Am. J. Hum. Genet. 60:1344–1353, 1997

Three Novel Homozygous Point Mutations and a New Polymorphism in the COL17A1 Gene: Relation to Biological and Clinical Phenotypes of Junctional Epidermolysis Bullosa

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Summary

Junctional epidermolysis bullosa (JEB) is a clinically and biologically heterogeneous genodermatosis, characterized by trauma-induced blistering and healing without scarring but sometimes with skin atrophy. We investigated three unrelated patients with different JEB phenotypes. Patients 1 and 2 had generalized atrophic benign epidermolysis bullosa (GABEB), with features including skin atrophy and alopecia. Patient 3 had the *localisata* variant of JEB, with predominantly acral blistering and normal hair. All patients carried novel homozygous point mutations (Q1016X, R1226X, and R1303Q) in the COL17A1 gene encoding collagen XVII, a hemidesmosomal transmembrane component; and, therefore, not only GABEB but also the *localisata* JEB can be a collagen XVII disorder. The nonsense mutations led to drastically reduced collagen XVII mRNA and protein levels. In contrast, the missense mutation allowed expression of abnormal collagen XVII, and epidermal extracts from that patient contained polypeptides of normal size, as well as larger aggregates. The homozygous nonsense mutations in the COL17A1 gene were consistent with the absence of the collagen from the skin and with the GABEB phenotype, whereas homozygosity for the missense mutation resulted in expression of aberrant collagen XVII and, clinically, in *localisata* JEB.

Introduction

Collagen XVII, also known as the 180-kD bullous pemphigoid antigen, or BP180, is a structural component of the epithelial hemidesmosomes in the skin, the mucous membranes, and the eye (Nishizawa et al. 1993). The cDNA structure predicts an unusual type II integral transmembrane molecule with several protein kinase consensus sequences in its cytoplasmic domain and with a large extracellular collagenous domain (Giudice et al. 1992), but the protein structure has not yet been fully characterized (Hirako et al. 1996). Because of the collagenous sequences in the ectodomain, the molecule has been designated "collagen XVII," a member of the collagen protein family (Li et al. 1993; Gatalica et al. 1997). As a transmembrane component of the hemidesmosomes, collagen XVII presumably plays a role in maintaining the linkage and integrity between the intracellular and the extracellular hemidesmosome components and in anchoring the epithelial cells to the underlying basement membrane (Hopkinson et al. 1995). This concept is evidenced indirectly by several pathological conditions. For example, in bullous autoimmune diseases, such as bullous pemphigoid, cicatricial pemphigoid, and herpes gestationes, the presence of autoantibodies reactive with collagen XVII is associated with diminished epidermal/dermal cohesion (Ishiko et al. 1993; Liu et al. 1993; Giudice et al. 1994; Balding et al. 1996). Furthermore, a hereditary blistering disorder of the skin, the generalized atrophic benign epidermolysis bullosa (GABEB), is associated with absence or altered expression of collagen XVII (Jonkman et al. 1995, 1996).

Junctional epidermolysis bullosa (JEB) refers to a group of genetic blistering diseases with characteristic features of skin fragility, ultrastructurally rudimentary hemidesmosomes, and separation of the basal keratinocytes from the basement membrane. JEB contains several subtypes, varying from lethal Herlitz JEB to mild localized acral subtype (Bruckner-Tuderman 1993; Gedde-Dahl and Anton-Lamprecht 1996). The severe forms exhibit onset of trauma-induced skin blistering at birth, but the milder forms occasionally have a later onset during childhood. In the course of the disease the skin

Received November 12, 1996; accepted for publication March 4, 1997.

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0002-9297/97/6006-0012$02.00

tends to become atrophic and fragile at sites of repeated blistering.

GABEB is characterized by generalized blistering and skin atrophy; alopecia of the scalp; partial absence of eyelashes, eyebrows, and pubic and axillary hair; nail dystrophy; and tooth anomalies (Hashimoto et al. 1976; Hintner and Wolff 1982). Recently, the first mutations in the gene encoding collagen XVII were determined in GABEB families (McGrath et al. 1995). Most of the mutations reported thus far (McGrath et al. 1995, 1996*a;* Darling et al. 1997; Gatalica et al. 1997) have been homozygous or heterozygous mutations leading to premature termination codons (PTC) and, in one case, to a missense glycine-substitution mutation (McGrath et al. 1996*b*). Because of the limited number of mutations, little is known about the genotype-phenotype correlations in these patients.

In the present study, we have identified three homozygous point mutations in the COL17A1 gene. Two of these resulted in PTC and, clinically, in the GABEB phenotype. The third was a missense mutation that led to expression of aberrant collagen XVII and to a partial loss of function of this protein, accompanying a clinically milder *localisata* JEB phenotype.

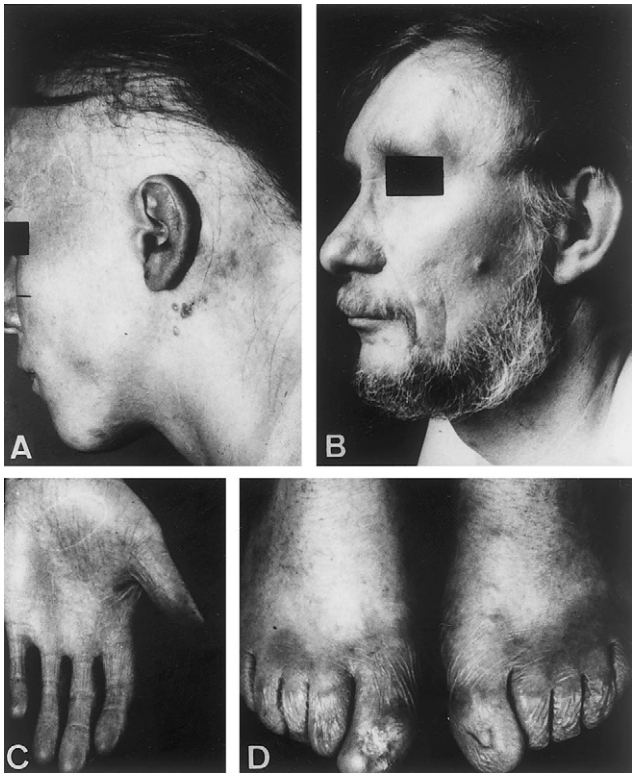

Figure 1 Clinical features of patient 1, with the GABEB phenotype, and patient 3, with the *localisata* phenotype. *A,* Generalized small blisters and typical parietal alopecia in patient 1. *B,* Normal hair of patient 3. *C,* Localized blistering and skin atrophy of the left hand of patient 3. *D,* Skin atrophy and nail dystrophy of the feet of patient 3.

