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Human Type VII Collagen: Genetic Linkage of the Gene (COL7A1) on Chromosome 3 to Dominant Dystrophic Epidermolysis Bullosa

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Summary

Epidermolysis bullosa (EB) is a heterogeneous group of heritable blistering disorders affecting the skin and the mucous membranes. Previous ultrastructural studies on the dystrophic (scarring) forms of EB have demonstrated abnormalities in the anchoring fibrils, morphologically distinct structures below the basal lamina at the dermal/epidermal basement membrane zone. Type VII collagen is the major collagenous component of the anchoring fibrils, and it is therefore a candidate gene for mutations in some families with dystrophic forms of EB. In this study, we performed genetic linkage analyses in a large kindred with dominant dystrophic EB. A 1.9-kb type VII collagen cDNA clone was used to identify a *Pvu*II RFLP to follow the inheritance of the gene. This RFLP cosegregated with the EB phenotype in this family, strongly supporting genetic linkage ($\hat{Z}$ = 5.37; $\hat{\theta}$ = .0). In addition, we assigned the type VII collagen gene (COL7A1) to chromosome 3 by hybridization to a panel of human × rodent somatic cell hybrids. These data demonstrate very close genetic linkage between the clinical phenotype in this family and the polymorphism in the type VII collagen gene mapped to chromosome 3. The absence of recombination between EB and the type VII collagen gene locus, as well as the observed abnormalities in the anchoring fibrils, strongly suggest that this collagen gene is the mutant locus in this kindred.

Introduction

Epidermolysis bullosa (EB) is a group of mechanobullous diseases characterized by a marked tendency to blistering of the skin and mucous membranes as a result of minor trauma. EB is characterized by considerable phenotypic variability, and the inheritance pattern can be either autosomal dominant or autosomal recessive. EB can be classified into three major categories based on the level of tissue cleavage within the basement-membrane zone at the dermal/epidermal interface: in the simplex, nonscarring forms of the disease, the blisters are intraepidermal; in the junctional forms, the cleavage occurs within the basement membrane at the level of the lamina lucida; and in the dystrophic—i.e., scarring—forms, the tissue separation occurs below the basement membrane, within the papillary dermis. (For reviews on EB, see Gedde-Dahl 1981; Kero 1984; Fine et al. 1991).

The dystrophic subtypes of EB can also be inherited in either a recessive or dominant pattern. Previous ultrastructural studies on dystrophic forms of EB have demonstrated abnormalities in the anchoring fibrils, morphologically distinct structures on the dermal side of the basement-membrane zone, which apparently provide stability for the attachment of the dermis to the basement membrane (Briggaman 1985; Tidman and Eady 1985). Recent studies have demonstrated that type VII collagen is a major component of the anchoring fibrils (Lunstrum et al. 1986, 1987; Sakai et al. 1986). This collagen has been shown to be absent

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in the skin of patients with severe generalized recessive dystrophic EB (Bruckner-Tuderman et al. 1989). Therefore, type VII collagen is a candidate gene in the dystrophic forms of EB. In the present study, we provide strong evidence for genetic linkage of the dominant dystrophic EB phenotype in a large kindred to the type VII collagen gene (COL7A1) that was localized on human chromosome 3.

Methods

Clinical

A large Finnish pedigree consisting of 20 affected and 22 unaffected living individuals in four generations was studied (fig. 1). The diagnosis of dominant dystrophic EB (the Cockayne-Touraine type) was made on the basis of clinical observations, as well as on the basis of histopathology and electron microscopy of the cutaneous lesions. Specifically, the affected patients demonstrated the presence of blisters and erosions, which healed with extensive scarring (fig. 2*A–C*). The tendency to blistering was noted either at birth or shortly thereafter. The affected individuals also had characteristic nail dystrophy (fig. 2*D*). Routine histopathology of the cutaneous lesions revealed blister formation below the basal lamina. Diagnostic electron microscopy of the nonblistered skin revealed a paucity of anchoring fibrils at the subbasal lamina region, and the few anchoring fibrils recognized were clearly hypoplastic and reduced in diameter. The inheritance in the family was consistent with an autosomal dominant pattern with complete penetrance (fig. 1).

