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Molecular mechanisms of phenotypic variability in junctional epidermolysis bullosa

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ABSTRACT

Background Junctional epidermolysis bullosa (JEB), a group of hereditary skin fragility disorders, is associated with a wide variety of phenotypes, although all forms are characterized by trauma-induced skin blistering and tissue separation at the dermal-epidermal junction zone. A subgroup, coined JEB-other, is associated with mutations in the *COL17A1* gene encoding collagen XVII or, more rarely, with mutations in the laminin 332 genes *LAMA3*, *LAMB3*, or *LAMC2*. The objective of this study is comprehensive genotype-phenotype analysis in JEB-other patients with *COL17A1* mutations and elucidation of disease mechanisms underlying different skin phenotypes.

Methods and results *COL17A1* mutations and their clinical and cellular consequences were systematically analyzed in 43 patients with JEB-other. Cell culture, RT-PCR and protein biochemistry were applied to assess the effects of splice-site mutations, i.e. the nature and amounts of transcripts and polypeptides synthesized and their association with the phenotypic outcome. Thirty-four distinct *COL17A1* mutations were disclosed, 12 of them novel. mRNA and protein analyses demonstrated that patients with only about 12-14% of the physiological collagen XVII levels had mild cutaneous involvement and a long life span.

Conclusions In contrast to complete null phenotypes, presence of minor amounts of collagen XVII protein in JEB skin is associated with mild phenotypic manifestations. The data have significant implications for design of molecular therapies for JEB, since they suggest that already a low extent of collagen XVII restoration will improve skin stability and alleviate symptoms.

INTRODUCTION

JEB is genetically and clinically heterogeneous, but in all forms trauma-induced tissue separation occurs at the level of the lamina lucida of the dermal-epidermal basement membrane^[1]. The mostly lethal JEB-Herlitz is caused by null mutations in the *LAMA3*, *LAMB3*, or *LAMC2* genes encoding laminin polypeptides and JEB with pyloric atresia by mutations in the integrin genes *ITGB4* and *ITGA6*. A phenotypically variable group, coined JEB-other, is associated with missense mutations in the above laminin genes or with mutations in *COL17A1* encoding collagen XVII^[2]. The *COL17A1* phenotypes exhibit a wide range of severity and, in addition to skin blistering, different degrees of mucosal involvement, enamel defects, dystrophy or loss of nails, and alopecia can occur. The causes for the phenotypic variability are not well understood. Although more than 60 *COL17A1* mutations have been reported in the literature (<http://www.hgmd.cf.ac.uk/ac/index.php>), the only clear genotype-phenotype correlation is

that patients with complete lack of collagen XVII in the skin exhibit severe, generalized blistering with mucosal and adnexal involvement, whereas patients with reduced amounts of normal or with mutated collagen XVII in the skin display more moderate symptoms^[3, 4].

The *COL17A1* gene (NM_000494.3) on chromosome 10q24.3 comprises 56 exons^[5]. Its product, collagen XVII, is a type II transmembrane protein. Each collagen XVII is a trimer of three 180 kDa $\alpha 1(\text{XVII})$ -chains with an intracellular N-terminal domain of 466 amino acids, a transmembrane stretch of 23 amino acids and an extracellular C-terminus of 1008 amino acids. The ectodomain contains 15 collagenous (COL) subdomains, interrupted by 16 non-collagenous sequences (NC) and is proteolytically shed from the cell surface by ADAMs proteinases, yielding a shorter form of the protein which remains stable in the extracellular matrix^[6]. Thus, functionally, collagen XVII plays a dual role as a cell surface receptor and as a matrix component. During steady-state homeostasis, it maintains adhesion of basal keratinocytes to the basement membrane. Shedding of the ectodomain releases the cell from some of its binding partners and allows it to become motile during epidermal differentiation or during wound healing and tissue regeneration^[7].

Recent pilot studies on causal therapies for genetic skin diseases have shown that relatively small biological changes, e.g. moderately increased levels of a missing protein in the skin, can have substantial clinical effects^[8]. Specifically, fibroblast-based cell therapies of collagen VII-deficient dystrophic epidermolysis bullosa demonstrated that about 35 % of physiological levels of the collagen confer greatly improved skin integrity^[9, 10] and, in a human pilot trial, gene therapy for JEB partially restored laminin 332 in the skin and significantly ameliorated the phenotype^[11]. This was corroborated by a recently described laminin gamma-2 hypomorphic mouse^[12], which has low levels of laminin 332 in the skin and a moderate phenotype, in contrast to the perinatally lethal laminin 332 knockout mice^[13, 14, 15]. These observations suggest that the multiprotein complexes at the dermal-epidermal junction are highly stable with robust interactions of the binding partners, which can compensate a significant, but not complete, loss of one component. On this background, we addressed the role in skin integrity of a major component of the epidermal adhesion complex, collagen XVII, in a large cohort of patients with collagen XVII-deficient JEB.

PATIENTS AND METHODS

Patients and tissue samples

Forty-three patients of Caucasian ancestry and European origin were investigated. They were suspected to have EB and presented at the Freiburg EB-center for clinical evaluation and diagnosis, or DNA and skin samples were provided by collaborating centers. Following informed consent, EDTA-blood and skin samples were obtained from the patients and, if available, from family members. The study was approved by the ethics committee of the University of Freiburg.

