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CLINICAL REPORT

Genotype–Phenotype Correlation in Chinese Patients with Dystrophic Epidermolysis Bullosa Pruriginosa

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Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare variant of dystrophic epidermolysis bullosa (DEB) due to dominant or recessive mutations in the *COL7A1* gene. More than 40 mutations in *COL7A1* have been described in DEB-Pr. The aim of this study was to understand the genotype–phenotype correlation in Chinese patients with DEB-Pr. Three Chinese families with typical clinical features of DEB-Pr were studied. The results were analysed in association with the eight Chinese DEB-Pr patients reported in the literature. In the three Chinese families with DEB-Pr, we found two dominant cases with G1773R and c.6900+1G>C mutations, and one case with heterozygous G2701W mutation of uncertain inheritance mode. In the 10 Chinese patients with dominant type of DEB-Pr, 7 glycine substitutions and three splicing site mutations of exon 87 skipping were identified. Glycine substitution mutations in the triple helix region and exon 87 skipping, leading to the in-frame deletion of 23 amino acid residues in the triple-helix, are often seen in Chinese patients with dominant DEB-Pr, although the glycine substitutions are also frequently present in dominant DEB. *Key words: dystrophic epidermolysis bullosa pruriginosa; genotype-phenotype correlation; Chinese patients.*

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Dystrophic epidermolysis bullosa (DEB) is a group of hereditary bullosa disorders characterized by mucocutaneous blistering induced by minor trauma, resulted in scarring and nail dystrophy. The blisters occur beneath the lamina densa of the epidermal-dermal junction due to congenital abnormalities of type VII collagen, the major component of anchoring fibrils (1). Mutations in *COL7A1* give rise to autosomal dominant or autosomal recessive forms of the disorder. Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare form of DEB, characterized by intense pruritus, nodular prurigo-like lesions and fragility of the skin, mainly in the extremities (2). The onset of these clinical features may be evident in early childhood, but in most cases the start of the symptoms is delayed until the second or third decade of life (3). Autosomal dominant, autosomal recessive and sporadic inheritance patterns have all been described in this disease (4). In this study, we conducted mutation detection of *COL7A1* in three unrelated Chinese patients with DEB-Pr. We also reviewed eight unrelated Chinese DEB-Pr patients in the literature in association with the three cases, in order to determine whether this form of DEB has relatively specific *COL7A1* genotypes.

MATERIALS AND METHODS

Patients

Proband 1, a 9-year-old Chinese boy, was found to have skin fragility, pruritus, and nodular prurigo-like lesions on his lower back and the extensor aspects of his legs. The pruritus and skin lesions were often aggravated during the summer. He had used topical corticosteroid ointment to control the itching, but without relief. His toenails had thickened since the age of 2 years (Fig. 1A–C). His mother and maternal grandfather also had mild prurigo-like lesions and toenail thickness, suggesting autosomal dominant inheritance of the disease. Proband 2, a 20-year-old Chinese male, had moderately to severely pruritic and widespread lichenoid lesions with a striking linear configuration, and thickened toenails from the age of 4 years. There was also a family history of these symptoms in four generations, characteristic of autosomal dominant inheritance (Fig. 1D–F). Proband 3, a 25-year-old Chinese female, had nodular prurigo-like lesions on her limbs and lower back, severe pruritus and thickened toenails since the age of 16 years with no family history of the disease (Fig. 1G–I). By using a variety of treatments, including oral traditional Chinese medicine, thalidomide and topical steroid ointment, her skin lesions improved only temporarily. Her father had psoriasis. Serum IgE was normal in the three patients. No atopic history was found in their families. Haematological and serum biochemical data were within normal ranges. Punch biopsy samples were obtained from lichenoid papules of the three probands for histopathological, indirect immunofluorescence and electron microscopy examinations.

Mutation detection

Genomic DNA samples extracted from peripheral blood lymphocytes were obtained from the three probands and their parents. The 118 coding exons and their flanking introns of *COL7A1* gene were amplified from the genomic DNA samples of the three probands by PCR using the previously reported primer pairs (5) with some modifications based on the human genome database in the webpage http://genome.ucsc.edu. The sequences of the PCR primer pairs are provided in Table SI (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1178). PCR products were directly sequenced on an automated DNA sequencer (ABI Prism 377 Sequencer) after purification of the products in 1.5% agarose gels.

