LETTERS TO THE EDITOR

Recurrent COL7A1 Mutations in Japanese Patients with Dystrophic Epidermolysis Bullosa: Positional Effects of Premature Termination Codon Mutations on Clinical Severity

To the Editor:

Epidermolysis bullosa (EB) is a group of heritable mechano-bullous disorders characterized by fragility of the skin and mucous membranes (Fine et al, 1991; Christiano and Uitto, 1996a). A special subset, the dystrophic forms of EB (DEB), is inherited either in an autosomal dominant (DDEB) or in an autosomal recessive (RDEB) fashion. Ultrastructurally, DEB is characterized by abnormalities in the anchoring fibrils, attachment structures extending from the lower portion of the dermal–epidermal basement membrane to the underlying dermis (McGrath et al, 1993). These structures can be morphologically altered, reduced in number, or entirely absent in patients with DEB.

Biochemical evidence has indicated that type VII collagen is the major, if not the exclusive, component of anchoring fibrils (Sakai et al, 1986; McGrath et al, 1993). This observation suggested that type VII collagen and the corresponding gene, COL7A1, are the candidate gene/protein systems for mutations in DEB (Uitto and Christiano, 1992). Subsequently, a number of distinct genetic lesions in COL7A1 have been demonstrated (Christiano and Uitto, 1996a, b; Hovnanian et al, 1997; Uitto et al, 1999).

Examination of the mutation database in DEB has suggested that, in general, the mutations are family specific, with relatively little evidence of recurrent mutations due to a founder effect or “hotspot” mutations (Uitto et al, 1999); however, careful examination of the database revealed that three mutations, 5818delC, 6573 + 1G→C, and E2857X occur in more than one unrelated family and that these mutations are present only in individuals of Japanese ethnic origin (Uitto et al, 1999). These observations suggested therefore that these three mutations may be restricted to the Japanese gene pool. In this study, we examined a cohort of 50 Japanese patients for the presence of these three mutations. All three patients were compound heterozygotes for two of these three recurrent mutations, respectively (see below). Examination of the current database consisting of 91 distinct COL7A1 mutations in 102 individuals with DEB failed to reveal the presence of these three mutations in non-Japanese patients (Uitto et al, 1999).

Clinically, DEB manifests with a spectrum of phenotypic severity. The most severe, recessively inherited form, the Hallopeau-Siemens variant (HS-RDEB), manifests with extreme fragility of skin leading to extensive scarring and fusion of the digits (pseudosyndactyly), associated with extracutaneous manifestations such as esophageal strictures and corneal erosions. Later in life, development of aggressively metastasizing squamous cell carcinomas can lead to a premature demise of the individual affected by HS-RDEB. In the milder, so-called mitis forms, M-RDEB, protracted blistering leads to less pronounced scarring with little or no tendency for pseudosyndactyly; however, the clinical phenotype presents with a continuum of severity, and some patients are classified as HS/M-RDEB.

Three patients in which recurrent mutations in both alleles were delineated were examined in detail.

Figure 1. Screening of Japanese DEB patients for recurrent mutations by restriction endonuclease digestions. Specifically, the 5818delC mutation destroys a restriction enzyme site for MspI (Christiano et al, 1995), whereas the mutation 6573 + 1G→C creates a new restriction enzyme site for MspI (Tamai et al, 1997).

Screening of 50 unrelated Japanese patients with RDEB revealed the presence of any of the three mutations in multiple individuals (Table I). Specifically, mutations 5818delC and E2857X were present in nine individuals (18%) each, whereas the mutation 6573 + 1G→C was found in six individuals (12%). None of the patients was homozygous for the respective mutation; however, three patients were compound heterozygotes for two of these three recurrent mutations, respectively (see below).

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Table I

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Restriction Enzyme</th>
<th>Digestion Products</th>
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<tbody>
<tr>
<td>5818delC</td>
<td>MspI</td>
<td>1 2 3</td>
</tr>
<tr>
<td>E2857X</td>
<td>MaeI</td>
<td></td>
</tr>
<tr>
<td>6573+1G→C</td>
<td>MspI</td>
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Manuscript received July 22, 1998; revised January 6, 1999; accepted for publication February 25, 1999.

Reprint requests to: Dr. Katsumi Tamai, Department of Dermatology, Hirosaki University School of Medicine, 5, Zaifu-cho Hirosaki, 036, Japan.
Patient 1 was a 39 y old Japanese man whose parents were unrelated and clinically unaffected. The clinical details of this patient consistent with the diagnosis of M-RDEB have been reported elsewhere (Fig 2A; Tamai et al, 1997). Patient 2 was a 40 y old Japanese male with generalized trauma-induced blistering since birth. He had lost all fingernails and toenails, and developed severe syndactyly (Fig 2B). The clinical presentation of this patient was clearly more severe than in Patient 1, but not as severe as in Patient 3. Clinically, this patient was classified as HS/M-RDEB.

Patient 3 was a 22 y old Japanese male with extensive and extremely severe blistering of the skin since birth. No other member of his family is affected, and his parents were not consanguineous. Marked scarring and ulcers were observed all over his body. Diffuse alopecia and almost complete syndactyly had developed (Fig 2C). The clinical diagnosis was HS-RDEB.