Southern Analyses

DNA was isolated from peripheral blood leukocytes of 37 members of the family and from 25 unrelated Caucasian individuals and was subjected to digestions with 15 different restriction enzymes. Southern analyses were performed as described elsewhere (Knowlton et al. 1989).

A 1.9-kb cDNA (K-131), identified as a human type VII collagen clone, was used as the probe in Southern hybridizations. The details of the clone are reported elsewhere (Parente et al. 1991). In brief, the cDNA was isolated from a human keratinocyte λgt11 cDNA library by immunoscreening with the IgG fraction separated from serum of a patient with EB acquisita, an autoimmune disease characterized by circulating antibodies to type VII collagen epitopes (Woodley et al. 1988). Dideoxynucleotide sequencing (Sanger et al. 1977) of the cDNA revealed that it encodes a 192-amino-acid collagenous domain consisting of 52 Gly-X-Y repeats. GenBank searches revealed that these sequences were distinct from any previously published collagenous genes. Antibodies selected by epitopes in the fusion protein encoded by this cDNA recognized type VII collagen epitopes in western analyses (Parente et al. 1991). Thus, this 1.9-kb human cDNA (K-131) was identified as a type VII collagen clone.

The K-131 cDNA was used to search for polymorphisms in the corresponding gene. Initially, DNA isolated from six unrelated healthy individuals was digested with 15 different restriction endonucleases and was screened in Southern hybridizations. RFLPs discovered were confirmed in hybridizations to DNA digests of 28 additional normal individuals and to DNA digests of members of the family with dystrophic EB (see above). Segregation of the informative COL7A1 *Pvu*II polymorphism was determined in members of the family with dominant dystrophic EB (fig. 1), and the linkage data were analyzed by the LIPED program (Ott 1974). To establish the chromosomal location of the corresponding gene in the human genome, the probe was used for hybridizations to DNA isolated from a panel of human × rodent cell hybrids containing subsets of human chromosomes (BIOS, New Haven, CT) (Sawamura et al. 1990).

Results

The newly isolated cDNA, identified as a type VII collagen clone (Parente et al. 1991), was first used in a search for RFLPs in the COL7A1 gene. Southern analyses of DNA from unrelated healthy individuals that was digested with 15 different endonucleases revealed evidence of polymorphism in *Pvu*II digests (fig. 3). Specifically, some individuals were apparently homozygous with respect to the presence of a DNA fragment of 3.6 kb (allele A), while others demonstrated the presence of two fragments, one of 1.9 kb and the other of 1.7 kb (allele B). Analyses of DNA from apparently heterozygous individuals revealed the presence of all three DNA fragments (AB genotype) (fig. 3). The allelic frequencies of the A and B alleles in 34 unrelated normal individuals were 65% and 35%, respectively. Segregation of the variable bands in three families was consistent with Mendelian inheritance (see fig. 1; authors' unpublished observations).

The K-131 cDNA was also used for chromosomal localization of the COL7A1 gene in the human ge-

