marriages, or originate from isolated populations [17]. c.193C>T and c.277C>T which are both nonsense mutations will lead to the early termination of protein translation, c.220delC which is a frameshift variant, will lead to the amino change p.H74Tfs\*31. To the best of our knowledge, the mutations of c.220delC and c.277C>T have not been reported previously. According to the 2015 ACMG guidelines [18], these mutations were preliminarily determined to be pathogenic. In addition, we used the SIFT, PolyPhen-2, Mutation Taster, GERP++, REVEL to predict the pathogenicity of variants, all results were shown as unknown. We also used the Swiss-model to predict the mutated protein structure, all mutations will lead to the loss of protein functional domain. Although the precise consequence of this mutation remains to be established, the N-terminal region of kindlin's is believed to be important for interaction with binding partners, such as integrins, misfiling, and integrin linked kinase [19,20].

## Conclusions

In summary, we have reported two Chinese Kindler syndrome patients, who had been molecularly confirmed to carry loss-of-function *FERMT1* mutations (c.193C > T and c.277C > T; c.220delC). These findings will expand the mutation spectrum of *FERMT1* and provide a detailed mutation repertoire of *FERMT1* in KNDLRS.

## Methods

### Molecular genetic analysis

For the Medical Whole Exome Sequencing (WES) V4, we prepared 1 to 3 µg of genomic DNA and used the Bioruptorsonicator (Diagenode, NJ, USA) to make about 180bp fragments. By using a DNA sample prep reagent set 1 (NEBNext, MA, USA), the paired-end sequencing libraries were prepared according to Illumina protocols. The amplified DNA was captured use GenCap Medical whole exome capture kit (MyGenosticsGenCap Enrichment technologies). The DNA probes were designed to tile along the exon regions of 23000~ genes. The DNA capture was conducted according to manufacturer's protocol. Finally, the high throughput sequencing was performed by Illumina HiSeq X sequencer for paired read 150bp. After sequencing, we used bioinformatics to screen and analyze the potential pathogenic mutations.

After the suspicious mutation highly related to the disease phenotype is determined, Sanger sequencing was conducted to verify with all the other family members available. Appropriate primer in pairs were designed for mutation sites (Forward- AAGAGTCTACAGGGCACAGG and Reverse-CTAATGCCATCCCAGTCCCT), and the amplified product is a 481bp fragment. The sequencing results was compared with *FERMT1* (NM\_017671) as in GRCh37/hg19 human reference sequence. Clinvar [21], HGMD [8] and the gnomAD [22] were used to verify if the mutation had been documented previously.

### Protein structure prediction

SIFT, PolyPhen-2, Mutation Taster, GERP++, Rare Exome Variant Ensemble Learner (REVEL) were used to predict the pathogenicity of variants.

Using SWISS-model, we predicted three-dimensional structure of both wild and mutated kindlin-1 proteins that encoded by *FERMT1*. Specific steps according to software requirements [23,24]. In brief, input the protein sequence to be predicted, and click build model first, then choose the right model. Finally, the predicted model is opened with the visual analysis software Swiss-pdbviewer.

### Literature review

The term, KNDLRS or Kindler syndrome, was used as the key words to search the documented publications in the PubMed [25] between 1984 and 2020. Pathogenic variations of *FERMT1* identified in KNDLRS patients were summarized in Fig. 5.

## **Declarations**

### **Ethics approval and consent to participate**

The study was approved by the Institutional Review Board of The First Affiliated Hospital of Soochow University and Peking University First Hospital and performed in accordance with the Declaration of Helsinki guidelines. Written informed consent forms from all individuals participated in this study were obtained. Peripheral venous blood samples were collected by standard procedures. Affected individuals were referred to this study by their dermatologists. In this study, two patients and four non-affected subjects from two KNDLRS families were investigated.

### **Consent for publication**

Written informed consent for publication of identifying images or other personal or clinical details was obtained from all the participants or the parents of any participant under the age of 18.

