Genetic Linkage Between the Collagen Type VII Gene COL7A1 and Pretibial Epidermolysis Bullosa with Lichenoid Features

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Pretibial epidermolysis bullosa is a rare form of dominant dystrophic epidermolysis bullosa. The disease was diagnosed after considerable delay in a large Belgian family and was remarkable for its late age at onset and its misleading clinical presentation in the proband, which strongly resembled keratosis lichenoides chronica. Both recessively and dominantly inherited forms of dystrophic epidermolysis bullosa have been shown to be linked to the collagen type VII gene, COL7A1. Two-point linkage analysis with two intragenic polymorphisms (*Pvu*II, *Alu*I) in COL7A1 was performed. Strong genetic linkage between the disease in this family and COL7A1 was demonstrated by a lod score of 4.45 (theta = 0) for the AluI polymorphism. The observed intrafamilial variability of clinical phenotypes contradicts the presently proposed classification of dominantly inherited dystrophic epidermolysis bullosa. *J Invest Dermatol 104:803–805, 1995*

Hereditary epidermolysis bullosa (EB) is a heterogeneous subgroup among the mechanobullous diseases. Classification is done on the basis of clinicopathologic criteria and inheritance patterns [1]. Pretibial EB is a rare variant of dominant dystrophic EB (DDEB) characterized by recurrent blistering and scarring, mainly in the pretibial area [1,2]. The great phenotypic variability even within a single family has created some doubts about the strict separation of different types of DDEB based on the above criteria [3,4]. Recent work at the molecular level supports the notion that various forms of DEB could be different expressions of the same gene defect [5,6].

Ultrastructural studies have demonstrated quantitative and/or qualitative alterations of the anchoring fibrils of the basement membrane zone in DEB [7]. Because collagen type VII is the major component of anchoring fibrils, the gene for collagen type VII, COL7A1 on chromosome 3p21.1, was a logical candidate gene for DEB. This assumption has proven to be correct, as different groups have demonstrated strong genetic linkage between both recessively and dominantly inherited DEB and the collagen type VII gene [4,8–11]. In this report, we demonstrate genetic linkage between the COL7A1 gene and a very peculiar phenotype of pretibial EB characterized by an age at onset after 10 years and by the presence of striking lichenoid lesions [12].

MATERIALS AND METHODS

Pedigree The pedigree of this Belgian family is presented in **Fig 1** and comprises 26 living individuals in three generations. The clinical characteristics of the affected family members have been reported elsewhere [12] and will be discussed only briefly. The diagnosis of pretibial EB in the proband (I-2) was delayed until the age of 68 years. She had suffered from slightly pruritic erythematosquamous plaques on both knees since she was 11 years old. Only at age 50, the lesions began to spread over the pretibial area and the lower third of the calves. The patient then denied the presence of similar or other skin lesions in family members.

Affected skin was characterized by the presence of multiple violaceous papules and plaques with some rare flaccid bullae. Isolated papules with the same characteristics were present on the trunk, the thighs, and the perineal region. Nail plates were dystrophic, and there was a severe pruritus that led to suicidal thoughts. On histologic analysis, multiple biopsy specimens showed a lichenoid infiltrate in the upper dermis with some rare subepidermal blisters that were apparently due to necrosis of the overlying basal keratinocytes. Direct immunofluorescence studies were negative. A diagnosis of keratosis lichenoides chronica (with secondary blistering) was made [13]. After multiple treatment failures, the condition of the patient improved with administration of etretinate. Unfortunately, the patient was lost to follow-up. She was hospitalized in the department of dermatology in 1989 because of the presence of multiple recalcitrant ulcers on both pretibial areas. A marked tendency for blistering after minor trauma became apparent. Because a hereditary mechanobullous disorder was suspected, all family members were examined clinically by two of us (JMN, HB). Six of eight affected family members (I-2, II-3, II-5, II-7, III-7, and III-10) had blistering and scarring predominantly on pretibial skin. Seven individuals (all affected members in the second and third generations) had atrophic scars on the elbows, knees, ankles, and dorsa of the hands. All eight affected members had dystrophic toenails. Lichenoid papules in the pretibial area were present in three individuals (I-2, II-3, and III-10). Besides the proband, only one of her sons (II-3, 40 years old) had lichenoid papules out of the main affected area (dorsa of the hands).

