TO THE EDITOR

Dystrophic epidermolysis bullosa (DEB) is a genetic blistering disorder associated with anomalies of collagen VII (C7), which builds the anchoring fibrils (AFs) at the dermal-epidermal junction. More than 900 COL7A1 mutations have been reported (accessible at https://www.deb-central.org). However, the pathomechanisms determining the clinical variability have been only partially discovered, and therapeutic needs are still unmet. Here, we have identified monoallelic large intragenic COL7A1 deletions, which eliminate parts of the triple-helical domain, as a molecular mechanism of mild DEB, previously unreported to our knowledge.

In our series of 272 DEB patients (Kern et al., 2006, 2009; van den Akker et al., 2011), COL7A1 mutations were found in 98.53% of patients; we failed to find COL7A1 mutations in four patients. After patients gave written informed consent, EDTA blood and skin samples were obtained, and the study was approved by the ethics committee of the University of Freiburg. Using multiplex ligation-dependent probe amplification, we found monoallelic intragenic large COL7A1 deletions in two patients in whom Sanger sequencing and whole-exome sequencing had failed to identify mutations. Patient 1 was an offspring of unrelated healthy Mongolian parents. At birth, she had skin defects on the lower legs and was considered to have transient bullous dermolysis of the newborn with intraepidermal deposition of C7 and remnants of AF (Fassihi et al., 2005). She developed acral blisters, which healed with milia and scars, and nail loss (Figure 1a). Her younger sister was similarly affected. Patient 2 was a German man who presented with blistering since early childhood; scars predominantly on elbows, knees, and lower legs; and nail loss in adulthood (Figure 1b). He was the only affected individual in his family.

The breakpoints of the COL7A1 deletions were shown by amplifications and Sanger sequencing over the deletion borders. In patient 1, the breakpoints were in exon 70 and 99 (c.5794_7515del) and were predicted to result in the in-frame deletion of 574 amino acids (p.Leu1932_Gly2505del). The deletion spans approximately one third of the collagenous domain and presumably results in functional C7p.Leu1932_Gly2505del could be, at least in part, functional. Based on the observed similar phenotype in patient 2, we have assumed that C7p.Leu1932_Gly2505del is produced and secreted together with the full-length protein in patients’ cells. In agreement with this, immunoreactivity for C7 was stronger in patient 1 and control skin samples (Figure 1h). Transmission electron microscopy showed shorter collagenous domain, we generated two deletion constructs by eliminating either exons 46–85 or exons 46–85. On the protein level, the deletion eliminates almost half of the collagenous domain and presumably spans approximately 717 amino acids (p.Pro1536_Glu2252del). The deletion occurred de novo, being excluded in both unaffected parents.

The consequences of p.Leu1932_Gly2505del were analyzed in keratinocytes and skin samples of patient 1. COL7A1 mRNA was increased by threefold, as was C7 abundance in cell lysates and conditioned media of mutant keratinocytes (Figure 1f and g). A band corresponding to a smaller protein was detected both in lysates and media, showing that C7p.Leu1932_Gly2505del is produced and secreted together with the full-length protein in patients’ cells. In agreement with this, immunoreactivity for C7 was similar in patient 1 and control skin samples (Figure 1h). Transmission electron microscopy showed shorter collagenous domain, we generated two deletion constructs by eliminating either exons 46–85 or exons 46–85. On the protein level, the deletion eliminates almost half of the collagenous domain and presumably spans approximately 717 amino acids (p.Pro1536_Glu2252del). The deletion occurred de novo, being excluded in both unaffected parents.

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Figure 1. Clinical and molecular findings in patients with large COL7A1 deletions. Scars and nail loss in (a) patient 1 and (b) patient 2. (c, d) Breakpoints of the large deletions. (e) Schematic of normal and truncated C7. Gray, triple-helical domain of C7 with pepsin fragments P2 and P1 and the hinge region; pink, non-collagenous domains 1 and 2 (NC1 and NC2). (f) COL7A1 quantitative real-time reverse transcriptase-PCR with cDNA obtained from mRNA of keratinocytes from patient 1 and a control sample. (g) Immunoblot analyses of lysates and conditioned media with the NC2-10 antibody to C7. Black arrow, normal C7; red arrow, truncated C7. (h) Immunofluorescence staining of skin samples from a control sample and patient 1. (i) Transmission electron microscopy of skin samples from a control sample and patient 1. Arrows, anchoring fibrils. Scale bar = 200 nm. aa, amino acid; C7, collagen VII.
c.4564_6750del, p.Pro1523_Gly2251del) or exons 57–99 (C7Δ57-99; c.5125_7521del, p.Val1709_Lys2507del) (Figure 2a). Using functional readouts, we determined the ability of the deletion mutants to bind collagen IV and to form stable triple helices (Bornert et al., 2016). Both deletions decreased the binding capacity of...
C7 to collagen IV and laminin 332 without disturbing the affinity (for collagen IV, dissociation constant $C7 = 12.15 \pm 4.9$ nmol/l vs. $C7^{Δ45,85} = 12.95 \pm 5.3$ nmol/l and $C7^{Δ57,99} = 10.36 \pm 3.9$ nmol/l) (Figure 2b and c, and see Supplementary Figure S1 online). This suggested that deletions in the collagenous domain could affect the flexibility of noncollagenous 1 domains of truncated C7 molecules by decreasing the ability to simultaneously interact with collagen IV or laminin 332 without changing the affinity. We then assessed the stability of the triple-helical structure of C7 by limited trypsin digestion. Both $C7^{Δ45,85}$ and $C7^{Δ57,99}$ could undergo trypsin digestion, showing that they can form stable trimeric structures despite large deletions in the collagenous domain (Figure 2d).

