

A novel missense mutation in the *COL7A1* gene underlies epidermolysis  
bullosa pruriginosa

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**Short Title:** *COL7A1* mutation in Epidermolysis Bullosa Pruriginosa

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**Abbreviations:** *COL7A1*, *COL7A1* gene; DEB, dystrophic epidermolysis bullosa;  
DDEB, dominant dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; PCR,  
polymerase chain reaction.

**Keywords:** *COL7A1* gene, epidermolysis bullosa pruriginosa, mutation

## **Abstract**

Epidermolysis bullosa (EB) pruriginosa is a subtype of dominant dystrophic epidermolysis bullosa (DDEB), characterized by severe pruritus and blistering localized to the extensor surface of the extremities. EB pruriginosa exhibits extensive clinical heterogeneity with variable expressivity and delayed age of onset. Mutations in the *COL7A1* gene, especially in glycine residues within of Gly-X-Y repeats, have been shown to cause this form of DDEB. Here, we report a novel *COL7A1* mutation in a Taiwanese pedigree with EB pruriginosa. Using polymerase chain reaction amplification and direct sequence analysis, we have identified a G-to-T transversion at nucleotide 7097 within exon 92 of *COL7A1*, converting a glycine residue to valine (G2366V). The mutation resides within a consecutive, uninterrupted 17 Gly-X-Y of the triple-helical domain of type VII collagen. Interestingly, the proband in our family also displayed elevated IgE levels, previously reported in some patients with this disorder. Our finding further implicates *COL7A1* mutation in the pathogenesis of EB pruriginosa and underscores the heterogeneous clinical symptoms of glycine mutations in DDEB.

## Report

Dystrophic epidermolysis bullosa (DEB) is a collection of hereditary bullous disorders characterized by blistering, scarring, and nail dystrophy.<sup>1</sup> The fragility is attributed to scarcity, or even the complete absence of anchoring fibrils composed mainly of type VII collagen.<sup>1</sup> Autosomal dominant and recessive inheritance patterns, as well as sporadic cases of DEB have all been described.<sup>2-4</sup> To date, several hundred pathogenetic mutations within the collagenous and noncollagenous domains of type VII collagen gene (*COL7A1*) have been identified in different forms of DEB.<sup>5-12</sup> One subtype of dominant dystrophic epidermolysis bullosa (DDEB) is EB pruriginosa associated with intense pruritus and nodular prurigo-like lichenified lesions localized mostly in the lower extremities and extensor forearms.<sup>2</sup> Specifically in EB pruriginosa, 13 mutations, 9 of which involve glycine substitution, have been reported in literature (Fig. 2).

Here, we have identified a two-generation EB pruriginosa kindred of Taiwanese descent (Fig. 1a). The proband is a 52-year old woman (II-8) who developed intense pruritic blisters in her lower extremities and extensor surface of both arms in her twenties. A detailed clinical description of her symptoms was reported previously in the Chinese literature.<sup>13</sup> Briefly, histology of biopsy showed a subepidermal cleft and mild perivascular mononuclear infiltration in the upper dermis. Upon electron microscopic

examination, the anchoring fibrils were found to be decreased in number and thinner than in normal control skin. Neither the parents nor the six other siblings of the index case developed any similar symptoms. Recently, at the age of 25 years, the son (III-1) of the proband developed similar pruritic blisters on both shins and extensor arms ([Fig. 2b](#)). Although the 22 year-old daughter of the proband (III-2) lacked blistering lesions, nail dystrophy, and other symptoms of the disease, she had reported localized pruritus on pretibial skin and not yet reached the age of onset of her mother and brother.

