Case Report

Novel and recurrent *COL7A1* mutations in Chinese patients with dystrophic epidermolysis bullosa pruriginosa

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ABSTRACT. Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare subtype of dystrophic epidermolysis bullosa (DEB). This disease is characterized by severe itching, lichenoid nodules or prurigo-like lesions, and linear scarring with a predilection for the extensor limbs. Pathogenic mutations in the type VII collagen alpha 1 (*COL7A1*) gene have been identified. We analyzed mutations in the *COL7A1* gene in a Chinese family including 5 affected individuals with typical DEB-Pr and in a patient previously reported with sporadic DEB-Pr. The entire coding region and exon-intron boundaries of *COL7A1* were detected by polymerase chain reaction and direct sequencing. We identified one novel heterozygote mutation (c.6842G>T, p.G2281V) and a second mutation (c.5443G>A, p.G1815R) reported previously in patients with DEB. Our findings contribute to the *COL7A1* mutation database and further reveal the genetic and phenotypic heterogeneity of DEB-Pr.

Key words: Dystrophic epidermolysis bullosa pruriginosa; *COL7A1* gene; Mutation analysis

INTRODUCTION

Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr; OMIM# 604129) is characterized by severe itching and lichenified or prurigo-like papules and nodules, with a predilection for the extensor aspect of the lower extremities (McGrath et al., 1994; Ee et al., 2007). Linear scarring, trauma-induced blistering, and nail dystrophy, as well as albopapuloid lesions on the trunk, were observed in some cases (McGrath et al., 1994; Ee et al., 2007). Skin lesions of DEB-Pr typically develop during early childhood, but occasionally occur in adulthood as well (Brick et al., 2012; Yang et al., 2012). Pathogenic mutations have been identified in the type VII collagen alpha 1 (*COL7A1*) gene on chromosome 3p21.3, which encodes the anchoring fibril protein of type VII collagen (Dang et al., 2007). Collagen VII is synthesized in keratinocytes and fibroblasts as a procollagen molecule pro-α1 (VII) polypeptide, and is a major structural component of the anchoring fibrils (Chen et al., 1997). Collagen VII may enable the adhesion of the epidermis and the dermis (Bruckner-Tuderman et al., 1999). More than 60 mutations in *COL7A1* have been described in DEB-Pr (Fortuna et al., 2013). Autosomal dominant, autosomal recessive, and sporadic inheritance patterns have been described in this rare disorder (Cambiaghi et al., 1997).

To obtain more information regarding the pathogenic mechanisms of *COL7A1* mutations, we performed a mutation analysis of the *COL7A1* gene in a Chinese family including 5 affected individuals with typical DEB-Pr phenotypes, and in a patient with sporadic DEB-Pr previously reported in 2009 (Fan et al., 2009).

MATERIAL AND METHODS

Patients and clinical examination

In this study, we investigated a 3-generation family with DEB-Pr (Figure 1A) and one patient with sporadic DEB-Pr, all recruited from the Guangdong Province of China. Informed consent was obtained from all subjects and relatives for clinical and genetic investigation. Permission was also obtained from the ethics commission of our hospital.

Case 1

A 23-year-old female proband presented with a 3-year history of pruritic papules on the extensor sides of the extremities. Nail dystrophy developed during childhood and a black patch appeared on the left foot at birth. She was otherwise in good general health, except for skin and nail lesions. At the age of 20 years, pruritic blisters developed spontaneously or after mild trauma on the legs and healed with scars and papules. The family included 5 affected individuals, and her parents were non-consanguineous. Clinical examination revealed multiple keratotic and excoriated papules and scars, and some milia on the extensor sides of the lower legs, forearms, and abdomen (Figure 1B). The patient's toenails showed obvious dystrophy, and a black patch with a few nodules was visible on the dorsal side of the left foot and 3 toes (Figure 1C). The leg papule exhibited hyperkeratosis, irregular acanthosis, and subepidermal splits, as well as dermal fibrosis, telangiectasias, and a mild perivascular lymphohistiocytic infiltrate in the papillary dermis. Affected family members also had mild prurigo-like lesions