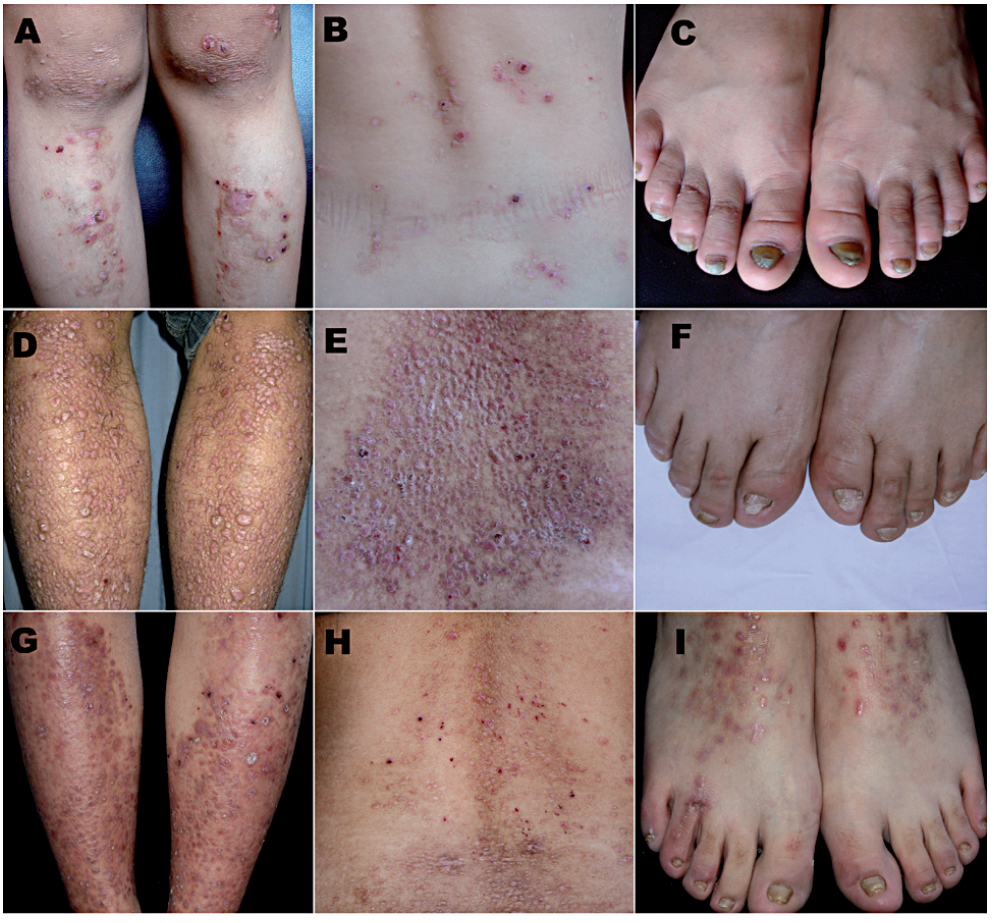

Fig. 1. Clinical appearance of the three dystrophic epidermolysis bullosa pruriginosa probands. (A–C) Proband 1, nodular prurigo-like papules on the extensor aspects of the legs, and on the lower back, with thick toenails. (D–F) Proband 2, widespread lichenoid lesions with a striking linear configuration on the legs and lower back, with thick toenails. (G, H) Proband 3, nodular prurigo-like papules on the extensor aspects of the legs, the lower back and dorsum of the feet, with thick toenails.

Proband 2 carried a c.6900+1G>C mutation in intron 87 of *COL7A1* gene. To examine the consequence of this mutation to mRNA splicing, we performed reverse transcription-PCR (RT-PCR) using total RNA extracted from the peripheral lymphocytes of proband 2. The nucleotide 6783-7083 of COL7A1 cDNA transcribed from exons 86–92 was amplified by PCR using exonic primers of sense primer: 5`-AGA TGG TGC CAG TGG AAA AG (6783-6802) and antisense primer: 5`-AGC CCC TTT CTG ACC ATC TT (7083-7062). The size of the normal PCR product is predicted to be 301 bp, but two PCR bands were found in the PCR product amplified from the cDNA of proband 2 (Fig. S1, available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1178). The lower band was cut, purified, and sequenced on an ABI Prism 377 sequencer (Fig. S2, available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1178).