Immunofluorescence staining

Immunofluorescence staining of the skin was performed using a panel of antibodies to components of the epidermal basement membrane zone as described^[16]. For diagnostic purposes, the following domain-specific collagen XVII antibodies were used: the polyclonal antibody NC16A, which binds to the NC16A domain of collagen XVII, and the monoclonal NC16A1 recognizing amino acids 513-525^[17, 18, 19]. The sections were observed with an Axiophot fluorescence microscope (Carl Zeiss, Göttingen, Germany). To quantify the collagen XVII expression *in situ*, 3 skin sections of each patient were stained with the NC16A1 antibody and photographed with the confocal laser scanning microscope (LSM510, Carl Zeiss, Göttingen, Germany). The fluorescence signal was quantified using the image processing program Image J^[20] version 1.43 (<http://rsbweb.nih.gov/ij/docs/index.html>), after background subtraction.

Keratinocyte cultures

Primary epidermal keratinocytes were isolated from skin biopsies of patients 28, 38 and 15 and of a normal control, and cultivated in keratinocyte growth medium (Invitrogen, Karlsruhe, Germany), as described^[21]. They were used for RNA and protein isolation in passage 2 at 80% confluence.

Mutation detection and RNA analysis

Genomic DNA (gDNA) was extracted from EDTA-blood using the QiAmp DNA mini kit (Qiagen, Hilden, Germany). For amplification of all *COL17A1* exons and exon/intron boundaries primers were used as described^[5, 22], and sequencing was performed on an ABI 7330XL DNA analyzer. Mutations were confirmed by resequencing in both directions.

Total RNA was extracted from subconfluent cells with QiaAmp RNA blood mini kit (Qiagen Hilden, Germany). Reverse transcription (RT) was performed with Advantage RT-for-PCR Kit (BD Biosciences, Heidelberg, Germany) with 0.5 µg of total RNA. The primers for RT-PCR of *COL17A1* were as follows: exon 18F 5'- gaggaggtgaggaagctgaa and exon 23R 5'- tccccttctctccagatcc, amplicon of 467bp; exon 50F 5'- attcagaggcatcgttgac and exon 52R

5'- ctgtgctcatggaagagctg, amplicon of 633bp; exon 51F 5'- gctgaaaacagcgacagctt and exon 54R 5'- gtcacgttgctgtaggcaga with a product of 532bp. The PCR products were separated on 1.5% agarose gels. The RT-PCR products were subcloned to TOPO TA-cloning vector (Invitrogen, Karlsruhe, Germany), and for each reaction, 10 to 15 clones were sequenced using the M13-RV primer.

Immunoblotting

For immunoblotting, cultured keratinocytes were extracted with a buffer containing 0.1 M NaCl, 20 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, Pefabloc and EDTA^[18, 23]. Protein content was quantified with a micro Lowry assay (DC Protein Assay, Bio-Rad, Munich, Germany). Normalized amounts of the proteins were subjected to SDS-PAGE, immunoblotted and incubated with the following anti-collagen XVII polyclonal antibodies: ecto-4, recognizing epitopes within the 50 most C-terminal amino acids and NC16A^[18, 24, 25]. The blots were incubated with antibodies to β -tubulin (Abcam, Cambridge, UK) or GAPDH (clone 6C5, Millipore, Temecula, California) to control loading. Densitometric quantification of the bands was performed with the software Gel-Pro Analyzer software (MediaCybernetics, Inc., Bethesda, MD, USA).

RESULTS

Mutation detection strategy and novel *COL17A1* mutations

Forty-three European patients with JEB-other were included in this study (Table 1). Most mutations were located in exons 51 and 52. Notably, splice-site mutations occurred preferentially in intron 51. Using this approach, the mutation detection rate reached 98.8 % (85/86 alleles). Thirty-four distinct mutations were disclosed, suggesting that most families have their own private mutation (Figure 1). The majority, 69%, were nonsense mutations, insertions and deletions predicted to lead to a premature termination codon (PTC). Missense mutations comprised 19%, and splice-site mutations 12% of the survey. The mutations were distributed over the entire *COL17A1* gene (Figure 1). Two mutations were found to be recurrent in European patients. The non-population-specific mutation p.Arg1226X^[3, 4, 26] was found in almost 10%, and p.Gly803X^[27] in approximately 7% of the mutated alleles.

Phenotypic variability

To assess disease severity the following clinical parameters were recorded during multiple patient visits: extent of skin and mucosal involvement, nail dystrophy and alopecia. Twenty-seven of the 43 patients had a disseminated blistering and a generalized disease phenotype^[28], whereas 11 had a localized phenotype. No clinical data was available of five

patients. All patients had dystrophic toe nails and the only patients with normal finger nails were 28, 35 and 36. For almost half of the patients, dental anomalies were reported, including enamel defects and subsequent susceptibility to caries and/or parodontitis^[29].

On molecular level, the individuals with severe clinical phenotypes were homozygous or compound heterozygous for PTC or splice-site mutations and exhibited the full spectrum of symptoms: generalized blisters, alopecia and nail anomalies^[1, 30] (Figure 2A-C). They were significantly younger than the group of patients with milder phenotypes, namely patients 28-38 (mean age 14.4 versus 48.5 years) (Figure 2D-J).