Our findings suggest that the deletion of multiple exons corresponding to the collagenous domain might result in truncated C7 that is secreted, forms triple-helix structures, assemblies in suprastructures, is deposited at the dermal-epidermal junction, and is at least partly functional. We found that deletion of an important part of the collagenous domain of C7 does not affect the stability of the C7 triple-helical structure or the binding capacity to collagen IV or laminin 332. Nevertheless, these shorter C7 variants, which also lack a small noncollagenous domain, called the hinge region, embedded in the collagenous domain, are probably less flexible than normal molecules and are not able to spatially interact with skin-stabilizing partners like the normal counterpart. This hypothesis is supported by our data showing that although the binding affinity to collagen IV and laminin 332 remains unchanged, the maximal binding capacity is drastically lower.

Exon-skipping therapies are a current focus of DEB research and have already reached the clinical stage in muscular dystrophy (Bornert et al., 2017; Lim et al., 2017). Although 107 of the 118 exons of COL7A1 are skippable, developing exon skipping remains challenging because of the large number of unique mutations. The use of minicollagens was proposed several years ago (Chen et al., 2000). A recombinant miniC7 with deletion of amino acids 1920–2603, almost overlapping the deletion found in patient 1, was secreted as correctly folded helical trimers resistant to protease degradation and was able to bind to ligands (Chen et al., 2000). A cDNA-encoding mouse mini proC7, lacking amino acids 1435–2476 in the collagenous domain, was used to study the biological consequences of amino acid substitutions (Brittingham et al., 2005; Colombo et al., 2003). A therapeutic approach that makes use of a minicollagen to alleviate disease severity in people with severe generalized recessive DEB is supported by our findings in patients with mild DEB.

Here, we have shown that naturally occurring large deletions of 500–700 amino acids within the C7 triple-helical region result in mild dominant DEB in patients in vivo. Thus far, glycosylation substitutions and small in-frame deletions accounted for the molecular background of dominant DEB and were associated with a broad spectrum of phenotypes with variable penetrance, ranging from solely nail dystrophy or milia to generalized skin and mucosal involvement (Almaani et al., 2011; Toyonaga et al., 2015; Varki et al., 2007). Therefore, it was surprising to find that loss of approximately 32–45% of the triple-helical region, including the hinge, allows C7 secretion and residual function and is clinically translated into a phenotype comprising nail loss and involvement of acral regions. Although large deletions can now be studied by various next-generation sequencing methods (Reuter et al., 2016), these technologies are usually not used to find COL7A1 mutations. Both cases illustrate the odyssey of patients with orphan diseases as they obtain a correct diagnosis and molecular classification of their disorders. Although DEB was a clear clinical diagnosis, the dilemma of the inheritance pattern could not be resolved without finding the disease-causing mutations.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
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REFERENCES
TO THE EDITOR

Seborrhoeic dermatitis is a chronic inflammatory skin disease with a complex etiology. The genetic predisposition for seborrhoeic dermatitis has not been studied, resulting in the absence of candidate genes involved in the pathogenesis of this condition. However, seborrhoeic dermatitis shares several clinical features with other chronic inflammatory skin diseases, in particular with psoriasis (PSO) (sometimes coined sebopsoriasis) and atopic dermatitis (AD), which are much better characterized genetically (Paternoster et al., 2010) (see Supplementary Materials). To discover new loci for seborrhoeic dermatitis, we conducted a GWAS for each cohort using the same model as for the SNP-based approach. The GWASs of the three separate cohorts were implemented in the ProbABEL package (Aulchenko et al., 2010), and the meta-analysis was carried out using metal (Willer et al., 2010) (see Supplementary Materials).

In total, 4,050 participants were available for analysis, of whom 609 (15%) had seborrhoeic dermatitis (see Supplementary Table S1 online). The SNP-based and gene-based CGAs did not yield any significant locus for seborrhoeic dermatitis after correcting for multiple testing (see Supplementary Tables S2–S5 online), although some of the findings suggested an overlap between seborrhoeic dermatitis and PSO and AD. For example, the LCE3 gene cluster, known to play a role in PSO and AD, showed suggestive associations with seborrhoeic dermatitis in the data of both the SNP-based CGA and the gene-based CGA (P = 0.0157 and P = 0.00869, respectively). MICB showed suggestive evidence in the gene-based CGA (P = 0.0063), and IL12B showed suggestive evidence in

Abbreviations: AD, atopic dermatitis; CGA, candidate-gene approach; GWAS, genome-wide association study; PSO, psoriasis; RS, Rotterdam Study; SNP, single-nucleotide polymorphism

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