After obtaining informed consent, DNA was isolated from peripheral blood lymphocytes in individuals II-7, II-8, and III-1 and from buccal cells in individual III-2, using PureGene DNA Isolation Kit (Minneapolis, MN). PCR amplification of the genomic sequence of *COL7A1* gene was performed with oligonucleotide primers and conditions described previously.<sup>6</sup> The PCR products were directly sequenced using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and the ABI Prism 310 automated sequencing system. Sequencing of affected individuals (II-8 and III-1) revealed a heterozygous GT transversion in exon 92 of the *COL7A1* gene at nucleotide position 7097 ([Fig. 1c](#)), leading to the conversion of a glycine residue (GGT) to a valine residue (GTT), designated G2366V. Although individual III-2 has not yet developed the blistering symptoms of EB pruriginosa, sequence analysis confirmed that she is a carrier of the missense mutation and thus predisposed to developing symptoms in the future. Mismatched PCR with a forward primer (5'-GAGCTC CTG TGA GCC AAT TC-3') and a reverse mismatch primer (5'-CTG GGT ACA CAT ACC TTG TA A-3') was

used to create a new recognition site for the restriction enzyme *MaeIII*. Restriction digest of the mismatched PCR product confirmed the mutation in affected and carrier family members, and excluded the mutation from 33 unrelated, unaffected control individuals, thus arguing against it being a common polymorphism.

To date, the etiology of severe pruritus in EB pruriginosa is unknown. Several studies had implicated an elevated IgE level and immune predisposition to atopy in the pathogenesis of the pruriginosa phenotype.<sup>4,15</sup> Interestingly, with no prior personal or family history of atopy, patient III-1 reported an episode of idiopathic atopic dermatitis in the extensor arms, waist, buttock, and pretibial areas immediately before the appearance of blisters on his pretibia. In accounting for pruritus in the family reported here, available blood chemistry of patient III-1 showed an elevated level of serum IgE (222 IU/milliliter; normal range 0-59 IU/milliliter), over three times the upper limit of normal range. Other causes of itching in III-1 have been ruled out, including thyroid dysfunction, uremia, and low ferritin levels. Our study supports the earlier observation by Mellerio et al<sup>4</sup> which found serum IgE levels to be elevated in seven out of nine EB pruriginosa patients. The pathogenesis of pruriginosa phenotype is poorly understood and how the immune response may participate in the development of the phenotype remains to be elucidated.

The mutation in residue G2366 occurs in the midst of 17 consecutive uninterrupted Gly-X-Y repeats in the triple helical domain of COL7A1. The missense mutation G2366V would likely destabilize the essential triple-helix formation of the collagenous domain. Glycine substitution mutations have been previously reported in various subtypes

of DDEB, including the Pasini, Cockayne-Touraine, pretibial, and pruriginosa subtypes.

<sup>3,4,8-10</sup> Interestingly, in the glycine residue 2366, one compound heterozygous patient with G2366S/G2063W mutations was diagnosed with recessive dystrophic EB,<sup>14</sup> highlighting the importance of residue G2366 (Fig. 2). Similarly, a heterozygous glycine-to-serine missense mutation (G2369S) in the adjacent Gly-X-Y repeat causes dominant EB pruriginosa (Fig. 2).<sup>4</sup> This observation is consistent with the notion that the specific position of a glycine substitution within a particular helical block, rather than its position along the entire collagenous domain, might be more critical in determining its impact on the overall stability of the collagen protein, disease phenotype, and mode of inheritance.<sup>3</sup>

In summary, we report the identification of a novel missense mutation, G2366V, within a Gly-X-Y repeat of type VII collagen in a family diagnosed with epidermolysis bullosa pruriginosa. This result furthers our understanding of both the clinical and allelic heterogeneity displayed in this subtype of DDEB and extends the body of evidence implicating *COL7A1* gene mutations in epidermolysis bullosa pruriginosa.

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## Legends for Figures

**Figure 1** (a) Pedigree of a Taiwanese family with epidermolysis bullosa pruriginosa.

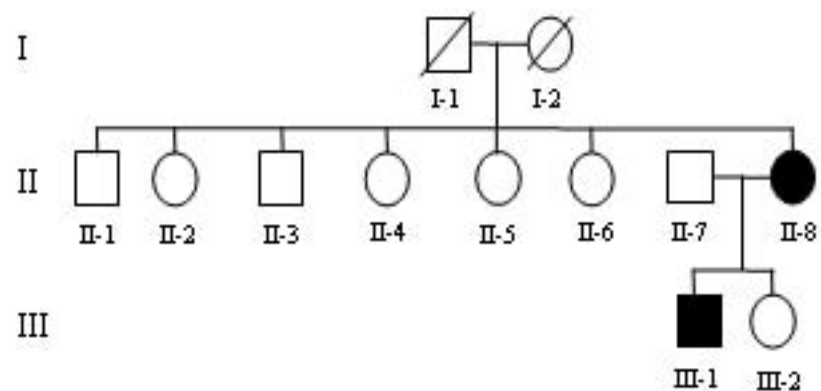
Affected individuals are indicated by filled figures. (b) Clinical appearance of individual

III-1 showing clusters of vesicles and prurigo-like nodules on bilateral pretibial skin. (c).