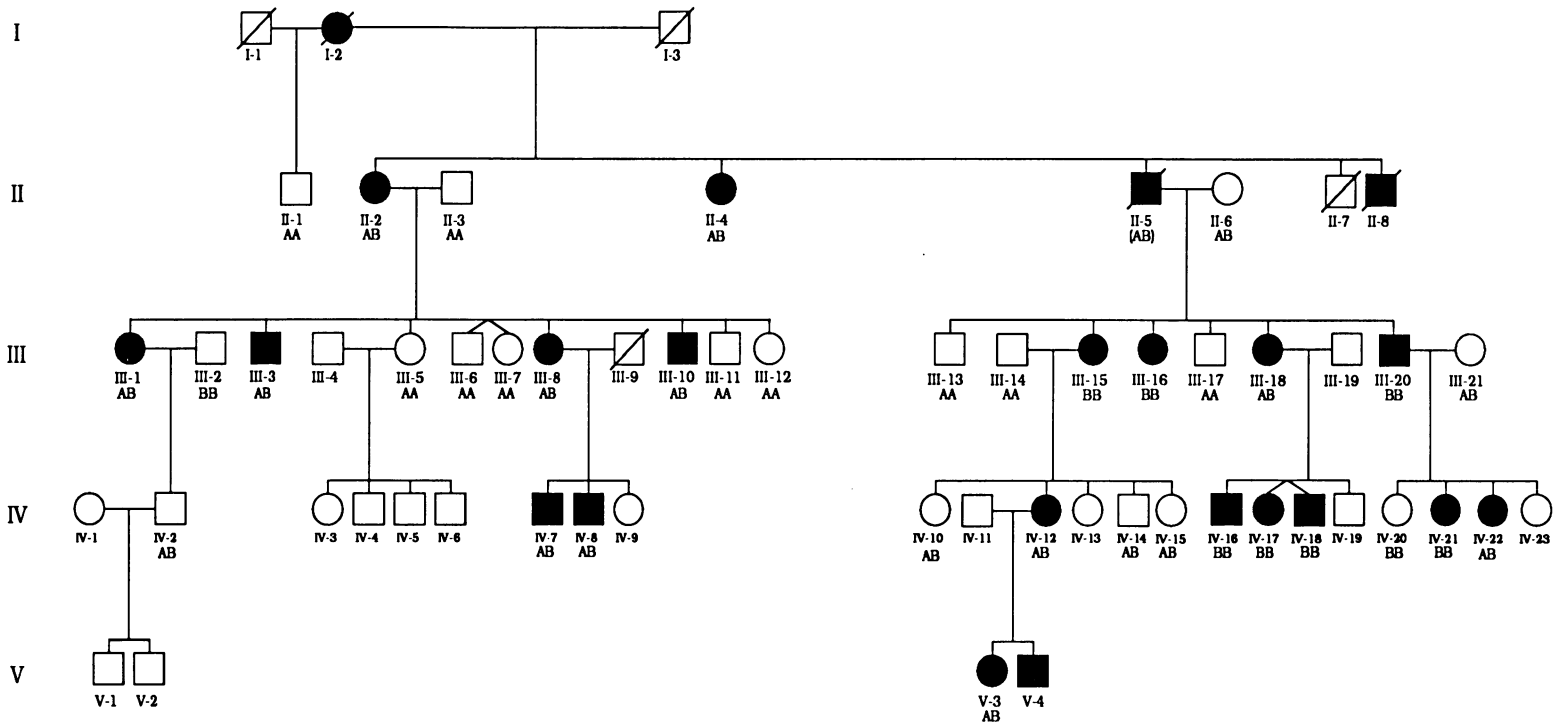

Figure 1 Pedigree with dominant dystrophic epidermolysis bullosa, with 20 affected and 22 unaffected living individuals in four generations. The genotype with respect to the inheritance of a type VII collagen allele, as described by inheritance of a *Pvu*II polymorphic marker (see fig. 3), is indicated under each figure (AA, AB, or BB). Individuals I-2 and II-5 were anamnestically found to have the disease. The genotype of individual II-5 (AB) is deduced from the children's genotype.