Patients, Material, and Methods

Patients

Patient 1.—The proband, an 18-year-old female, is the only child of healthy parents, and her clinical features at a young age were described by Zortea-Caflisch (1985). There was no family history of related skin diseases or similar genetic disorders. There was no known history of consanguineity, but both parents originated from the same region in the central European Alps. The proband presented at birth with multiple blisters of the arms and legs. Mechanically induced and spontaneous blisters continued to appear, the extremities, head, and face being most often affected. The blisters healed without scars but with some atrophy and hyperpigmentation. Periungual blistering and paronychia led to dystrophy of all nails. Beginning at the age of 6 years, patchy alopecia of the scalp developed, leading to loss of all parietal hair (fig. 1*A*). The oral mucosa and the tongue were regularly involved with erosions. This is the characteristic phenotype of GABEB. Electron microscopy showed junctional blistering and hypoplastic hemidesmosomes (Zortea-Caflisch 1985).

Patient 2.—The proband, a 9-year-old female, is the older of two daughters of nonconsanguineous German parents. No other family member had a related skin disorder or a genodermatosis. She has had blistering of the skin and the mucous membranes since birth. Mechanically induced blisters often were localized to the extremities and the face. They healed without scarring but with skin atrophy. Blistering of the scalp led to diffuse alopecia. Electron microscopy showed junctional blistering and severely hypoplastic hemidesmosomes in the basal keratinocytes at the blister roof.

Patient 3.—The proband, a 53-year-old male, is the only offspring of a consanguineous marriage, his parents being third cousins. The proband has a clinically unaffected daughter. The family history was negative for skin diseases or genetic disorders. The proband had had trauma-induced blistering of the skin since school age but developed an overall milder phenotype, with blistering predominantly at the distal extremities and, occasionally, of the oral mucosa. During the course of the disease, all nails were lost, and mild skin atrophy developed on the extremities. He had slight alopecia of androgenetic pattern (fig. 1*B–D*). Electron microscopy showed slight structural alterations of the hemidesmosomes (not shown).

Informed Consent

Informed consent was obtained from all patients and their relatives before biopsies or blood samples were taken.

Mutation Detection

Genomic DNA was isolated from peripheral blood by use of the Easy-DNA™ kit according to the manufacturer's instructions (Invitrogen). Amplification of exons 45, 51, and 52 of COL17A1 was performed by use of primers placed on the flanking intronic sequences (Giudice et al. 1992; Gatalica et al. 1997) (Genbank accession M91669). Primers used for amplification of exon 45 were sense primer 5′-CTCAGACCATGGAAGCTATT-3′ and antisense primer 5′-CTTGGCAAAGAGACT-GTATC-3′. The size of the PCR product was 272 bp. Primers used for amplification of exon 51 were sense primer 5′-TTTCTCTCCTCCCATCACCC-3′ and antisense primer 5′-TGTCCCTTTAAGTGCCTCCC-3′. The size of the amplification product was 374 bp. Primers used for amplification of exon 52 were sense primer 5′-CAAGTCTTTCTCTCCACCGA-3′ and antisense primer 5′-CCACAAACAAGAAAGCCAGT-3′. The size of the amplification product was 526 bp. PCR was performed under standard conditions (Christiano et al. 1994), and heteroduplex analysis was performed as described elsewhere (Ganguly et al. 1993; Gatalica et al. 1997). If a heteroduplex band was detected, the PCR products were purified with a PCR purification kit according to the manufacturer's recommendations (QIAGEN), followed by sequencing by an automated sequencer (Applied Biosystems).

A Novel Polymorphic Site in the COL17A1 Gene

A G/A sequence variation at position 2212 of the cDNA within exon 27 was detected in DNA samples of unaffected individuals. PCR products spanning exon 27 and flanking intronic sequences were made by use of sense primer 5′-TGAGGGTCTGATGGACACGA-3′ and antisense primer 5′-TCTTGGTCCAGGTCCTGTGC-3′. The size of the PCR product was 306 bp. The *Bsr*I restriction enzyme (NCCAGT) was used according to the manufacturer's (New England Biolabs) recommendations. In the case of homozygosity for the base G at position 2212 of the cDNA , the PCR product was cut into 173-bp and 133-bp fragments. In case of homozygosity for the base A, a single 306-bp fragment remained undigested, indicating absence of the *Bsr*I site. The allele frequencies in 80 chromosomes were 78% G and 22% A; the PIC value was .28.

Northern Blotting

Total RNA was isolated from cultured JEB and control keratinocytes by use of the guanidine isothiocyanate method (Chromczynski and Sacchi 1987). For northern blotting, the RNA was separated on formaldehyde-containing agarose gels (Sambrook et al. 1990) and then was vacuum transferred onto a nitrocellulose filter and hybridized with a ^{32}P-dCTP- and ^{32}P-dGTP-radiolabeled COL17A1 cDNA (McGrath et al. 1995). cDNA of glyceraldehyde phosphate dehydrogenase was used as a reference probe to correct for small differences in RNA loading. The radioactive cDNA-mRNA hybrids were visualized by autoradiography by exposure of the filters to x-ray films (X-Omat; Eastman Kodak).