Particular emphasis was put on various localized phenotypes, which may be difficult to recognize. Missense mutations were typically associated with modest-mild phenotypes, as seen in patients 29, 30, 32-34, and 37. Patients 29, 30, 33 and 34, who harboured the same mutation Arg1303Gln, exhibited a somewhat unusual phenotype with mild, predominantly acral blistering and mucosal involvement, which was associated with light fibrotic changes such as lacrimal duct obstructions, microstomia, esophageal stenoses or finger contractures (Figure 2I-J).

Intriguing was the constellation observed in patients 28, 35, 36 and 38 with minimal, localized blistering and toe nail dystrophy (Figure 2D-H). They had had mild trauma-induced blistering since childhood, but only sought medical attention when age-related health conditions aggravated the blistering. This was first misinterpreted as an acquired bullous disease, but recalcitrance to therapies finally led to the diagnosis of JEB at the advanced age of 67 - 80 years.

In order to explain the phenotypic variability, we focused on biological consequences of splice site mutations, which are delineated in the following.

Splice-site mutations associated with mild phenotypes in elderly individuals

The 80 year-old patient 28 presented with blisters mostly on the gluteal area and the thighs, as she was restricted to bed because of joint problems, and with toe nail dystrophy (Figure 2 E-F). Mucous membranes and hair were normal. Her two sisters, 79 and 82 years-old (patients 35, 36), were very mildly affected, with toe nail dystrophy as the only symptom (Figure 2D). These 3 patients were the only ones in our cohort, who did not have dystrophic finger nails.

In this family, the novel splice-site mutation c.1744-2A>C at the acceptor splice site of exon 20 was combined with the nonsense mutation p.Arg1169X. RT-PCR with primers spanning exon 18-23 revealed three abnormal transcripts ([Figure 3A](#)). The PCR products were subcloned, the clones sequenced and the transcripts identified. Transcript 1 (12.5% of the clones) contained in-frame skipping of exons 21 and 22 (90 nucleotides) and resulted in the deletion of 30 amino acids, spanning part of the COL15 domain. In transcript 2 (25% of the clones), three nucleotides in exon 21 were skipped leading to the deletion of Gly582 within the COL15 domain. The T2 band presumably also contains the transcripts that do not bear the splice-site mutation, but correspond to the allele carrying the p.Arg1169X mutation. Transcript 3 (12.5% of the clones) led to activation of a cryptic acceptor splice-site in intron 20 and to partial out-of-frame retention of 95 nucleotides of this intron ([Figure 3A](#)). 50% of the clones contained the normal sequence, which probably resulted from the allele with the p.Arg1169X mutation. It is likely that mutated collagen XVII is translated from transcripts 1 and 2. The first would contain a partial deletion of the COL15 domain and the latter a single amino acid deletion of Gly582 ([Figure 3B](#), lower panel). Immunoblotting of keratinocyte lysates of patient 28 showed that only about 3-4% of collagen XVII was present, as compared to control cells ([Figure 3B](#), upper left panel). Since mutated polypeptides are often sensitive to proteolytic degradation, we subjected a skin biopsy of patient 28 to immunofluorescence staining using collagen XVII antibodies. This revealed a reduced signal, corresponding to approximately 14% of collagen XVII levels in control skin ([Figure 3B](#), upper right panel). Taken together, the data indicate that a fraction of the translated polypeptides was degraded, but also that about 14% of normal collagen XVII levels confer reasonable skin stability and a mild blistering phenotype *in vivo*.

The 67 year-old male patient 38 presented with blisters mostly on thighs and hands ([Figure 2G](#)). He had nail dystrophy ([Figure 2H](#)), but no mucosal involvement. The frontal alopecia was of androgenetic type and not associated with JEB. Mutation screening disclosed two novel mutations, the splice-site mutation c.4156+1G>A at the donor splice site of exon 52 and the nonsense mutation p.Arg169X. RT-PCR with primers spanning exons 51-54 generated four aberrant transcripts ([Figure 4A](#)). Transcript 1 (25% of the clones) led to partial, out-of-frame skipping of exon 52 and to a PTC after 155 codons. In transcript 2 (25% of the clones), 210 nucleotides of exon 52 were skipped resulting in the deletion of amino acids 1316 - 1385 of the COL3 and NC3 subdomains. Transcript 3 (50% of the clones) comprised in-frame skipping of exon 52 (390 nucleotides) and led to the deletion of 130 amino acids and absence of the COL4 and COL3 subdomains ([Figure 4A](#)). The fourth, minor one could not be identified. Immunoblotting of patient keratinocyte lysates revealed small amounts of a shorter, truncated collagen XVII polypeptide of about 165 kDa, likely a

product of transcript 3 ([Figure 4B, upper left panel](#)). Semiquantitative analysis of band intensity showed that this corresponded to about 4 % of controls. Immunofluorescence staining of a skin biopsy revealed reduced collagen XVII staining, corresponding to about 12% of controls ([Figure 4B, upper right panel](#)).

The novel splice-site mutation c.3766+5G>A associated with complete loss of collagen XVII and a generalized phenotype

In contrast to the mutations described above, the outcome of the novel splice-site mutation c.3766+5G>A, at the donor splice site of exon 51 was associated with a severe phenotype in the 1 year-old patient 15. He suffered from congenital and persisting generalized blistering, mucosal involvement and nail dystrophy. In accordance, three of the four transcripts representing more than 80% of the clones, resulted in frame-shift and PTC. One minor transcript could not be identified in the analyzed clones. Only transcript 4 (18% of the clones) led to in-frame skipping of exon 51. This deletion is predicted to encompass amino acids 1207-1255 and to eliminate the NC5, COL5 and NC6 domains of the protein ([Figure 5](#)). However, the truncated collagen XVII was obviously unstable and susceptible to degradation, since neither immunofluorescence staining of the skin (not shown) nor immunoblotting of keratinocyte extracts revealed presence of collagen XVII protein ([Figure 5](#)).