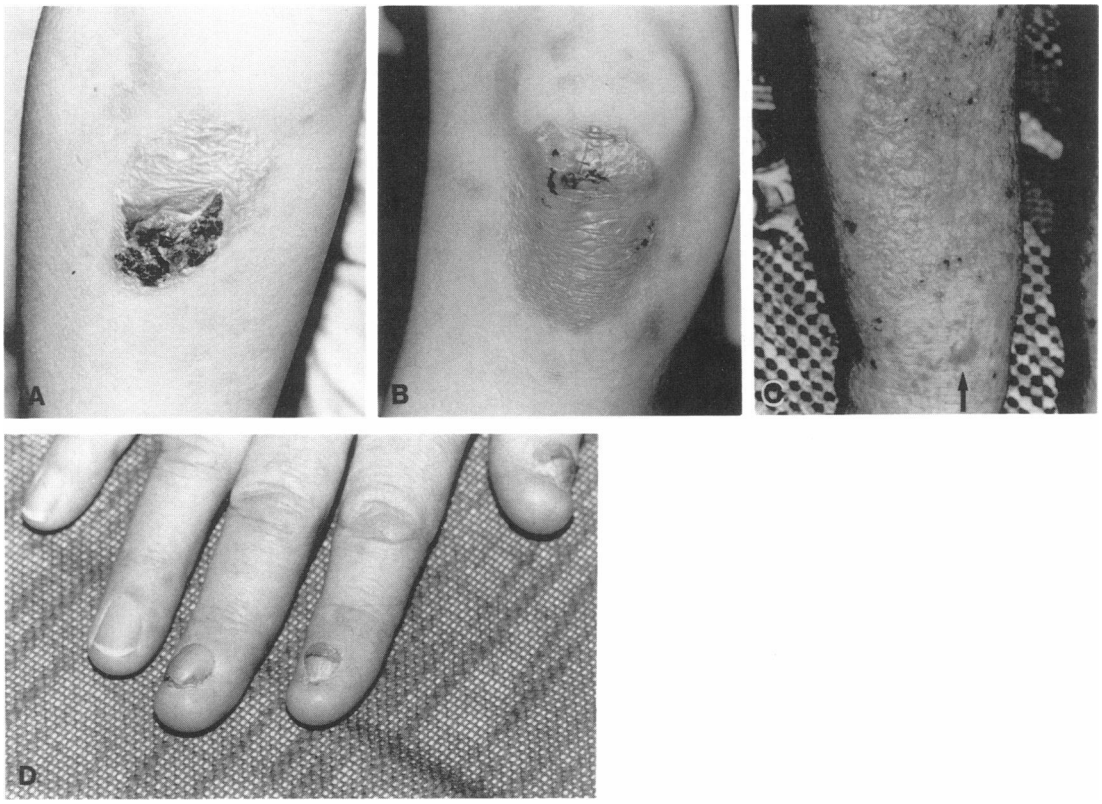

Figure 2 Clinical features of patients with dominant dystrophic EB in pedigree shown in fig. 1. Note the erosions and atrophic scarring on the pressure points (*A* and *B*.) The patients presented with tense blisters (arrow) and extensive scarring (*C*). All individuals affected by the skin lesions also demonstrated nail dystrophy (*D*).

nome by utilizing a panel of human × rodent somatic cell hybrids. The results of Southern hybridizations indicated concordance with human chromosome 3, while the data excluded all other chromosomes (table 1).

The large kindred was then analyzed for genetic linkage of dominant dystrophic EB and COL7A1 by using the *Pvu*II RFLP as a marker. Hybridizations of genomic DNA revealed cosegregation of the *Pvu*II polymorphism with the EB phenotype in the pedigree, with strong support for linkage (peak LOD score [$\hat{Z}$] = 5.37; peak recombination fraction [$\hat{\theta}$] = 0) (see Appendix). The upper confidence limit on the recombination fraction (θ) is .10, and the data indicate that the EB mutation is closely linked to the COL7A1 RFLP on chromosome 3. Because no recombination between EB and the COL7A1 locus was observed, the data are consistent with COL7A1 being the mutant gene.

Discussion

EB is clinically and genetically a heterogeneous group of blistering disorders that affect the basement-membrane zone at the dermal/epidermal interface. The cutaneous basement-membrane zone contains at least 20 different macromolecules, many of which have been characterized by immunologic, biochemical, and DNA cloning methods (Fine 1988; Uitto et al. 1989). None of these molecules has been directly implicated at the molecular level in any form of EB, and the low abundance and poor solubility of these macromolecules in human tissues preclude direct biochemical analyses.