Keratinocyte Cultures

Keratinocytes were obtained by trypsinization of control and JEB skin biopsies and were cultured in serum-

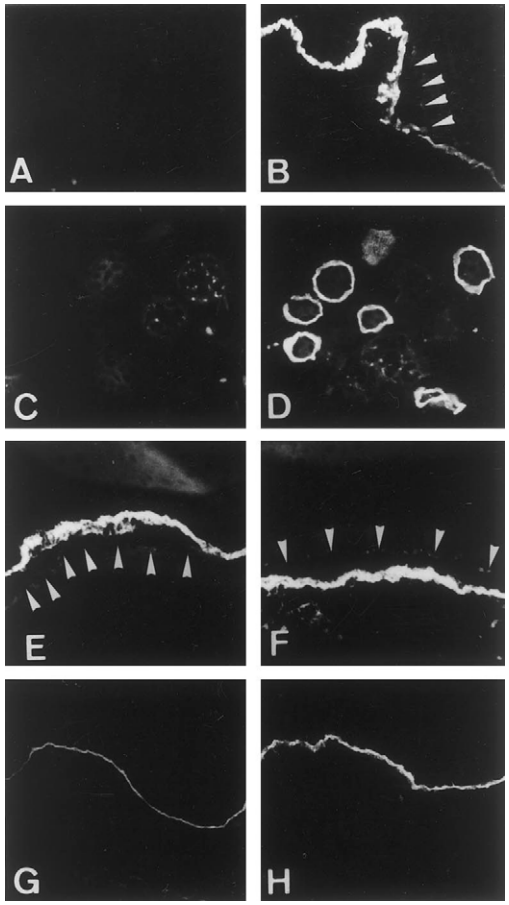

Figure 2 IF staining of the patients' and control skin with antibody 1D1 to the extracellular domain of collagen XVII (*A, C, E,* and *G*) and, as a control, to collagen VII (*B, D, F,* and *H*). Staining with antibodies to collagen XVII showed lack of reaction in the skin of patient 1 (*A*). In the skin of patient 2, the epidermis was detached, but the basement membrane of the sweat glands demonstrated lack of reactivity with the collagen XVII antibody (*C*). In the skin of patient 3, staining with antibodies to collagen XVII was positive and localized to the blister roof at separated areas (*E*). In panel *G,* control staining of normal skin with antibodies to collagen XVII is shown. IF staining of the biopsies with the antibody 1A8C to the intracellular domain of collagen XVII exhibited the same staining pattern as above (not shown); in comparison, staining with antibodies to collagen VII, another component of the anchoring complex, was positive in all biopsies (*B,* patient 1; *D,* patient 2; *F,* patient 3; and *H,* normal control). The staining was localized to the blister floor in patients 1 and 3, confirming junctional blistering. The arrowheads point to the blister roof (in panels *B* and *F*) and to the blister floor (in panel *E*).

free keratinocyte growth medium containing 0.09 mM calcium supplemented with bovine pituitary extract and epidermal growth factor (KGM™; Gibco), as described elsewhere (König and Bruckner-Tuderman 1992).

Antibodies and Immunodetection of Proteins

The monoclonal antibodies 1A8C and 1D1, recognizing the intra- and extracellular domains of collagen XVII, respectively, were produced and characterized as described elsewhere (Kitajima et al. 1992; Nishizawa et al. 1993). Autoantisera from patients with cicatricial pemphigoid that were strongly reactive with collagen XVII were used for immunoblotting (Schumann and Bruckner-Tuderman 1996). For antigen mapping (Hintner et al. 1981), the following antibodies were employed: cytokeratins (Dako), BP230 (a high-titer autoantiserum), α6 and β4 integrins (Gibco Life Technologies), collagen IV (Dako), laminin 5 (monoclonal antibody BM-165, a kind gift from Dr. R. Burgeson, CBRC, Harvard Medical School, Cambridge, MA), and polyclonal antibodies against collagen VII (Bruckner-Tuderman et al. 1995). Immunofluorescence (IF) staining of cryosections of skin or of cultured keratinocytes was performed with standard techniques, by use of either fluorescein isothiocyanate (FITC)–labeled second antibodies (Dako) or amplification with biotin-labeled second antibodies and FITC-streptavidin (Boehringer-Mannheim). Immunoblotting of keratinocyte and epidermal extracts was performed essentially as described elsewhere