RESULTS

Histopathology, immunofluorescence and electron microscopy studies

Light microscopic examination of the 3 probands' skin biopsies from lichenoid plaques showed hyperkeratosis, mild acanthosis, dermal-epidermal separation, and dense fibrosis in the upper dermis. Electron microscopy showed abundant collagen bundles beneath the dermal–epidermal junction; anchoring fibrils were also reduced in number in perilesional skin. Indirect immunofluorescence study using monoclonal antibody LH 7:2 recognizing type VII collagen revealed normal or nearly normal linear fluorescence located in the dermal–epidermal junction (data not shown).

Mutations in the 3 probands

In proband 1, we found a heterozygous transversion mutation of c.5317G>C in exon 61, which results in the amino acid change of G1773R (Fig. S2A). This mutation also presented in his clinically affected mother, but was not found in the 50 unrelated normal controls.

In proband 2, we detected a heterozygous transversion mutation of c.6900+1G>C at the donor end of intron 87 (Fig. S2B), which is a splicing site mutation. We further confirmed the abnormal splicing of COL7A1 mRNA in this proband by RT-PCR using total RNA from peripheral leukocytes. This mutation caused in-frame skipping of exon 87 in COL7A1 mRNA (Fig. S2D), resulting in a deletion of 23 amino acid residues in the Gly-X-Y triple repeat region of the protein. This mutation was not found in the 50 normal controls.

In proband 3, we detected a heterozygous transversion mutation of c.8101G>T in exon 109, which caused the amino acid change of G2701W (Fig. S2C). Her clinically unaffected mother had the same mutation, and her father was clinically normal. This mutation was not found in the 50 normal controls.

DISCUSSION

In this report we have identified three pathogenic *COL7A1* mutations (G1773R, splicing site mutation of c.6900+1G>C, and G2701W) in the 3 DEB-Pr families. G1773D (c.5318G>A) mutation was previously described in a dominant DEB patient (6), which locates at the same residue site as the mutation in our proband 1, but with different amino acid change. The splicing site mutation of c.6900+1G>T was originally reported in a DEB-Pr case (7), which is similar to the mutation in our proband 2, but with different nucleotide change. The G2701W mutation has not been reported previously.

Proband 3 had a heterozygous mutation of c.8101G>T (G2701W) in exon 109. Her mother carried the same heterozygous mutation without any DEB manifestations. One possibility is that c.8101G>T (G2701W) is a dominant mutation with reduced penetrance, and her mother carries this mutation without DEB phenotype. Individuals with identified *COL7A1* mutations but without signs of DEB have been reported (8, 9). The other possibility is that c.8101G>T (G2701W) is a recessive mutation, and the other mutation in the proband/her father has not yet been detected.

The splicing site mutation of c.6900+G>T in proband 2 was further confirmed by RT-PCR using total RNA from the patient's blood leucocytes. Skin tissue contains abundant COL7A1 mRNA, but peripheral leucocytes also express small amount of COL7A1 mRNA (http://bioinfo2.weizmann.ac.il/cgi-bin/genenote/home_page.pl), which is easily available for screening abnormalities in COL7A1 mRNA. This approach may be used as the first step to screen mutations in *COL7A1* gene.

We have reviewed the 8 reported and unrelated Chinese DEB-Pr patients in association with the three cases in the present study (Table I). The area of skin lesions seems to be relatively widespread, and the pruritus is more severe in patients with splicing site mutations. Pruritus can also occur in DEB patients and other forms of epidermolysis bullosa, but is always less severe than that in DEB-Pr. There is no evidence of other specific causes of pruritus in Chinese DEB-Pr patients. Elevated serum IgE and/or atopic history have been reported in DEB-Pr patients, but only one Chinese patient showed an increase in serum IgE (3). Therefore, the higher serum IgE seems not to be closely related to Chinese DEB-Pr cases. Most cases are of dominant inheritance, and the inheritance mode is uncertain in one case (proband 3 in this study). In the 10 dominant DEB-Pr (DDEB-Pr) patients, seven mutations locate at the first glycine position in Gly-X-Y repeats in the triple helical region of the protein. All the three splicing site mutations locate near the 3' end of exon 87, resulting in exon 87 skipping, which causes in-frame deletion of 23 amino acid residues in the triple helical region. Therefore, glycine substitution mutations in the triple helix region and exon 87 skipping leading to the in-frame deletion of 23 amino acid residues in the triple-helix are often seen in Chinese patients with DDEB-Pr, although the glycine substitutions are also frequently present in DDEB. Glycine substitution mutations in the triple helix region and in-frame deletion of 23 amino acid residues in this region may influence the adhesive function of collagen VII, resulting in the instability of anchoring fibril structure. Activation of inflammatory responses due to the chronic skin damage and repeated wound healing may play a part in the characteristic presentations of DEB-Pr. The improvement of DEB-Pr after treatment with immunomodulatory drugs, such as cyclosporine, thalidomide and tacrolimus, suggests the involvement of additional immune-mediated factors in the pathogenesis of DEB-Pr.