Genetic approaches have suggested the chromosomal location of mutated genes in dominant simplex forms of EB. For example, linkage (LOD score [$\hat{Z}$] = 10.98; $\hat{\theta}$ = .05) to the glutamic-pyruvic-transaminase (GPT) locus on chromosome 8 has been demonstrated in a family with the rare "Ogna" variety of dominant simplex EB (EBS1) (Olaisen and Gedde-Dahl 1973). However, only a single pedigree demonstrating this genetic linkage has been identified thus far. More recently, several studies have provided suggestive evidence for the linkage of the generalized dominant simplex, the "Koebner" type of EB (EBS2), to the long arm of chromosome 1 (Mulley et al. 1984; Humphries

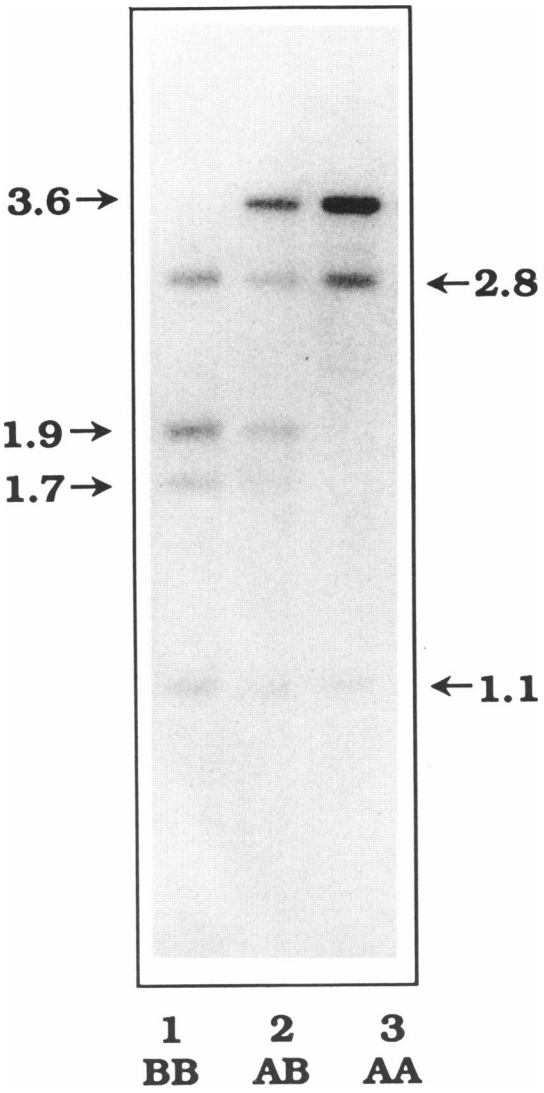

Figure 3 Demonstration of *Pvu*II polymorphism in type VII collagen gene. The Southern blot demonstrates hybridization of *Pvu*II-digested DNA from three unrelated individuals with a radioactively labeled type VII collagen cDNA (K-131). Note the presence of variable bands of 3.6, 1.9, and 1.7 kb. Lane 1, DNA homozygous for 1.9/1.7-kb bands (BB genotype). Lane 2, Heterozygous DNA, demonstrating all three bands (AB genotype). Lane 3, DNA homozygous for 3.6-kb band (AA genotype). Two invariable bands, of 2.8 and 1.1 kb, are also present.

et al. 1990*a*, 1990*b*), but the mutated gene has not been identified.

In the present study, we provide evidence for strong linkage between the type VII collagen locus on human chromosome 3 and the clinical phenotype in a large pedigree with dominant dystrophic EB. Previous studies have suggested that the anchoring fibrils may be altered in dystrophic forms of EB. In particular, the recessive dystrophic form is characterized by the absence of both type VII collagen and the anchoring fibrils. Alternatively, their ultrastructural morphology is so severely perturbed that they cannot be detected by electron microscopy (Tidman and Eady 1985; Bruckner-Tuderman et al. 1989). Furthermore, in some cases with recessive dystrophic EB, immunofluorescence with antibodies to type VII collagen has demonstrated retention of the protein epitopes within the basal keratinocytes, suggesting a secretion defect (Fine et al. 1990; Smith and Sybert 1990). The electron-microscopic observations on dominant dystrophic EB have been somewhat variable, but ultrastructural evidence for abnormal anchoring fibrils has been obtained in several cases (Ghadially 1988).