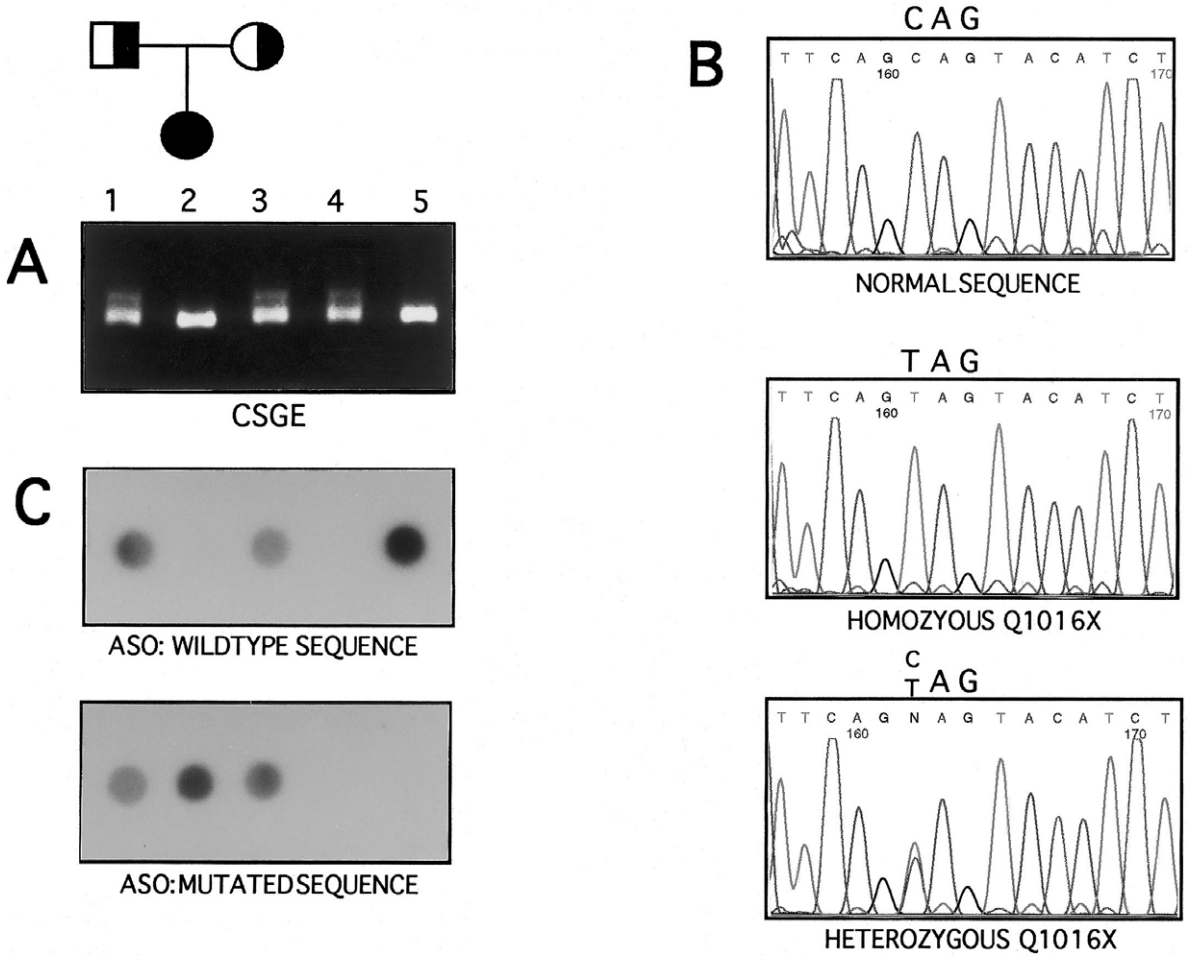

Figure 3 Mutations in the COL17A1 gene in patient 1 and the parents. *A,* Conformation-sensitive gel electrophoresis (CSGE) analysis of the PCR product containing exon 45, which revealed heteroduplex bands in both parents' DNA (lanes 1 and 3) and in the proband's DNA, when mixed with the same PCR product from an unrelated control individual (lane 4). The proband's DNA (lane 2) and control DNA (lane 5) showed homoduplex bands when tested alone. *B,* Automated sequence analysis of the patient's DNA, which revealed a homozygous C→T transition at nucleotide position 3151 of the cDNA, designated "Q1016X" (*middle panel*). The mutation was present in the heterozygous state in the parents (*lower panel*) but was absent from a control (*upper panel*). *C,* Mutation as verified with allele-specific–oligonucleotide hybridization, as described elsewhere (Christiano et al. 1994). Hybridization with oligonucleotides with the wild-type sequence (*upper panel*) showed a strong signal with a control DNA (lane 5) and a weaker signal with DNA of both heterozygous parents (lanes 1 and 3). No signal was detectable with the patient's DNA (lane 2). Hybridization with the mutated sequence revealed a strong signal with the patient's DNA (lane 2), a weaker signal with the parents' DNA (lanes 1 and 3), and no signal with control DNA (lane 5).