### **Availability of data and materials**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### **Competing interests**

The authors declare that they have no competing interests

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### **Author contributions**

Experimental design (SM and LZ); Clinical data collected (LL, SY, and LJ); Molecular experiments (LL, SY, and LJ); Data analysis and interpretation (SM and LZ); and Manuscript writing (LM, LW, and ZD).

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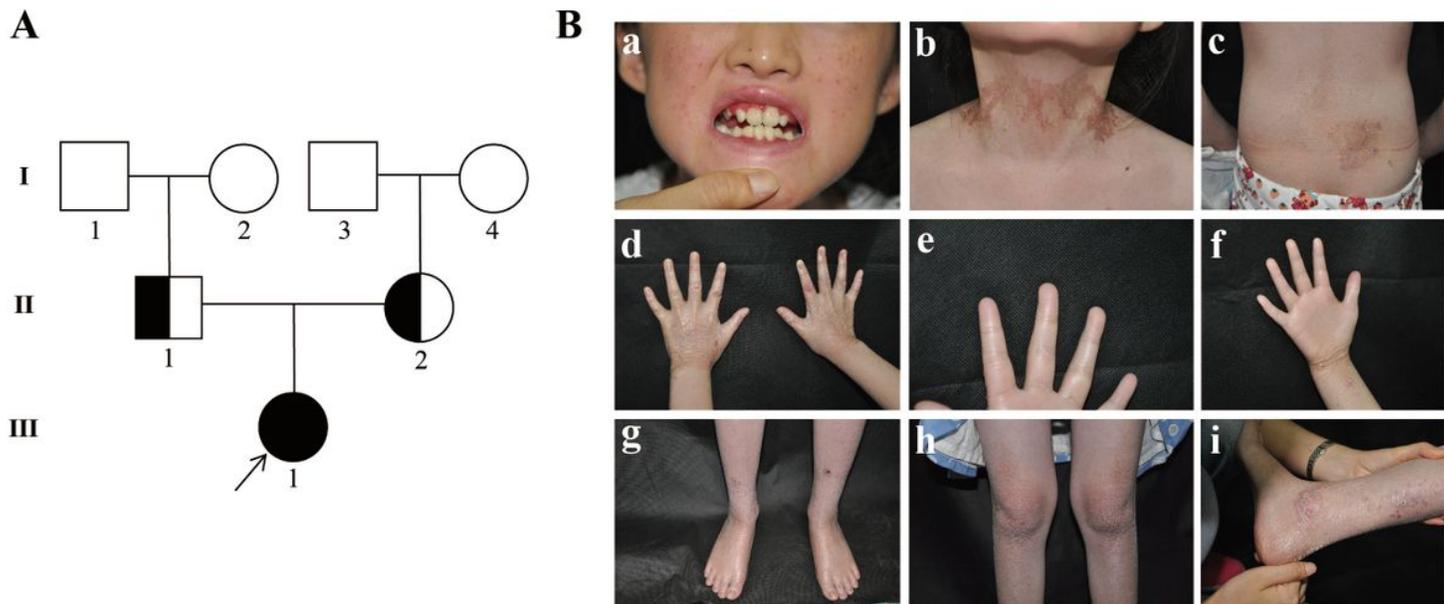
We would like to thank all the family members for their participation in this study.