The anchoring fibrils are morphologically distinct structures which extend from the upper papillary dermis to the basement membrane at the dermal/epidermal interface. A major component of the anchoring fibrils is type VII collagen, a homopolymer consisting of three individual α-chains, α1(VII). These α-chains contain a large, ~145-kD collagenous domain and a ~145–150-kD NC-1 globular domain (Woodley et al. 1988). It has been suggested that type VII collagen molecules assemble into anchoring fibrils by formation of antiparallel dimers through association of overlapping NC-2 (Morris et al. 1986). This arrangement allows the NC-1 domains to interact with type IV collagen in the basement membrane either at the dermal/epidermal interface or within the dermis with basement-membrane-like structures, the so-called anchoring plaques (Keene et al. 1987). These types of delicate interactions would then literally anchor the basement membrane to the underlying dermis, thus providing stability to the dermal/epidermal interface. A defect in the anchoring fibrils could lead to instability of the cutaneous basement-membrane zone, manifesting clinically as easy blistering and separation of the tissue at the dermal side of the basement membrane. These features were demonstrated in the family examined in the present study. Specifically, routine histopathology revealed tissue cleavage below the basal lamina, diagnostic electron microscopy showed a paucity of anchoring fibrils, and the few recognizable fibrils were hypoplastic and reduced in diameter.

It is reasonable to postulate that, with compelling evidence for close linkage, type VII collagen is the gene harboring the genetic mutation in this family with dominant dystrophic EB. We are currently testing this hypothesis through detailed characterization of the candidate COL7A1 allele in this family. Meanwhile, the RFLP marker in this collagen gene provides a means for prenatal diagnosis of EB in this family. It will also be important to test for linkage to EB in other

Table 1

Segregation of Type VII Collagen Gene with Human Chromosomes in Human x Rodent Hybrid Cell Lines

		Presence of Human Chromosome[a]																							
Cell Line	COL7A1[b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
862	−					+				+															
1049	−					+						+													
937	−	+				+									+	+		+				+			
1079	+			+		+					45%					10%	+								
756	−					D	+	+					+	+	45%					+	+	+			+
904	−					D	+						+				5%					+			+
212	−					Dq																			+
867	−	60%				+								+	+				+	+					
854	−		+			+																			
423	+			+																					
860	+			15%		+	+				15%									45%		+			
803	−				+	+			+			15%											+	+	
209	−					D	+		+						+									+	
1006	−				55%	+		+	+					+		+				+		+			
811	−								+									+	+						
967	−					+			+								+								
734	−					+				+									+						
968	−					+				+				+										+	
683	−					+						+	45%		+					+		+	+		
507	+			+		+							+		+						40%		25%		+
750	−					D								+	+					+					
1099	−		+			D								+						+		+	+		
324	−																		+						
940	−					+															+				
983	−					+					+														
% Discordancy		28	20	0	24	56	24	24	36	28	12	20	17	40	33	29	17	24	32	36	20	36	24	24	24

[a] A plus sign (+) indicates the presence of human chromosomes, as determined by karyotype analysis; percentages are of cells (when noted to be less than 100%) that contain the corresponding human chromosome; D = deletion at p15.1-15.2: Dq = multiple deletions in 5q.

[b] Presence (+) or absence (−) of the human COL7A1 gene in hybrid cell line DNA was determined by Southern hybridization with the K-131 cDNA.

families to determine which forms of the disease are caused by a mutation in the same gene.

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Appendix

Z Values for Linkage of COL7A1 and Dominant Dystrophic EB

Z values were calculated for assumed values of θ ($\theta_f = \theta_m$) by the LIPED program (Ott 1974). $\hat{Z}$ is 5.37 at $\theta = .0$, with a confidence limit of $\theta = .10$ (Conneally et al. 1985).

5.37 at $\theta = 0$
5.28 at $\theta = .01$
4.89 at $\theta = .05$
4.38 at $\theta = .10$
3.31 at $\theta = .20$
2.15 at $\theta = .30$
.92 at $\theta = .40$

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