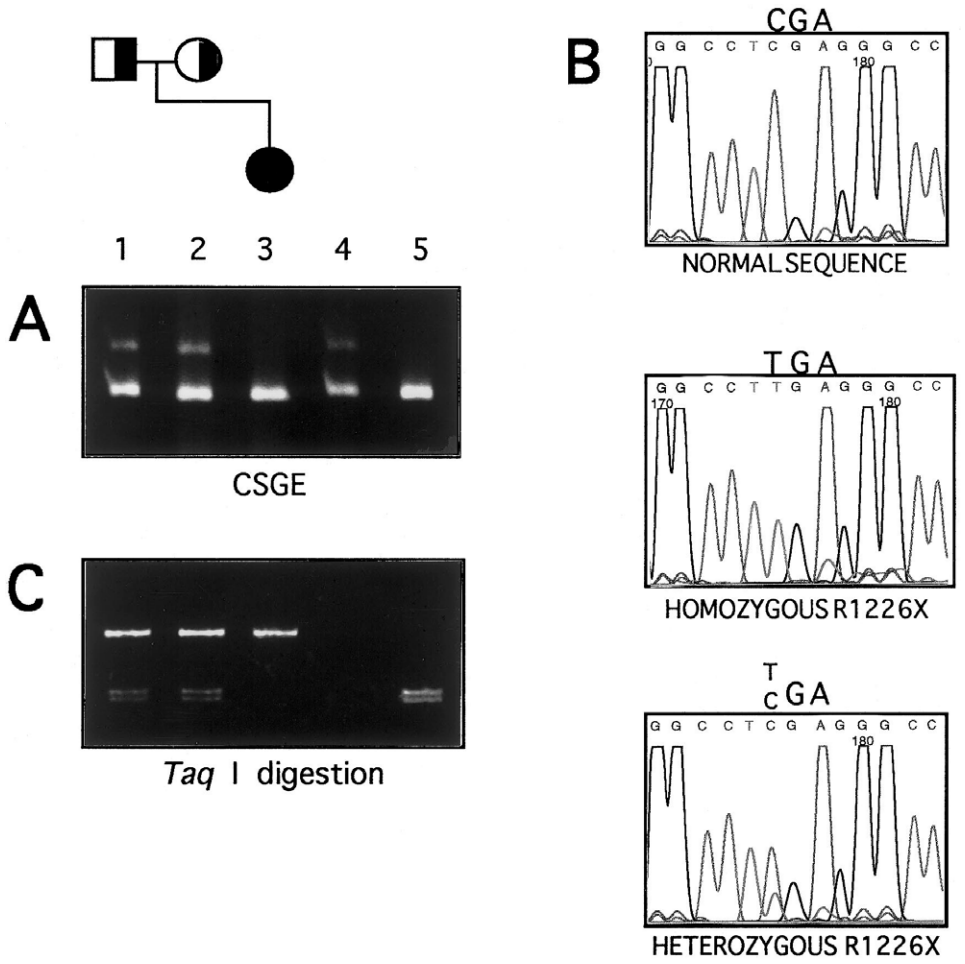

Figure 4 Mutations in the COL17A1 gene in patient 2 and parents. *A,* CSGE analysis of the PCR product containing exon 51, which revealed heteroduplex bands in both parents (lanes 1 and 2) and in the proband's DNA, when mixed with the same PCR product from an unrelated control individual (lane 4). The proband's DNA (lane 3) and control DNA (lane 5) showed homoduplex bands when tested alone. *B,* Automated sequencing disclosing a homozygous C→T transition in the proband, at nucleotide position 3781 of the cDNA, designated "R1226X" (*middle panel*). The mutation was present in the heterozygous state in the parents (*lower panel*) but was absent from the control (*upper panel*). *C,* Mutation leading to loss of a *Taq*I restriction-enzyme site. The control PCR product (lane 5) was digested to 185-bp and 157-bp bands, whereas in both heterozygous parents (lanes 1 and 2) the mutation resulted in an additional, uncleaved 342-bp band. The homozygous patient showed only the uncleaved 342-bp band (lane 3).

(König and Bruckner-Tuderman 1992; Bruckner-Tuderman et al. 1995), with minor modifications (Sonnenberg et al. 1991).

Results

Immunohistological Phenotypes

Staining with antibody 1A8C to the intracellular domain and with antibody 1D1 to the extracellular domain of collagen XVII revealed that the skin of patient 1 was negative for most of the basement-membrane zone (fig. 2*A*), interrupted by a few short stretches of positive yet attenuated staining. The skin of patient 2 was completely negative (fig. 2*C*), whereas the skin of patient 3 was positive (fig. 2*E*). In spontaneously blistered areas of skin from all three patients, the antibodies to cytokeratins, BP230, and α6 and β4 integrins stained the blister roof, and the antibodies to laminin 5, collagen IV, and collagen VII stained the blister floor. This staining pattern therefore confirmed junctional tissue separation in all three patients.

Identification and Verification of Mutations

On the basis of sequence information on the COL17A1 intron-exon organization (Gatalica et al. 1997), balanced primer pairs were designed to allow PCR amplification of exons directly from genomic DNA. The primers were placed ⩾24 bp away from the intron-exon borders. In family 1, exon 45 was affected; in family 2, exon 51; and, in family 3, exon 52. After mixing patients' DNA with control DNA, a heteroduplex band similar to that of carri-