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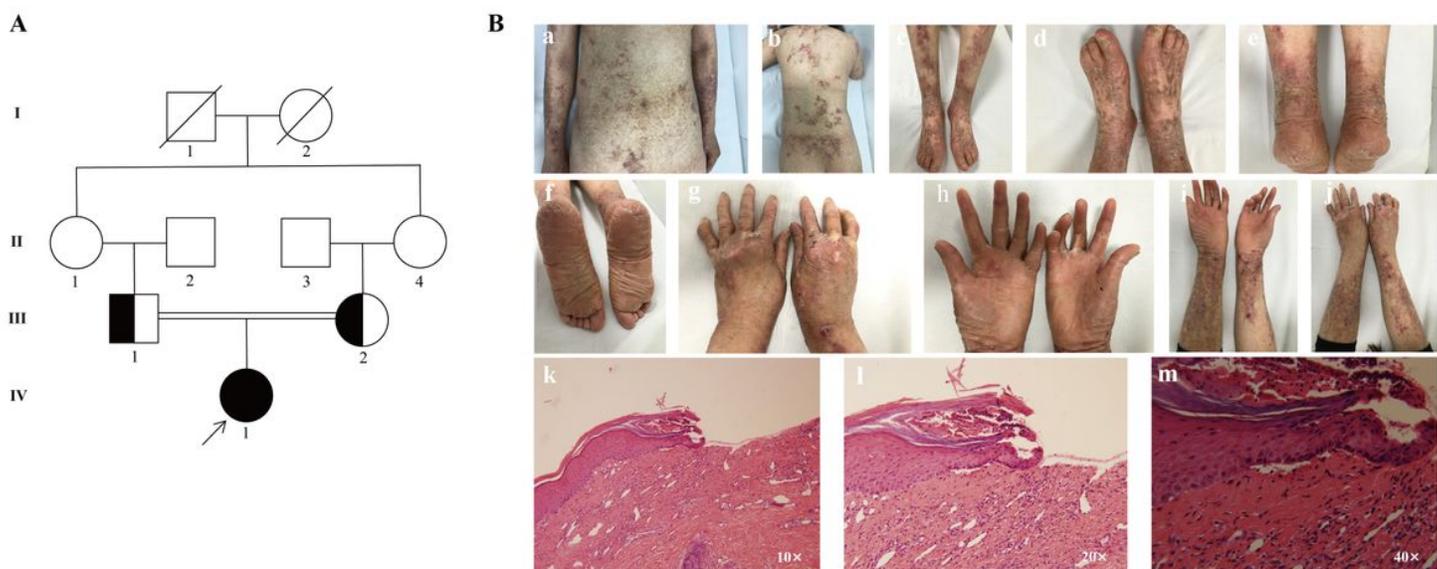
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## Figures