DISCUSSION

Here we describe genotype-phenotype correlations in a cohort of 43 European JEB patients with collagen XVII mutations. The group exhibited a wide spectrum of clinical variability ranging from mild, predominantly acral blistering to severe phenotypes with generalized blistering, mucosal involvement, extensive nail dystrophy and universal alopecia. In general, the milder pathological manifestations were associated with missense or splice-site mutations and the presence of at least some collagen XVII in the skin. The hallmark of the severe phenotypes was total lack of collagen XVII in the skin, but different mutation mechanisms were found to underlie the deficiency. Delineation of such mechanisms is a pivotal for understanding the molecular pathology and a prerequisite for development of novel, biologically valid therapies.

Particularly interesting was the observation that splice-site mutations allowing low expression of collagen XVII generate mild disease manifestations, as has been suggested in individual case reports^[31, 32]. Illustrative examples of this were the elderly patients 28, 35, 36 and 38, who had had very few symptoms during past several decades. Occasional skin fragility had been suspected to represent an acquired condition. However, with advancing age, diseases such as diabetes or increasing physical immobility aggravated the blistering,

and the patients were finally diagnosed with JEB in their 7th or 9th decade of life. These observations suggest that comorbidities are modifying factors of JEB and should alert medical professionals to critically re-evaluate individuals suspected to have an acquired skin blistering disorder, which does not respond to therapy.

Based on the data in the present study, we predict that both the percentage of transcripts not causing PTCs and the location of the splice-site mutation play a role in the outcome on protein level. All splice-site mutations described here targeted collagenous subdomains of the collagen XVII molecule. Some, but not all of them had devastating effects on the stability of collagen XVII and led to degradation of unstable molecules by tissue proteinases. Mutations within collagenous domains are known to perturb the G-X-Y repeat motif and affect the stability of the ectodomain, leading to unfolding and degradation of the collagen XVII molecule^[25, 33, 34]. Biochemical analyses of transcripts and mutant polypeptides, as performed here, may become useful prognostic markers for determining the course of the disease at an early stage and be helpful for counselling the patients relating to genetic and environmental contributions to their disease and quality of life. As reported for other EB forms, modifying factors might influence the phenotype^[35, 36]. Our results suggest that environmental conditions related to physical activity and trauma, as well as comorbidities are aggravating circumstances. Modifier genes and epigenetic factors are certainly involved in modulation of phenotypic variations. In the case of missense and truncating mutations, misfolded proteins are subjected to intracellular degradation, processes which are likely to be subject of inter-individual variations^[37].

In terms of therapeutic perspectives, an important finding in this study is that already a low percentage of functional collagen XVII translated from the transcripts of splice-site mutations can confer a mild skin phenotype, in contrast to the null variants. The natural history of JEB in four aged individuals with 2-4% of collagen XVII *in vitro* and about 12 - 14 % of normal collagen XVII levels *in vivo* demonstrated that for successful biological therapies it will not be necessary to achieve 100 % restoration of collagen XVII in the skin, but already smaller increases will improve dermal-epidermal stability and alleviate symptoms in JEB.

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Figure legends

Figure 1. Distribution of collagen XVII mutations in this study. Schematic representation of the collagen XVII molecule with delineation of the corresponding domains. The intracellular (IC) N-terminal domain of 466 amino acids is depicted in blue and the short transmembrane (TM) stretch of 23 amino acids in grey. The extracellular (EC) C-terminus of 1008 amino acids contains 15 collagenous subdomains, depicted in green and 16 non-collagenous domains in pink. The mutations underlined in red boxes are recurrent and those in green boxes are previously undescribed. The other mutations have been reported previously. Missense and nonsense mutations are described on protein level, whereas frameshift and splice site mutations on cDNA level.

Figure 2. Phenotypic variability in JEB patients with *COL17A1* mutations.

(A-B) Severe phenotype in the 26 year-old patient 8 (compound heterozygous for p.Met1Thr and p.Arg1226X). (A) Blisters, erosions and atrophic skin on the lower legs; (B) blistering and nail dystrophy of the right fingers. (C) The 9 year-old, severely affected patient 4 (compound heterozygous for p.Arg154X and p.Gly803X) presented with blisters, dystrophy and loss of toenails on the right foot. (D-F) The very mildly affected patients 28 and 35 (compound heterozygous for p.Arg1169X and the splice-site mutation c.1744-2A>C) are siblings. The lower legs (D) of the 82 year-old patient 35; (E) normal fingernails and (F) dystrophic toenails of the 80 year-old patient 28. (G-H) The 67 year-old patient 38 (compound heterozygous for p.Arg169X and the splice-site mutation c.4156+1G>A) has a mild phenotype consisting of: (G) occasional blistering on the lower legs and (H) dystrophic fingernails. (I-J) The 53 year-old patient 33 (homozygous for p.Arg1303Gln) presented with: (I) microstoma and (J) finger contractures in the right hand.