DNA sequence from an unrelated, unaffected control individual and an affected individual (II-8) are shown. The arrows indicate nucleotide position 7097, which is mutated from G-to-T in the heterozygous sequence of affected and carrier individuals, resulting in the missense mutation G2366V.

**Figure 2** Compilation of mutations in *COL7A1* resulting in EB pruriginosa (modified from Mellerio *et al*, 1999). G2366V described in this study is shown in bold. G1791E, 5532+1GA/7786delG, G2242R, 6863del16, G2369S, and G2713R (Mellerio *et al*, 1999); G2239D and G2242E (Tamai *et al*, 1998); G2037E (Jonkman *et al*, 1998); G2034R (Chen *et al*, 2000); G2028R (Murata *et al*, 2000), and 6899AG (Jiang *et al*, 2002). Compound heterozygote G2366S/G2063W resulted in recessive dystrophic epidermolysis bullosa (Hashimoto *et al*, 1999).

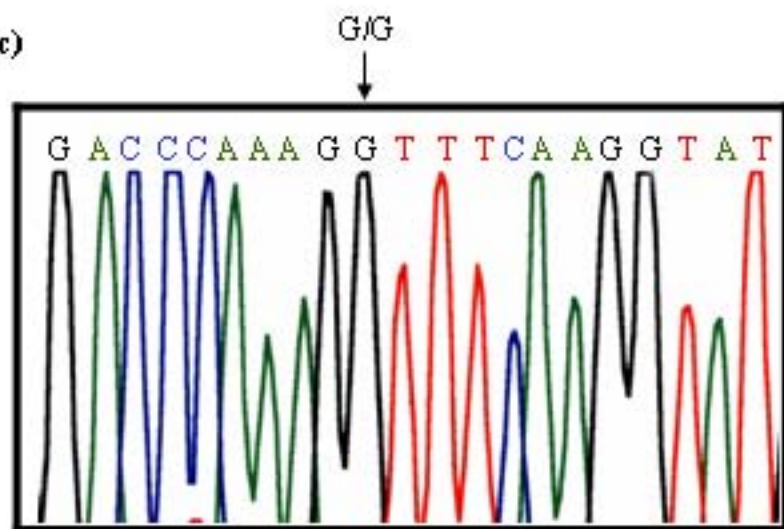
(a)



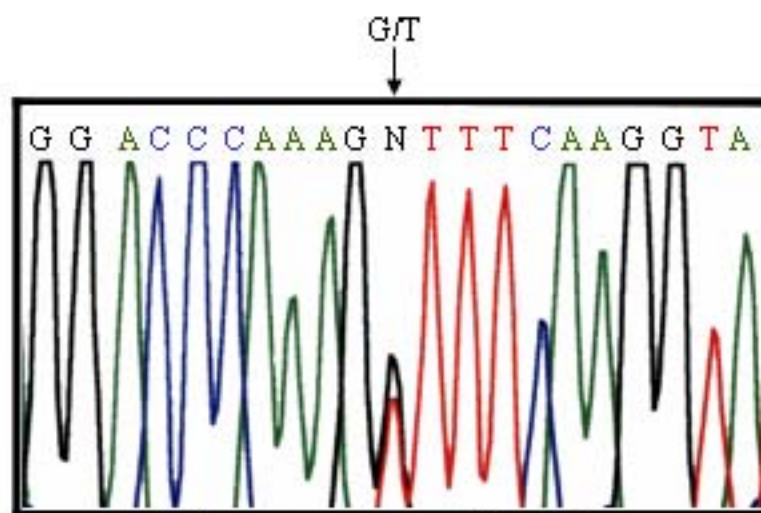
(b)



(c)



Wild-type sequence  
Exon 92



Heterozygous sequence G2366V  
Exon 92