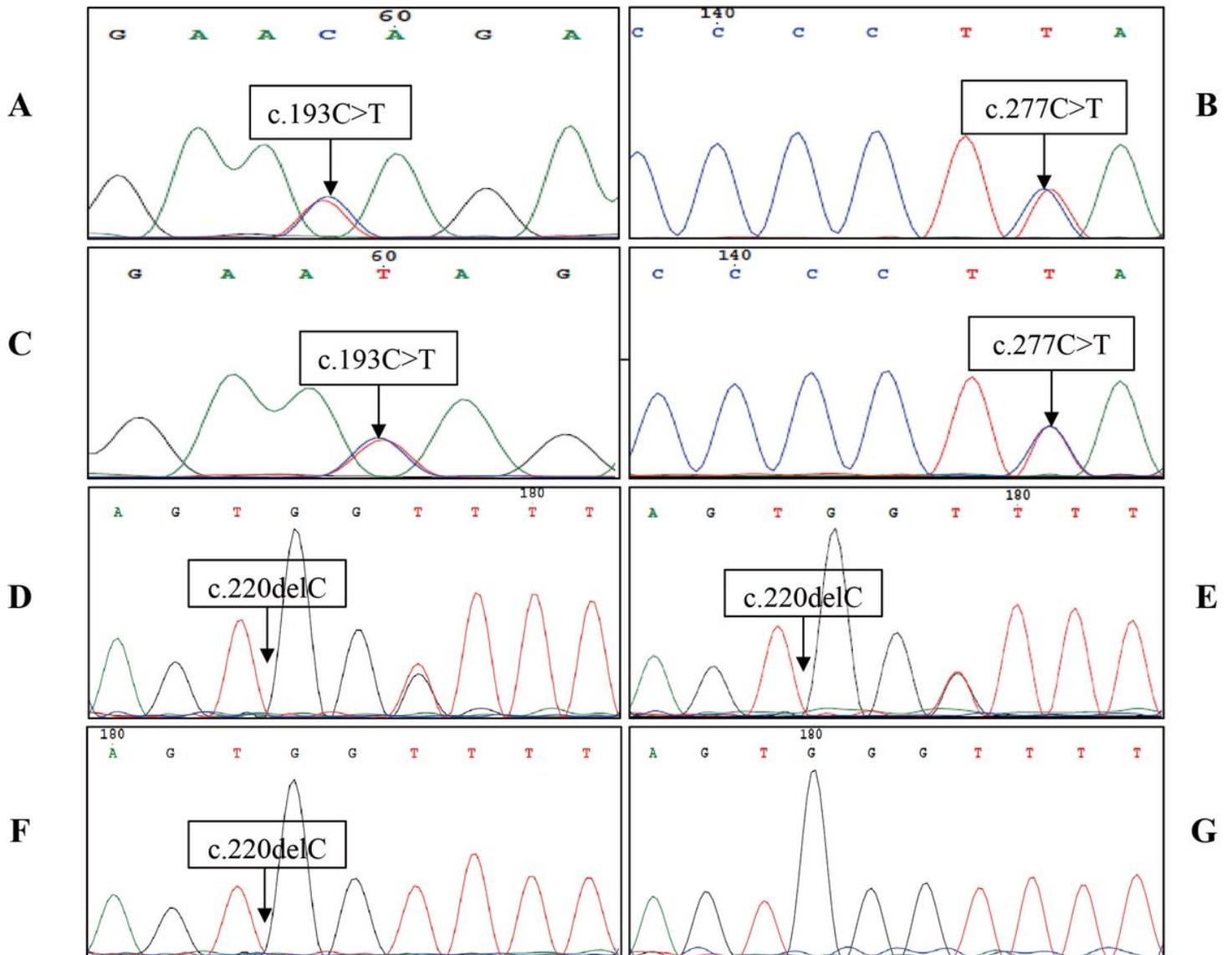
**Figure 1**

Pedigree of family 1 and the clinical features of KNDLRS patient. Pedigree of the family1 (circle indicated female, square indicated male, black indicated carrier of FERMT1 mutation and blank indicated normal). The arrow represents the proband III-1 (A). B. The phenotype picture of a 11-year-old female proband in family 1. a. Vulnerable gums, erosion on the gingivitis; b. Skin blisters in the neck; c. Blisters and heterochromatism in the lower abdomen; d-f. Atrophy of skin of hands and blisters of extremities; g-i showed the skin pigment of both lower limbs is abnormal, and atrophic, spreading from the knee to the extremities.