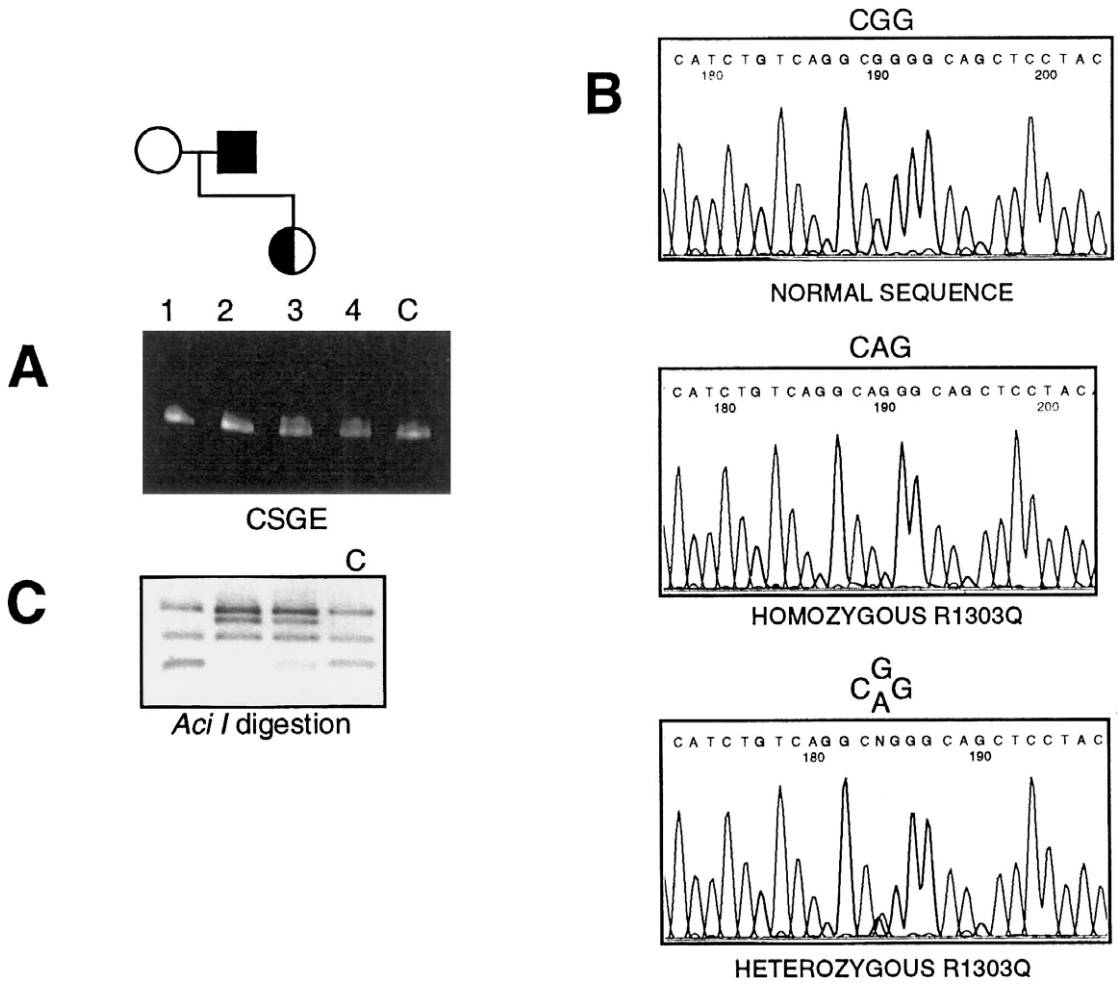

Figure 5 Mutations in the COL17A1 gene in patient 3 and his daughter. *A,* CSGE analysis of the PCR product containing exon 52, showing heteroduplex bands in the daughter (lane 3) and in the proband's DNA, when mixed with the same PCR product from an unrelated control individual (lane 4). The proband's DNA (lane 2), as well as the DNA of his wife (lane 1) and of an unrelated control (lane C), showed homoduplex bands when tested alone. *B,* Homozygous G→A transition at nucleotide position 4013 of the cDNA, designated "R1303Q," disclosed by automated sequence analysis in the proband (*middle panel*). The mutation was present in the heterozygous state in the daughter (*lower panel*) but was absent from the control (*upper panel*). *C,* Mutation that abolished an *Aci*I restriction-enzyme site. The control's and the unaffected wife's PCR products (lanes C and 1) were digested to 165-bp, 106-bp, 69-bp, 65-bp, and 59-bp fragments. The proband's DNA showed an additional 134-bp band and the loss of the 69-bp and 65-bp bands (lane 2). The heterozygous daughter (lane 3) had both the 134-bp and the 69-bp and 65-bp bands.

ers was detected (figs. 3–5). This electrophoretic pattern indicated heterozygosity of the parents of patients 1 and 2 and of the daughter of patient 3, as well as homozygosity for all three patients. Patient 1 was homozygous for a 3151C→T transition (Q1016X), and the parents were heterozygous (fig. 3). Patient 2 was homozygous for a 3781C→T transition (R1226X), and each parent was heterozygous (fig. 4). Patient 3 was homozygous for a 4013G→A transition, and his daughter was heterozygous (fig. 5).

Expression of COL17A1 mRNA

Total cellular RNA was isolated from cultivated keratinocytes of controls and patients 1 and 2. In northern blot analysis, a strong signal of collagen XVII mRNA was found with control keratinocytes. Collagen XVII mRNA isolated from the JEB keratinocytes produced a very weak signal, suggesting nonsense-mediated mRNA decay in these cells (fig. 6).

Synthesis of Collagen XVII in Vitro

IF staining of keratinocytes of patient 1 with the antibody 1A8C revealed a positive, diffuse intracellular IF indicating synthesis and intracellular accumulation of immunoreactive protein fragments (fig. 7). In contrast, in control keratinocytes the positive reaction was observed as a perinuclear ring corresponding to the rough endoplasmatic reticulum and, in the cell periphery, to

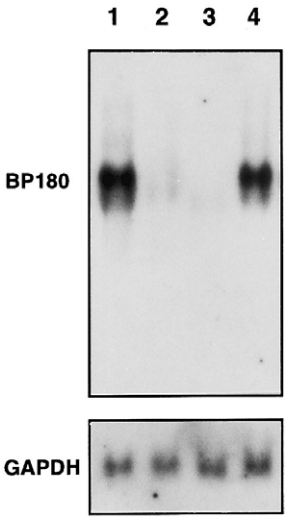

Figure 6 Northern blotting of mRNA isolated from GABEB keratinocytes. A strong signal of collagen XVII mRNA was found with control keratinocytes (lanes 1 and 4); in contrast, collagen XVII mRNA isolated from keratinocytes of patients 1 (lane 3) and 2 (lane 2) produced a very weak signal, suggesting nonsense-mediated mRNA decay in these cells. The intensity of the GAPDH signal was similar in all lanes.

the transmembrane location of collagen XVII (fig. 7). Staining with the antibody 1D1 showed a barely visible fluorescence in the patient's keratinocytes but a positive membrane-associated staining in control cells. Results

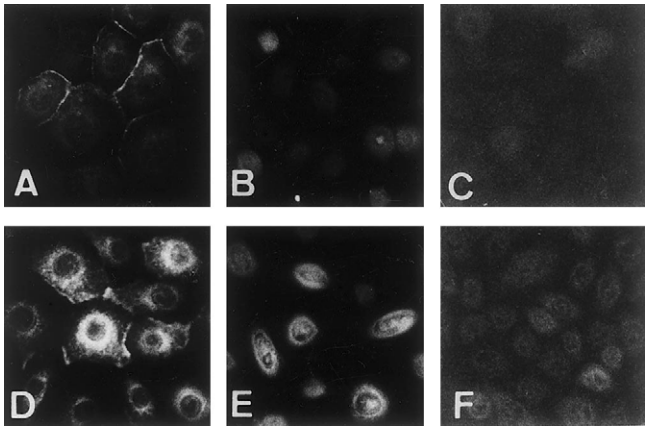

Figure 7 IF staining of GABEB keratinocytes with domain-specific antibodies to collagen XVII. In control cells, the antibody 1D1 to the extracellular domain showed a positive IF in the cell periphery, corresponding to the transmembrane localization (*A*), and the antibody 1A8C to the intracellular domain showed a strongly positive intracellular IF (*D*). The antibody 1D1 produced a barely visible staining in the keratinocytes of patient 1 (*B*) and no reaction with cells of patient 2 (*C*). Staining of keratinocytes of patient 1 with the antibody 1A8C (*E*) revealed a weak, diffuse intracellular IF. The staining, with the latter antibody, of keratinocytes of patient 2 (*F*) was comparable to that of the negative control.