The clinical diagnosis of pretibial EB was confirmed by histologic and ultrastructural studies that demonstrated the dermolytic nature of the blisters. Of note, most anchoring fibrils in affected skin had a normal ultrastructural appearance with cross-banding and fanning, but they were irregularly distributed along the basement membrane zone. Five children (III-9, III-14, 15, 16, and 17), all younger than 10 years, are still too young to show manifestations of the disease.

DNA Studies and Linkage Analysis Genomic DNA was isolated from leukocytes from eight affected and 21 nonaffected family members according to standard procedures [14]. Two previously described intragenic polymorphisms in the COL7A1 gene were used for linkage analysis [15]. The first polymorphism (A) was detected with the restriction enzyme *Pvu*II.

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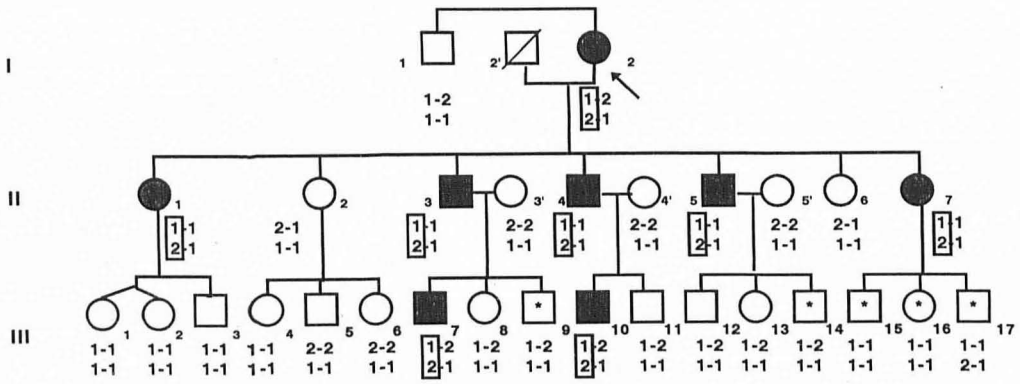

Figure 1. Pedigree of Belgian family with pretibial EB with lichenoid lesions. *Dark symbols* indicate clinically affected individuals. *Upper line*, *Pvu*II haplotypes; *bottom line*, *Alu*I haplotypes; *arrow*, proband; *asterisks*, siblings less than 10 years. The EB allele segregating with affected individuals boxed.

Absence of the restriction site corresponds to the A1 allele and presence of the restriction site to the A2 allele. The allelic frequency of A1/A2 is 0.60/0.40. The second polymorphism (B) was detected with the restriction enzyme *Alu*I. Allele B1 represents absence and allele B2 presence of the restriction site. The allelic frequency of B1/B2 is 0.75/0.25.

A polymerase chain reaction technique was used to detect the two polymorphisms. Polymerase chain reaction was performed with two sets of primers spanning the region of both polymorphic sites under conditions described elsewhere [15]. Segregation of the alleles of the *Pvu*II and the *Alu*I polymorphisms of the COL7A1 gene was determined. Two-point linkage analysis was performed using the LINKAGE program (version 5.2) [16]. We assumed autosomal dominant inheritance with full penetrance after the age of 10 years and zero penetrance before 10 years and a gene frequency of 0.00001 for this rare form of DDEB.

RESULTS AND DISCUSSION

The haplotypes obtained for the two polymorphic markers (*Pvu*II and *Alu*I) are shown in the pedigree **(Fig 1)**.