Examination of the mutations in these three patients revealed that Patient 1 was a compound heterozygote for 6573 + 1G→C/E2857X, the two most 3' mutations of these three PTC mutations (Fig 2A). In contrast, Patient 3 was compound heterozygote for 5818delC/6573 + 1G→C, the two most 5' mutations (Fig 2C). Patient 2 with intermediate severity was compound heterozygote for 5818delC/E2857X mutations (Fig 2B). These results clearly indicate that mutation 6573 + 1G→C, when compared with the more 5' mutation 5818delC, both superimposed on the common mutation E2857X in the other allele, results in a milder phenotype.

On the other hand, mutation E2857X, by comparison with the mutation 5818delC in the other allele, results in a milder phenotype (Fig 2). These observations clearly suggest that the positions of the PTC mutations along the type VII collagen polypeptide can influence the clinical severity, and in general, the spectrum of genetic lesions in COL7A1 can underlie different clinical variants of DEB. The combinations and types of mutations as well as their positions along the type VII collagen molecules, superimposed on genetic background of the affected individual, collectively reflect the phenotypic variability observed in DEB.

Table I. Recurrent mutations in Japanese patients with RDEB

<table>
<thead>
<tr>
<th>Mutation</th>
<th>5818delC</th>
<th>6573 + 1G→C</th>
<th>E2857X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (n = 50)</td>
<td>9</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Per cent</td>
<td>18</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Non-Japanese RDEB patients (n = 120)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The authors thank Carol Kelly for excellent secretarial assistance and Yasuyoshi Shikama and Komaki Hanada for skilled technical help. This study was supported by grants from the Rare and Intractable Disease Research Committee (Ministry of Health and Welfare, Japan); the Ministry of Education, Science, Sports and Culture of Japan (07670927); the Dystrophic Epidermolysis Bullosa Research Associations of U.K. and America; and the United States Public Health Service, National Institutes of Health (P01-AR38923).

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Figure 2. Clinical features of Patients 1, 2, and 3. The positions of the premature termination codon-causing mutations (PTC) along the type VII collagen molecule in the corresponding cases are shown on the right-hand side. Open boxes (□) correspond to the triple-helical regions, whereas the solid bars (■) correspond to noncollagenous domains within the type VII collagen molecule. During diagnostic work-up, indirect immunofluorescence of the skin within a monoclonal LH7.2 antibody recognizing type VII collagen showed clearly positive staining in Patient 1 (A) at the dermal–epidermal junction, whereas the basement membrane zone staining in Patient 2 (B) was markedly attenuated. The staining in Patient 3 (C) was entirely negative. Transmission electron microscopy showed clearly detectable, yet thin, anchoring fibrils in Patient 1, whereas the anchoring fibrils in Patient 2 were markedly reduced in number. No anchoring fibrils were seen at the dermal–epidermal junction in Patient 3 (not shown).
Native Type I Collagen is Not a Substrate for MMP2 (Gelatinase A)

To the Editor:

A recent JID paper by Herouy et al. (1998) presented some interesting new data on lipodermatosclerosis. Their interpretations of the data, however, are based upon a single paper in the MMP literature (Aimes and Quigley, 1995), which claims that MMP2 (gelatinase A) is an interstitial collagenase, a claim that we have not been able to confirm. We were the first laboratory to purify, characterize, and clone human MMP2 (Seltzer et al., 1981; Collier et al., 1988). We found that this proteinase had absolutely no activity against helical collagen. Native helical collagen is defined by its resistance to trypsin cleavage and characteristic viscosity in solution. Subsequent work from our laboratory has shown that MMP2 is an extremely opportunistic proteinase against any collagenous sequence in which the helicity is not perfect. For example, MMP2 cleaves helical type VII collagen within the helical portion of the molecule, but in an area that has relaxed helicity (Seltzer et al., 1989). When the Aimes and Quigley paper was published we confirmed our original observations using pure TIMP-free MMP2 and helical collagen shown to be trypsin resistant. Interstitial type I collagen in which the helix was relaxed enough to render it susceptible to trypsin digestion was indeed susceptible to digestion by MMP2. Intact helical collagen was not. Ohuchi et al. (1997) have recently confirmed our original findings.

Cleavage of interstitial collagen by MMP1 yields two characteristic fragments, which lose their helicity at 37°C and become soluble. Therefore, solubilization of type I collagen is not indicative of MMP2 activity, but is of MMP1 activity. Herouy et al. clearly show active MMP1 in their western immunoblots, indicating that cleavage of labeled type I collagen by their extracts can definitely occur.

It is important to point out that TIMP-MMP complexes have been previously described, but are separated when subjected to SDS-PAGE electrophoresis (Goldberg et al., 1989; Wilhelm et al., 1989). The high molecular weight band shown in the immunoblots (Fig 2) by Herouy et al. (1998) either are a new type of complex, or represent an artifact of antipeptide antibodies.

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References


Reply

Lipodermatosclerosis is characterized by distinct morphologic features. Prominent perivascular fibrin cuffs and deposition of hemosiderin pigments due to red blood cell extravasation are major tissue changes occurring in patients with severe chronic venous insufficiency. Perivascular cuffs are highly organized structures primarily composed of fibrin, laminin, fibronectin, tenascin and type I and III collagens (Herrick et al., 1992). Fibrous scar tissue associated with fragmentation of elastic fibers and loss of papillary structures at the dermal–epidermal junction are further histologic findings. The dermal–epidermal junction is built up by an extraordinarily complex network of interconnecting proteins such as different collagen subtypes and laminin (Burgese and Christiano, 1997). To understand the molecular basis of these morphologic alterations

Manuscript received January 18, 1999; accepted for publication March 7, 1999.