**Figure 2**

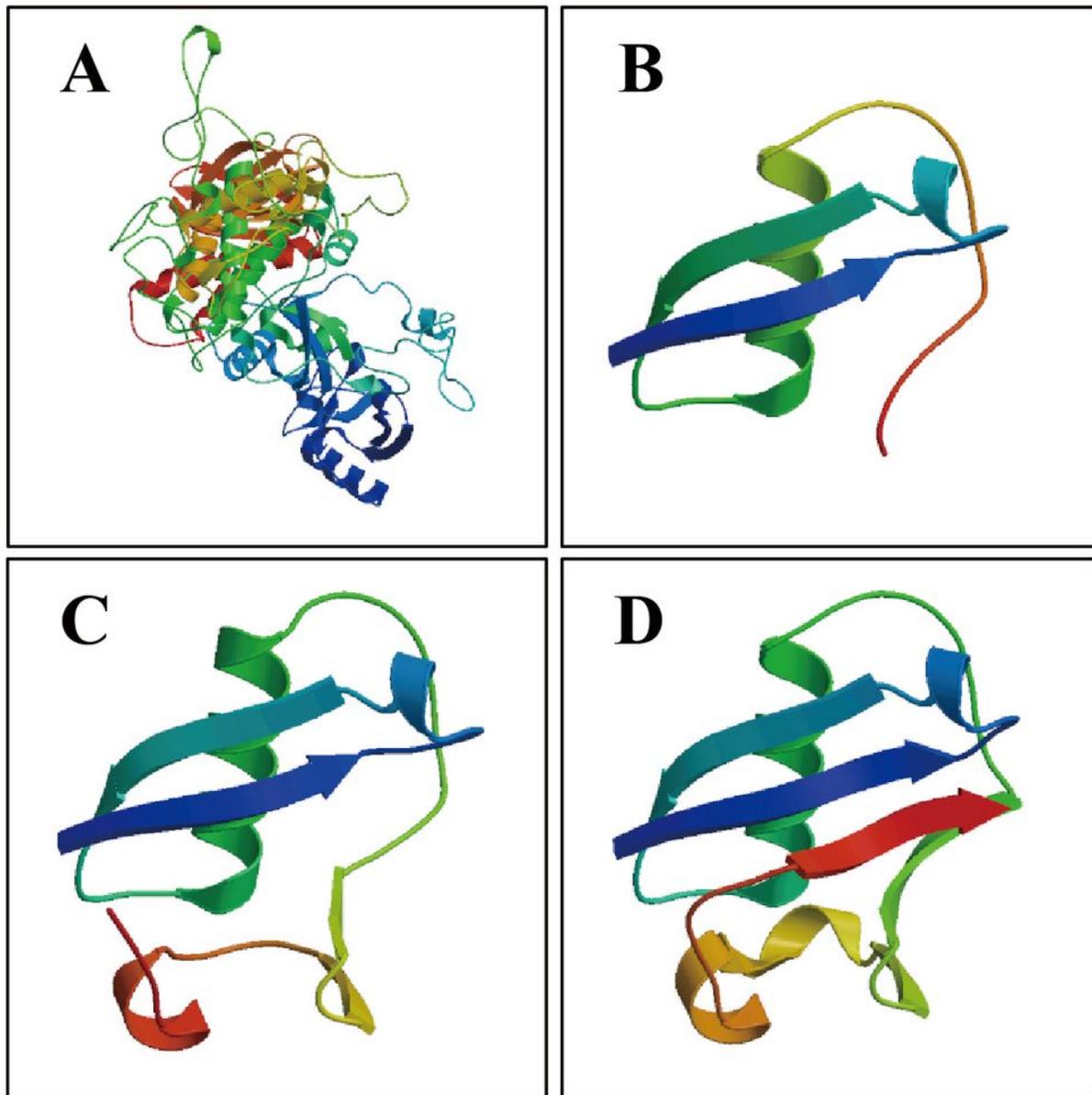
Pedigree of family 2 and the clinical features of KNDLRS patient. Pedigree of the family 2 (circle indicated female, square indicated male, two lines indicated marriage between close relatives, black indicated carrier of FERMT1 mutation and blank indicated normal). The arrow represents the proband IV-1 (A). B. The phenotype picture of a 30-year-old female proband in family 2. a-b showed diffuse skin blisters and heterochromatism in trunk and back. c-j showed the four limbs of patient. It showed that limb skin atrophy is obvious, limb blisters, skin peeling with cracks. k-m. Routine hematoxylin and eosin (H&E) stains showed atrophy of epidermis, hyperkeratosis, and dilated blood vessels in upper dermis, and microbubbles at the dermis and epidermis junction.



**Figure 3**

Sanger sequencing results of family 1 and 2. Sanger sequencing showed c.193C>T and c.277C>T compound heterozygous mutations in FERMT1 of the proband III-1 (C). A c.193C>T mutation came from the mother (A). A c.277C>T mutation came from the father (B). (D, E and F) Sanger sequencing showed a