All affected members of generation II are homozygous for the A1 allele of the *Pvu*II polymorphism, reducing the number of informative meioses considerably. The *Alu*I polymorphism is highly informative, and cosegregation of the B2 allele with the disease was observed **(Fig 1)**.

Table I shows lod scores of the two-point linkage analysis at different recombination fractions for both the *Pvu*II and *Alu*I polymorphisms. We obtained a lod score of 4.45 at theta = 0.0 for the *Alu*I polymorphism. No recombination was seen in any of the family members. These findings demonstrate genetic linkage of this particular form of DDEB with the COL7A1 gene. The lod score of 4.45 at theta = 0 and the absence of recombinants strongly suggest that COL7A1 is the causal gene for the disease in this family. Previous reports have provided evidence for linkage between COL7A1 and the Pasini and Cockayne Touraine types of DDEB and/or Bart's syndrome [4,8–10].

From 10 informative families with DDEB [10], a combined lod score of 26.97 at theta = 0 was calculated, favoring linkage between the COL7A1 locus and the disease with a probability of approximately 10^{27}. Because no recombination was detected, one may safely conclude that in most if not all families, the DDEB phenotype is due to a mutation in the COL7A1 gene [10].

Pretibial EB is classified as a rare form of DDEB [1]. The disease in our family clearly shows clinical variability, as has been observed by others [4,8]. The presence of lichenoid papules in three members is striking and explains the delay in diagnosis in the proband for almost 2 decades. The condition in this female patient was initially diagnosed as keratosis lichenoides chronica. This diagnosis remained uncontested after presentation at an international clinical meeting and publication in a journal with peer review [13]. The misleading nature of this clinical phenotype with a striking resemblance to various acquired inflammatory dermatoses has been described in other families and/or in sporadic cases of pretibial EB [2,17]. As a rule, affected patients have dystrophic nail plates. In most of these patients, the disease has a late onset. Therefore, III-17, aged 4 years, who carries the disease-associated allele B2, will probably develop the disease at a later stage (paternal inheritance of B2 is not formally excluded, as his father was unavailable for study).

That pretibial EB is linked to COL7A1 supports the notion that collagen type VII is crucial in the pathogenesis of dystrophic EB in general [5]. It is now known that different mutations in a single gene, COL7A1, can cause a clinical phenotype with dominant or recessive inheritance. Recently, DDEB in a large Finnish family has been shown to be due to a point mutation causing substitution of a glycine residue by the bulkier amino acid serine in the helical domain of the collagen type VII molecule. Heterozygosity for such a mutation results in the synthesis of one normal and one abnormal alpha 1 (VII) chain. Because of random association of the alpha-chains during trimer formation, it is expected that only one eighth (approximately 15%) of molecules will be normal [6]. This is consistent with the observed structural and/or quantitative abnormalities of the anchoring fibrils. The authors stressed the fact that this single point mutation underlay both the Cockayne Touraine and the Pasini phenotypes in the same family.

Characterization of the mutation in the present family with pretibial EB and lichenoid features is in progress. We expect that a single mutation in COL7A1 is responsible for the great phenotypic variability in this family, supporting the hypothesis that the present classification of DDEB is somewhat artificial. Ultrastructural study of the anchoring fibrils in affected skin showed only minor quantitative and absent or very subtle structural alterations in this family [12]. However, the fact that the disease is linked to COL7A1 indicates that the anchoring fibrils must have some functional deficiency. Therefore, characterization of the mutation in COL7A1 in this family could shed further light on the structure-function relation in the collagen type VII molecule. One hopes that work at the molecular level will also improve understanding of still unexplained clinical phenomena such as the predilection for the pretibial area, the presence of lichenoid papules, or the severe pruritus.

Table I. Lod Scores at Different Values of Theta

	Theta						
Marker	0.00	0.05	0.10	0.15	0.20	0.30	0.40
*Pvu*II	1.80	1.64	1.48	1.31	1.12	0.72	0.28
*Alu*I	4.45	4.09	3.72	3.32	2.90	1.98	0.93

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