and toenail thickness. No family members had a history of skin cancer or other abnormalities.
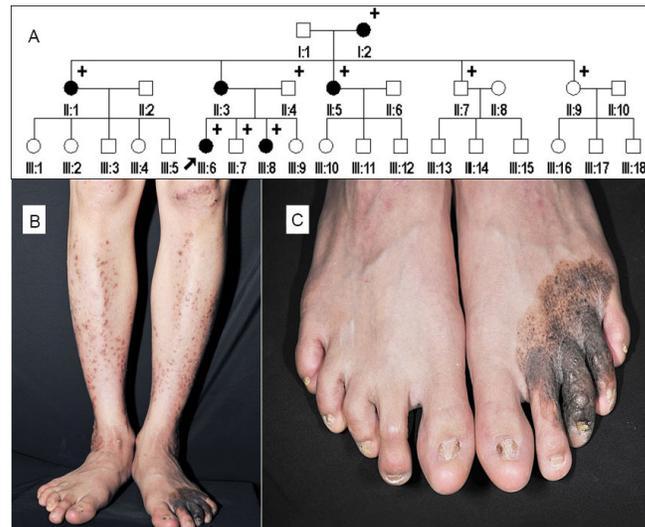
Figure 1. A. Pedigree of DEB-Pr family studied and clinical features in Case 1. **B.** Multiple keratotic and excoriated papules and scars, as well as some milia on the extensor sides of the lower legs can be seen. **C.** Nail dystrophy on the feet and a congenital melanocytic nevus on the dorsal side of the left foot and 3 toes are evident.

Case 2

An 18-year-old man was the first-born of unrelated parents and had an unremarkable family history. Spontaneous or trauma-induced pruritic blisters and subsequent papules and scars developed on the legs and feet at the age of 3 years. From the age of 8 years, albopapuloid lesions and milia occurred on the trunk with decreasing blistering frequency. Scalp papules and pustules with non-scarring healing commenced 2 years prior (Fan et al., 2009).

Mutational analysis

Genomic DNA was extracted from peripheral blood lymphocytes in a DEB-Pr family (5 patients, 4 healthy family members), 1 patient with sporadic DEB-Pr, and 100 unrelated healthy Chinese people. Genomic DNA was used to amplify the exons of the *COL7A1* gene with intronic flanking sequences by polymerase chain reaction (PCR) with the primers described elsewhere (Christiano et al., 1997). PCR products were purified using a QIA quick PCR Purification kit (Qiagen; Hilden, Germany), and the *COL7A1* gene was sequenced using the ABI PRISM®3730 automated sequencer (Applied Biosystems; Foster City, CA, USA). Sequence comparisons and analysis were performed using the Phred-Phrap-Consed version 12.0 program.

RESULTS

We identified 2 heterozygous mutations in the *COL7A1* gene. One (c.6842G>T) was

a novel mutation in patient with sporadic DEB-Pr (Figure 2A and B); another (c.5443G>A (rs121912841) was in a family with DEB-Pr (Figure 2C and D) and has been previously reported in dystrophic epidermolysis bullosa (DEB), but not in DEB-Pr. Both mutations were missense mutations, leading to amino acids substitutions (p.G2281V and p.G1815R). No mutations were found in the 100 healthy controls or unaffected family members.
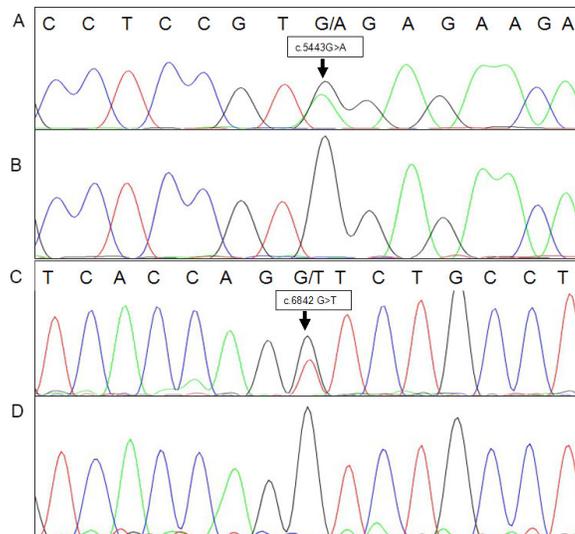
Figure 2. Mutation analysis of *COL7A1* gene in patients. **A.** Heterozygous missense mutation c.5443G>A in exon 63 in Case 1. **B.** Sequence of exon 63 of the *COL7A1* gene in normal subjects. **C.** Heterozygous missense mutation c.6842G>T in exon 87 in Case 2. **D.** Sequence of exon 87 of the *COL7A1* gene in normal subjects.