We also summarized the mutations in *COL7A1* in DDEB-Pr reported to date in the literature. Fig. 2 demonstrates that most mutations locate in the triple helical region and most mutations are G substitutions at the first position of Gly-X-Y repeats in this region (6–21). Of the splicing site mutation, all are found in the triple helical region, and most of them locate in the 3' end region of exon 87 or near the donor site of intron 87, probably resulting in the in-frame skipping of exon 87 (3, 16, 17). Substitution mutations in the triple-helix region, especially the substitution of glycine residues in Gly-X-Y repeats, are the characteristic molecule bases underlying the pathogenesis of DDEB (22). The glycine residues in Gly-X-Y repeats locate along the central axes of the triple helices, where the tight packing of

Table I. *Mutations in COL7A1 gene and their consequences in 11 Chinese dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) families*

Pat. No.	Diagnosis	Mutation	Exon/intron	Amino acid change	Consequence	Reference
1	DDEB	c.5317G>C	Exon 61	G1773R	GS	This study
2	DDEB	c.6100G>A	Exon 73	G2034R	GS	Chen et al. (10)
3	DDEB	c.6724G>A	Exon 85	G2242R	GS	Lee et al. (11)
4	DDEB	c.6724G>T	Exon 85	G2242W	GS	Shi et al. (12)
5	DDEB	c.6752G>A	Exon 86	G2251E	GS	Ee et al. (8)
6	DDEB	c.6900+1G>C	Intron 87	6900+1G>C	in-frame skipping	This study
7	DDEB	c.6899+2A>G	Intron 87	6899+2A>G	in-frame skipping	Jiang et al. (13)
8	DDEB	c.6900+1G>T	Intron 87	6900+1G>T	in-frame skipping	Ren et al. (7)
9	DDEB	c.7097G>A	Exon 92	G2366V	GS	Chuang et al. (14)
10	DDEB	c.7877G>A	Exon 106	G2626D	GS	Wang et al. (15)
11	Uncertain	c.8101 G>T	Exon 109	G2701W	GS	This study

DDEB: dominant DEB; GS: glycine substitution mutation.

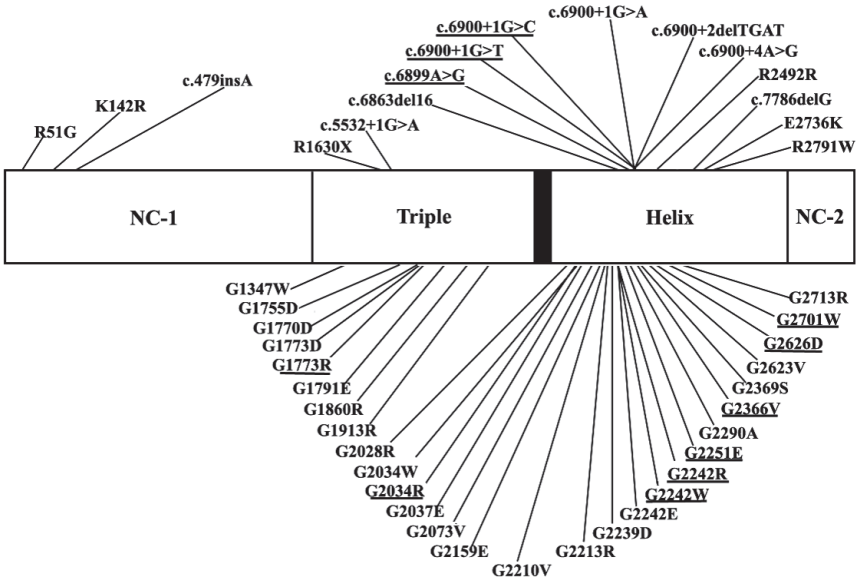

Fig. 2. COL7A1 mutations reported in patients with dominant DEB-Pr (DDEB-Pr). The mutations in Chinese patients (including 3 patients in this study) are underlined.

the protein strands cannot accommodate amino acids other than glycine. Interestingly, most heterozygous splicing site mutations near the 3' end of exon 87 are reported in DDEB-Pr. Accordingly, mutations leading to skipping of exon 87 may be closely correlated with the phenotype of DDEB-Pr.

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