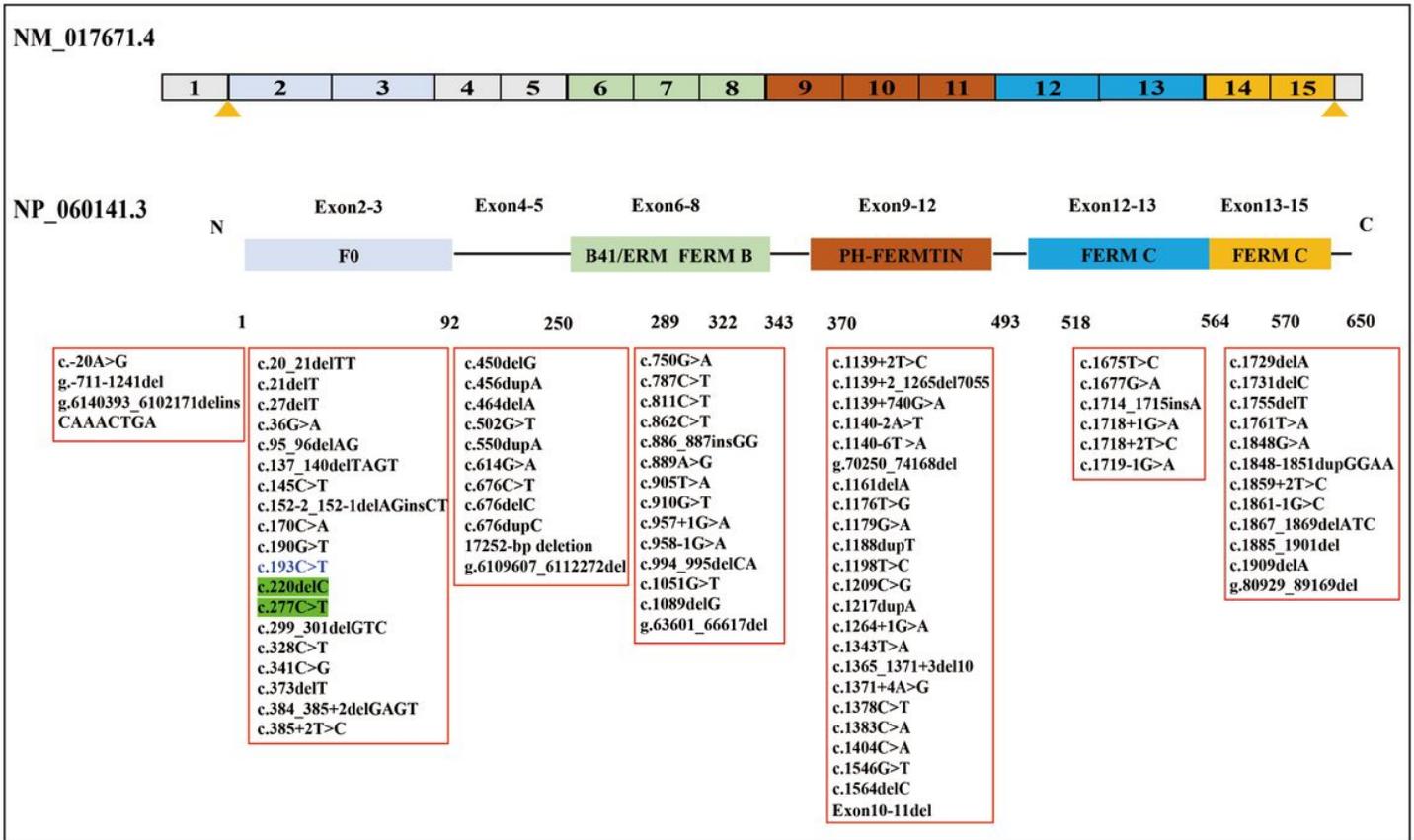
1 bp deletion was existed in the exon3 of FERMT1 for proband's parents (D, E) and proband IV-1 (F) (NM\_017671; c.220delC (p.H74Tfs\*31)).



**A: WT; B:p. Q65X; C:p. Q93X; D: p. H74Tfs**

**Figure 4**

Three-dimensional structure prediction of SWISS-model software. A. Three-dimensional structure of WT FERMT1 encoded protein generated by SWISS-model. B. Three-dimensional structure of FERMT1 mutation protein (p.Q65X). C. Three-dimensional structure of FERMT1 mutation protein after early termination mutants (p.Q93X) at amino acids 93. D. Three-dimensional structure of FERMT1 mutation protein (p.H74Tfs\*31).



**Figure 5**

Summary of FERMT1 mutations in KNDLRS. To date, about 91 different pathogenic FERMT1 mutations have been reported. These mutations were scattered throughout FERMT1, including exons 1 to 15, introns (1, 7 to 11, 13 to 14) and regulatory regions without obvious hotspots or clustering. A mutation (c.193C>T) reported previously was in BLUE, while two novel mutations (c.277C>T and c.220delC) reported in present study were highlighted in GREEN.