DISCUSSION

DEB encompasses a heterogeneous group of inherited blistering dermatoses. DEB-Pr, a rare subtype of DEB, also shows genetic heterogeneity, as autosomal dominant and recessive inheritance patterns have been previously reported (Jiang et al., 2012). *COL7A1* gene mutations have been demonstrated to cause the entity (Christiano et al., 1997). The *COL7A1* gene encodes a 2944-amino acid protein (type VII collagen), one of the major components of anchoring fibrils protein (Christiano et al., 1994). Mutations in this gene may result in a decreased quantity or abnormal function of type VII collagen. Mutation carriers often present with clinical manifestations of DEB-Pr during early childhood. To date, including our study, 63 mutations in the *COL7A1* gene have been reported to be associated with DEB-Pr, of which 36 mutations are glycine substitutions in the first amino acid of the Gly-X-Y repeats located in the triple-helix region (Fortuna et al., 2013).

In our study, we first observed a novel glycine substitution mutation, p.G2281V, in a sporadic Chinese DEB-Pr patient that has been previously reported (Fan et al., 2009). In addition to the clinical characteristics of DEB-Pr, such as trauma-induced blistering, prurigo-like lesions, milia, and albopapuloid lesions, the patient showed multiple follicular papules and pustules on the scalp, but no nail dystrophy. Whether or not the novel mutation p.G2281V is related to the unique phenotype of this case should be further examined.

By contrast, the mutation p.G1815R (rs121912841) was previously detected in DEB patients with toenail dystrophy (Sato-Matsumura et al., 2002). Similarly, all of the affected individuals in the 3-generation family with DEB-Pr showed typical manifestations of DEB-Pr and toenail dystrophy. The inheritance of all the familial patients was consistent with an autosomal dominant pattern. These data indicate that the p.G1815R mutation accounts for toenail dystrophy and that the same mutation results in various manifestations.

The phenotypic disparity of DEB-Pr is currently unknown. Identical mutations in the *COL7A1* gene can lead to DEB-Pr and the classical form of DEB (Almaani et al., 2009). Although 63 mutations in the *COL7A1* gene have been identified in different pedigrees of DEB-Pr, these mutations and their relationship between genotypes and phenotypes remain unclear. Theoretically, *COL7A1* gene mutations can influence the biosynthesis of collagen VII, resulting in different phenotypes of DEB-Pr (Drera et al., 2006; Dang and Murrell, 2008). Furthermore, although additional immune-mediated factors may be involved in the pathogenesis of DEB-Pr (Jiang et al., 2012), it is unclear whether other genetic, epigenetic, metabolic, or environmental factors contribute to the DEB-Pr phenotype (Almaani et al., 2009).

In conclusion, one novel and another previously reported mutations of *COL7A1* were identified in two Chinese families with DEB-Pr. These 2 mutations may be the underlying causes of DEB-Pr in the familial and sporadic Chinese cases, but they are not common polymorphisms. Our findings enrich the *COL7A1* mutation database and provide information regarding the genetic and phenotypic heterogeneity of DEB-Pr. The correlation between genotypes and phenotypes in DEB-Pr should be further examined.

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