Figure 3. Splice-site mutation c.1744-2A>C associated with mild phenotype in patient 28 (compound heterozygous with p.Arg1169X). (A) The outcome on cDNA level. RT-PCR amplification revealed one transcript in control keratinocytes (C) and several aberrant transcripts in patient's keratinocytes (P28). The numbers on the left side indicate the size in bp. The schematic representation depicts the normal transcript (C) and the aberrant transcripts (T1-T3). Boxes represent exons, while black bars show introns. Transcripts resulting in premature termination codon are noted with PTC. (B) The outcome of the non-PTC transcripts on protein level is shown. The upper left panel shows immunoblot with lysates of control (C) keratinocytes and patient (P28) keratinocytes and the NC16A antibody to collagen XVII. β -tubulin was used to control loading. Numbers on the left side indicate the molecular weight in kDa. Arrows indicate the position of the bands. The upper right panels show immunofluorescence stainings of control skin (C) and patient's skin (P28) with the NC16A1 antibody for collagen XVII (green). The nuclei are stained in blue with DAPI. The schematic representation below shows the predicted consequences of the mutation at protein level within the collagen XVII molecule. Red bars indicate deletion of amino acids.

Figure 4. Splice-site mutation c.4156+1G>A associated with mild phenotype in patient 38 (compound heterozygous with p.Arg169X). (A) The outcome on cDNA level. RT-PCR amplification revealed one transcript in control keratinocytes (C) and several aberrant transcripts in patient's keratinocytes (P38). The numbers on the left side indicate the size in bp. The schematic representation depicts the normal transcript (C) and the aberrant transcripts (T1-T3). Boxes represent exons. Transcripts resulting in premature termination codon are noted with PTC. (B) The outcome of the non-PTC transcripts on protein level is shown. The upper left panel shows immunoblot with lysates of control (C) keratinocytes and patient (P38) keratinocytes and the NC16A antibody to collagen XVII. β -tubulin was used to control loading. Numbers on the left side indicate the molecular weight in kDa. Arrows indicate the position of the bands. The upper right panels show immunofluorescence stainings of control skin (C) and patient's skin (P38) with the NC16A1 antibody for collagen XVII (green). The nuclei are stained in blue with DAPI. The schematic representation below shows the consequences of the mutations at protein level within the collagen XVII molecule. Red bars indicate deletion of amino acids.

Figure 5. The novel splice-site mutation c.3766+5G>A leading to severe phenotype in the 1 year-old patient 15. The left panels show the outcome on cDNA level. RT-PCR amplification revealed one transcript in control keratinocytes (C) and several aberrant transcripts in patient's keratinocytes (P). The numbers on the left side indicate the size in

bp. The schematic representation depicts the normal transcript (C) and the aberrant transcripts (T1-T4). Boxes represent exons, while black bars show introns. Transcripts that result in premature termination codon, are noted with PTC. The outcome of the non-PTC leading transcript on protein level is shown on the right. The upper panel shows the immunoblot with lysates of control (C) keratinocytes and patient (P) keratinocytes and the NC16A antibody to collagen XVII. GAPDH was used to control loading. Numbers on the left side indicate the molecular weight in kDa. The schematic representation below shows the consequence of the mutation at protein level within the collagen XVII molecule. Red bars indicate deletion of amino acids.

Table 1. The cohort of 43 JEB patients: *COL17A1* mutations, collagen XVII expression and phenotype

No.	Mutations c.DNA ¹	Mutations protein	IFM: COLXVII staining ²	Mucosal involvement	Alopecia ³
Generalized severe JEB-o⁴					
1	c.[3676C>T]+[1826G>A]	p.[Arg1226X]+ [Gly609Asp]	strongly red	oral	-
2	c.[2993dupC]+ [2993dupC]	p.[Gly999fs]+ [Gly999fs]	strongly red	oral	NA ⁵
3	c.[2560_2564del]+ [2560_2564del]	p.[Asn854fs]+ [Asn854fs]	neg	NA	NA
4	c.[460C>T]+[2407G>T]	p.[Arg154X]+ [Gly803X]	neg	oral, nasal, genital	+
5	c.[3865dupA]+ [3865dupA]	p.[Asp1289fs]+ [Asp1289fs]	neg	NA	+
6	c.[3548delC]+ [3548delC]	p.[Arg1183fs]+ [Arg1183fs]	NA	NA	+
7	c.[1616G>A]+ [2561_2562delTT]	p.[Gly539Glu]+ [Asn854fs]	neg	NA	+
8	c.[2T>C]+[3676C>T]	p.[Met1Thr]+ [Arg1226X]	neg	oral, anal	+
9	c.[3676C>T]+[3676C>T]	p.[Arg1226X]+ [Arg1226X]	neg	oral, genital, anal	+
10	c.[3676C>T]+[2564T>G]	p.[Arg1226X]+ p.[Leu855X]	neg	oral, nasal	+
11	c.[3046C>T]+[3046C>T]	p.[Gln1016X]+ [Gln1016X]	neg	oral	+
12	c.[2860delG]+ [2860delG]	p.[Gly954fs]+ [Gly954fs]	neg	oral	+
13	c.[2860delG]+ [2860delG]	p.[Gly954fs]+ [Gly954fs]	neg	oral	NA
14	c.[2723dupC]+ [3766+1 G>C]	p.[Gly909fs]+SS⁶	neg	oral, nasal, genital, anal	-
15	c.[3766+5G>A]+ [3766+5G>A]	SS	neg	oral, anal	-
16	c.[2407G>T]+ [2407G>T]	p.[Gly803X]+ [Gly803X]	strongly red	anal	+
17	c.[2383C>T]+ [2383C>T]	p.[Arg795X]+ [Arg795X]	red red	NA	+
18	c.[2383C>T]+[2383C>T]	p.[Arg795X]+ [Arg795X]	red	NA	-
19	c.[1834G>A]+ [3800dupC]	p.[Gly612Arg]+ [Gly1267fs]	red	oral, nasal, genital, anal	+
20	c.[433C>T]+ [1898G>A]	p.[Arg145X]+ [Gly633Asp]	red	anal	-