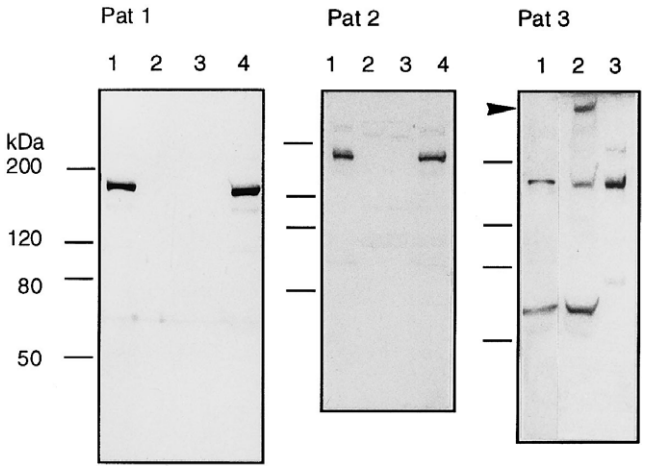

Figure 8 Immunoblotting of keratinocyte and epidermis extracts. Keratinocyte extracts of patients 1 (Pat 1) and 2 (Pat 2) were blotted with polyclonal antibodies to collagen XVII. Compared with control extracts (lanes 1 and 4, in both panels), the 180-kD collagen XVII band was not detectable in the patients' cells (lanes 2 and 3, in both panels). Since keratinocytes were not available from patient 3, epidermis extract was immunoblotted (Pat 3). The extract contained immunopositive collagen XVII polypeptides. In lane 1, normal epidermis extract containing the 180-kD α1(XVII) chain is shown. In addition, a second immunoreactive, diffuse band of ~65 kD was observed occasionally. The identity of this band has not been characterized. In lane 2, the epidermis extract of patient 3 containing the normal 180-kD α1(XVII) chain and an additional large molecular-weight band of ~300 kD (*arrowhead*), which was not present in normal epidermis, is shown; also in the patient's epidermis extract, the 65-kD band was present. In lane 3, control keratinocyte extract is shown. The migration positions of molecular-weight standards are indicated next to each blot. The molecular weights are, from top to bottom, 205 kD, 116 kD, 82 kD, and 46 kD.

of staining of keratinocytes of patient 2 with either antibody were similar to those for negative controls, suggesting either that the cells did not synthesize immunoreactive collagen XVII or that the nascent truncated polypeptide chains were subjected to intracellular degradation (fig. 7). Keratinocyte cultures could not be initiated from the skin biopsies of patient 3.

Immunoblotting confirmed the results of the IF staining of keratinocytes of patients 1 and 2. The cells were cultured in the presence of ascorbic acid, were extracted with nonionic detergents, and were immunoblotted by use of polyclonal antibodies against collagen XVII. A single 180-kD band representing the α1(XVII) polypeptide chain of collagen XVII was found in control extracts, but with JEB extracts neither α1(XVII) polypeptides nor any distinct fragments were demonstratable (fig. 8). In contrast, epidermal extracts of patient 3 revealed immunoreactive collagen XVII in immunoblots. The normal size α1(XVII) polypeptide chains were detected, together with a larger immunoreactive band of an apparent molecular weight close to 300 kD (fig. 8). In extracts of normal epidermis, the larger band was

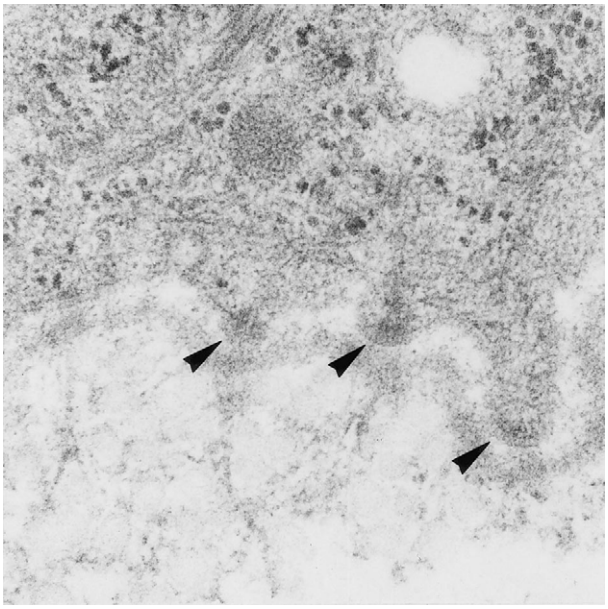

Figure 9 Electron microscopy of clinically uninvolved skin of patient 1. Ultrastructural analysis showed rudimentary, narrow hemidesmosomes (*arrowheads*), with lack of distinct dense plates. Other basement-membrane structures appeared normal.

not seen. In both normal and JEB epidermis extracts, a diffuse ~65-kD band occasionally was seen. The band is likely to represent an unspecific reaction, but the identity of the band has not been characterized.

Electron Microscopy

Ultrastructural analysis of a biopsy obtained from clinically uninvolved skin of the upper thigh of patient 1 showed abnormal hemidesmosomes, with rudimentary structure and lack of a distinct dense plate (fig. 9). Other basement-membrane structures, the lamina lucida, lamina densa, anchoring filaments, and anchoring fibrils appeared normal. No dermal/epidermal separation was observed in the sample. A fresh biopsy from patients 2 and 3 was not available for electon-microscopic examination.