21	c.[4305_4308dup]+ [4305_4308dup]	p.[Gln1437fs]+ [Gln1437fs]	pos	oral, anal	-
22	c.[3865dupA]+ [3865dupA]	p.[Asp1289fs]+ [Asp1289fs]	neg	oral	-
23	c.[2407G>T]+NA	p.[Gly803X]+NA	neg	oral, nasal, genital	-
24	c.[415_416delAG]+ [415_416delAG]	p.[Glu139fs]+ [Glu139fs]	neg	oral	NA
25	c.[415_416delAG]+ [415_416delAG]	p.[Glu139fs]+ [Glu139fs]	red	oral	-
26	c.[2336-2A>G]+ [2336-2A>G]	SS	neg	oral, anal, genital	+
27	c.[3676C>T]+ [2336-2A>G]	p.[Arg1226X]+SS	neg	oral, anal	-
Localized JEB-o					
28	c.[3505C>T]+ [1744-2A>C]	p.[Arg1169X]+ SS	red	-	-
29	c.[3908G>A]+ [3908G>A]	p.[Arg1303Gln]+ [Arg1303Gln]+	pos	oral, eyes	-
30	c.[3908G>A]+ [3908G>A]	p.[Arg1303Gln]+ [Arg1303Gln]+	pos	oral, oesophagus, eyes	-
31	c.[3676C>T]+ [53_1224del]	p.[Arg1226X]+ [18_407del]	strongly red	anal	-
32	c.[1834G>A]+ [1834G>A]	p.[Gly612Arg]+ [Gly612Arg]	red	anal, genital	-
33	c.[3908 G>A]+ [3908G>A]	p.[Arg1303Gln]+ [Arg1303Gln]+	NA	oral, eyes	+
34	c.[3908G>A]+ [3908G>A]	p.[Arg1303Gln]+ [Arg1303Gln]+	NA	oral, eyes	-
35	c.[3505C>T]+ [1744-2A>C]	p.[Arg1169X]+ SS	NA	-	-
36	c.[3505C>T]+ [1744-2A>C]	p.[Arg1169X]+ SS	NA	-	-
37	c.[1861G>A]+ [1601_1602insG]]	p.[Gly621Ser]+ [Ser534fs]	NA	NA	NA
38	c.[505C>T]+ [4156+1G>A]	p.[Arg169X]+SS	strongly red	-	-
No clinical data available					
39	c.[2993dupC]+ [2993dupC]	p.[Gly999fs]+ [Gly999fs]	neg	NA	-

40	c.[1392G>A]+ [1392G>A]	p.[Trp464X]+ [Trp464X]	neg	NA	NA
41	c.[3676C>T]+ [2407G>T]	p.[Arg1226X]+ [Gly803X]	NA	NA	NA
42	c.[3676C>T]+ [2407G>T]	p.[Arg1226X]+ [Gly803X]	NA	NA	NA
43	c.[3800dupC]+ [3800dupC]	p.[Gly1267fs]+ [Gly1267fs]	strongly red	NA	NA

Legend: ¹ cDNA numbering from first A of start codon ATG ([NM_000494.3](#))
² IFM, immunofluorescence mapping; COLXVII, collagen XVII; red, reduced; neg, negative; pos, positive
³ +, present; -, absent
⁴ JEB-o, junctional EB-other
⁵ NA, not available
⁶ SS, splice site affected
The previously unreported mutations are depicted with **bold**

Figure 1

Collagen XVII

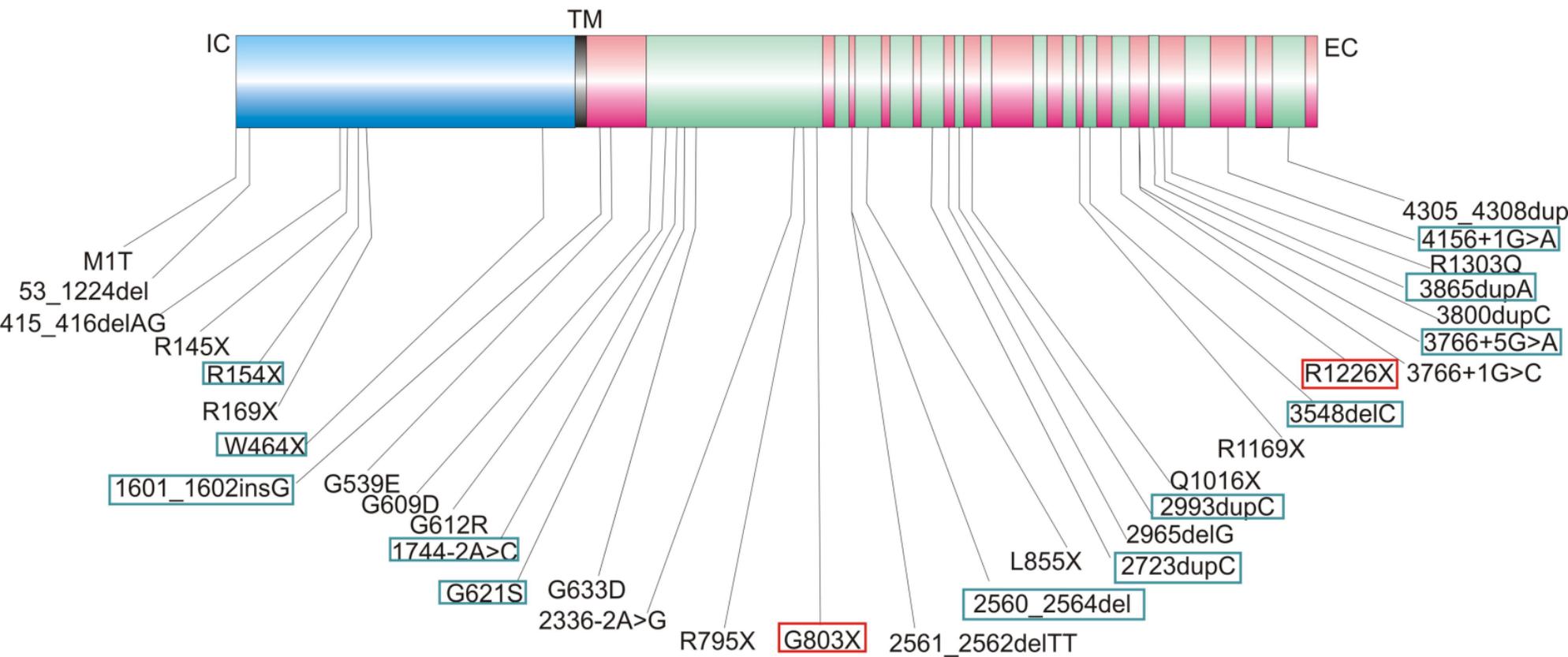
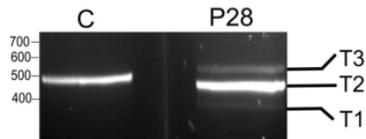
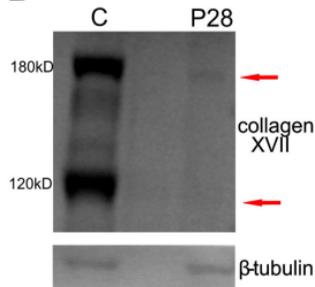
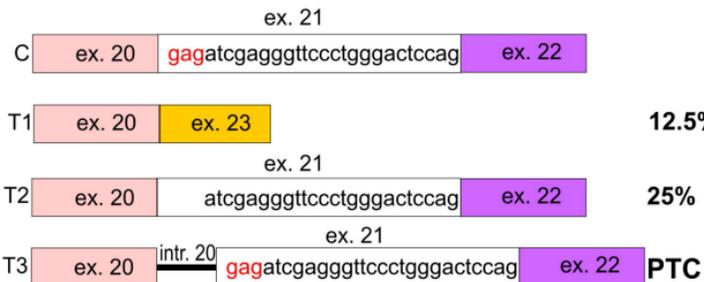
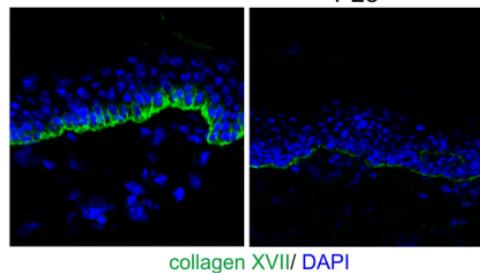