Discussion

Collagen XVII presumably functions as both a structural macromolecule and a cell-adhesion receptor. Its cDNA structure predicts a transmembrane protein in type II orientation, with an intracellular N-terminus and an extracellular C-terminus containing an interrupted collagenous domain (fig. 10). Probably because of the inaccessibility of the protein to standard biochemical analyses, its molecular structure and functions have not been investigated in detail. Nevertheless, cross-linking experiments have suggested a trimeric structure (Hirako et al. 1996), and transfection experiments with truncated collagen XVII cDNAs have implicated molecular interactions with α6 integrin (Hopkinson et al. 1995). Indirect demonstration of collagen XVII's role in epidermal adhesion has been provided by its deficient expression in GABEB patients (Jonkman et al. 1995, 1996). Subsequently, homozygous or compound-heterozygous nonsense mutations in the COL17A1 gene have been disclosed in seven GABEB families (McGrath et al. 1995, 1996*a;* Darling et al. 1997; Gatalica et al. 1997). A glycine-substitution mutation in combination with a PTC was found in one patient with a phenotype somewhat different from classical GABEB (McGrath et al. 1996*b*).

In the present study, we describe three homozygous COL17A1 mutations in unrelated central European JEB patients. One of them, R1226X, previously had been found in heterozygous form in a GABEB patient (McGrath et al. 1995). The authors of that study did not define the genealogical origin of their proband, and therefore it remains unclear whether the mutation has a common founder or represents a mutational hot spot, similar to that in the LAMB3 gene associated with JEB Herlitz (Kivirikko et al. 1996; Pulkkinen et al. 1996).

The nonsense mutations described here resulted in lack of collagen XVII from the skin; however, at the cellular level, the phenotypes differed. Immunoreactive collagen XVII epitopes were observed in keratinocytes of patient 1 but not in those of patient 2. The mutation Q1016X is located in a noncollagenous domain, whereas R1226X is in a collagenous domain (fig. 10). It is feasible that, in spite of nonsense-mediated mRNA

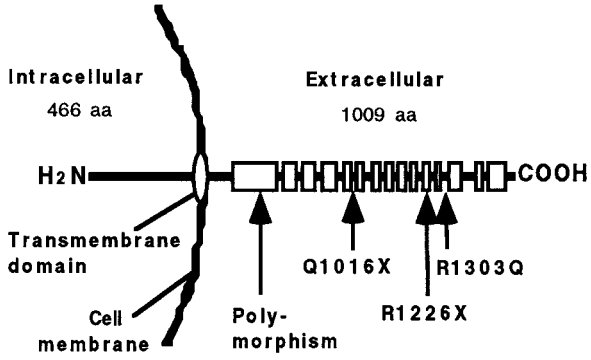

Figure 10 Schematic representation of the predicted structure of collagen XVII. The cDNA sequence (Giudice et al. 1992) predicts a type II transmembrane protein, with an amino-terminal intracellular domain of 466 amino acid residues in length, a 22-amino-acid-long transmembrane domain, and a carboxy-terminal, rodlike collagenous extracellular domain of 1,009 amino acids in length. The intracellular domain contains a cluster of cysteins and several protein kinase consensus sequences, and the ectodomain contains a repetitive collagenous –Gly–X–Y– sequence with multiple interruptions. The solid black line indicates noncollagenous sequences, and the open boxes indicate collagenous sequences. The localization of the mutations and the polymorphic site in the present study are indicated by arrows.

decay, small amounts of nascent truncated polypeptide chains are synthesized and accumulate intracellularly prior to degradation through default pathways. Mutations in the noncollagenous domains may yield more stable mutant polypeptides than are produced by those in the collagenous domains.

Interestingly, the missense mutation R1303Q led to a different biological and clinical phenotype, the *localisata* variant of JEB. This observation indicates that collagen XVII mutations underlie not only GABEB but other junctional EB subtypes as well. Collagen XVII was present at the dermal/epidermal junction in the proband's skin and localized into the blister roof after trauma-induced splitting. The mutation caused an Arg→Gln substitution in the serine-rich NC-4 domain of collagen XVII, within a –S–S–S–V–R–R/Q–G–S–S–Y–S–S– sequence in the most carboxy-terminal region of the protein (fig. 10). It alters a dibasic serine proteinase cleavage site, –X–R–R–, which might play a role in the posttranslational modification of collagen XVII. It also abolishes a consensus sequence (RRGS) for phosphorylation by a cAMP- or cGMP-dependent protein kinase. It appears more likely, however, that a binding site for a physiological ligand is weakened or eliminated by this genetic alteration. Immunoblots with the proband's epidermal extracts revealed, in addition to the normal 180-kD α1(XVII) polypeptides, a larger immunoreactive collagen XVII band (fig. 8), which is likely to represent an aggregate with intra- or intermolecular cross-links. This assumption is supported by the fact that the mutation results in an Arg→Gln substitution, thus providing a novel site for transglutaminase cross-links. Recently, transglutaminase has been shown to cross-link structures of the skin basement-membrane zone, including hemidesmosomes (Raghunath et al. 1996). If some of the α1(XVII) polypeptides in the proband's skin are subjected to transamidation at an abnormal site in the carboxy-terminus of the molecule, ligand interactions may be perturbed. The nature of these interactions remains elusive at present, but it seems likely that collagen XVII is a flexible longitudinal molecule that interacts with one or several components of the hemidesmosome-anchoring filament complex (Ishiko et al. 1993; Hopkinson et al. 1995). Mutations affecting different binding sites may lead to a variety of phenotypes. Characterization of such mutations and their phenotypic consequences at the cellular and clinical level will help in the dissection both of the physiological functions of collagen XVII and of its assembly into hemidesmosomes, as well as shed light on pathogenetic mechanisms underlying both heritable and acquired blistering diseases.

Acknowledgments

The authors thank Ms. M. Schubert for expert technical assistance and thank Drs. Biljana Gatalica, John McGrath, and Marcel Jonkman for helpful discussions. This investigation was supported by grants Br 1475/1-1, Br 1475/2-1, and SFB 293 Münster/B3, all to L.B.-T., from the Deutsche Forschungsgemeinschaft and by U.S. Public Health Service NIH grant P01-AR38923 to J.U. H.S. was fellow of the Deutscher Akademischer Austauschdienst, and L.P. was supported by the Dermatology Foundation.

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