Figure 2



Figure 3**A****Patient 28****B****C****12.5%****25%****PTC**

TM

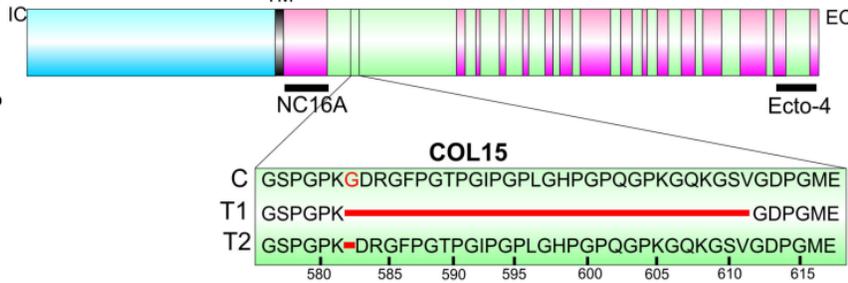


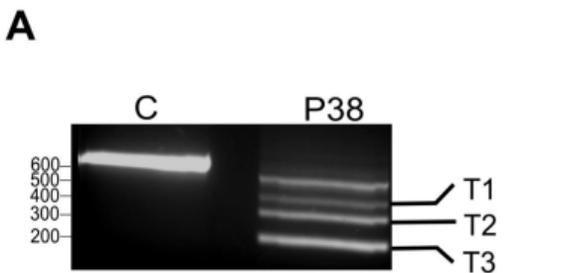
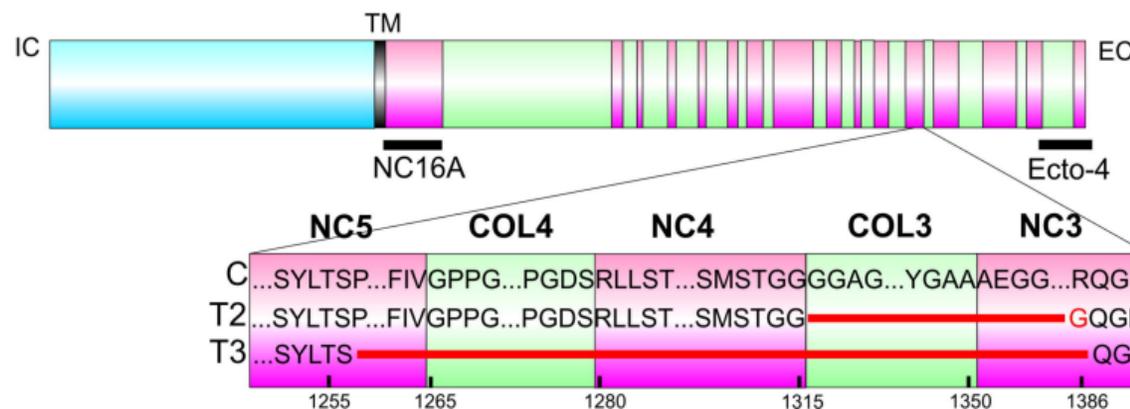
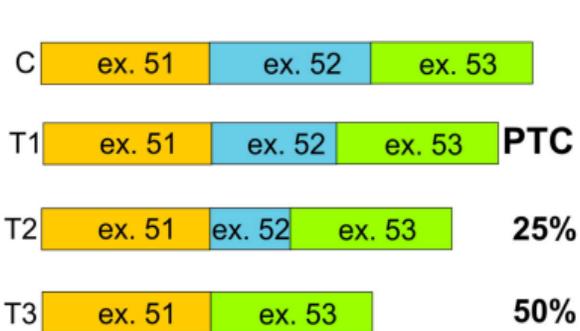
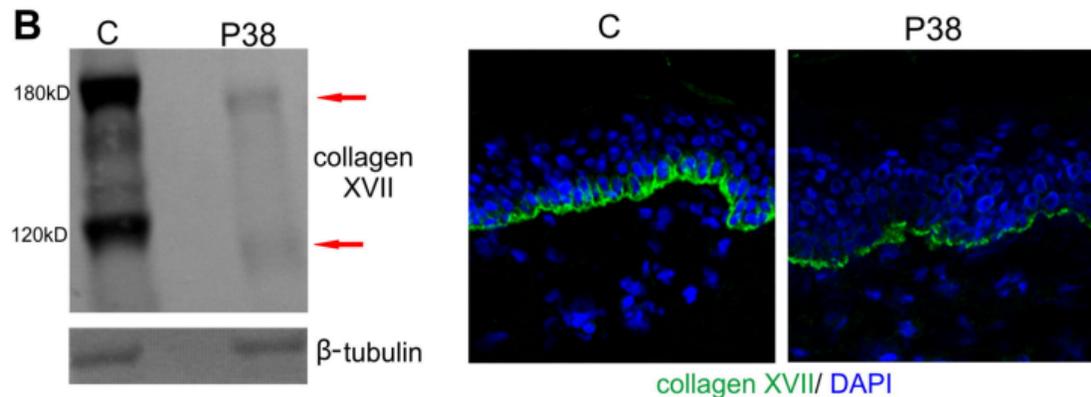
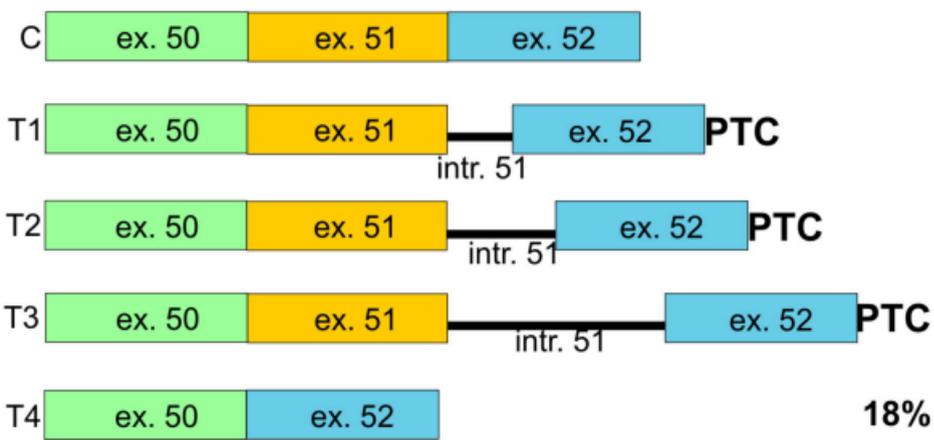
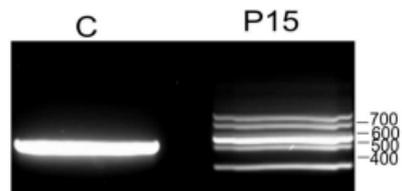
Figure 4**Patient 38**

Figure 5



Patient 15

