Cold cases in epidermolysis bullosa: not the usual suspects
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Epidermolysis bullosa simplex caused by distal truncation of BPAG1-e: an intermediate generalized phenotype with prurigo papules

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Basal epidermolysis bullosa simplex (EBS) represents a heterogeneous group of hereditary mechanobullous diseases characterized by an intraepidermal cleavage plane. The majority of basal EBS cases (75%) arise from mutations in KRT5 or KRT14. Rare subtypes result from mutations in PLEC, COL17A1, ITGB4, EXPH5, KLHL24 or DST. DST (dystonin) encodes, among other tissue isoforms, the epithelial isoform of bullous pemphigoid antigen 1 (BPAG1-e). To date, only a few pathogenic DST mutations have been reported involving the epidermal isoform (Figure 1a), and not the muscle and nerve isoforms. They were homozygous nonsense mutations leading to absence of the BPAG1-e protein. The clinical phenotype manifested as non-pruritic generalized skin fragility and mild, predominantly, acral skin blistering. Herein, we report a homozygous nonsense mutation in the epidermal isoform of DST, resulting in a truncated BPAG1-e protein and an intermediate generalized phenotype with blisters and remarkably prurigo papules.

The index patient is a 39-year-old Syrian man born from a consanguineous union (Figure 1b). Clinical history included generalized skin fragility and blistering since his earliest childhood recollections. Tense blisters arose predominantly on his feet, legs, and trunk upon mechanical trauma and increased skin temperature. Skin defects often healed with post inflammatory hyperpigmentation, but without scarring or milia formation. In addition, he experienced severe generalized pruritus and developed prurigo papules. Hair, nails and mucous membranes were unaffected. An older sister had similar clinical features with blisters and prurigo papules, she was, however, not available for consultation or further testing; no other family members were affected.

Given the context of severe prurigo and tense blisters it was important to exclude the possibility of a concomitant autoimmune bullous disease, lichen planus or eczema. Immune- and histopathological studies on skin and serum excluded the above mentioned differential diagnosis and fitted with prurigo in epidermolysis bullosa (Figure 2 e-i). Direct immunofluorescence (DIF) showed no depositions of immunoglobulins IgA, IgG and/or C3c in patient’s skin. Also, no circulating autoantibodies were found by Western blot (both BPAG1-e, BPAG2), ELISA (NC16A domain), and indirect immunofluorescence (IIF) studies using both human salt-split-skin and monkey oesophagus. To identify the underlying genetic mutation, we applied our diagnostic next generation sequencing gene panel test consisting of 33 genes associated with or mimicking EB. The test is based on targeted SureSelect enrichment (Agilent Technologies Inc) and subsequent sequencing on a MiSeq sequencer (Illumina Inc). A novel homozygous nonsense mutation c.6559 C>T, p. Gln2187* was identified in exon 24 of the epidermal isoform of DST (GenBank NM_ 001723.5) and confirmed by Sanger sequencing (Figure 1a, c).
This mutation was not found in the Genome of the Netherlands, 1000 genomes (http://www.internationalgenome.org/1000-genomes-browsers/), or the ExAc Browser databases (http://exac.broadinstitute.org/).

Immunofluorescence staining with monoclonal antibody (mAb) R815 against the rod domain of BPAG1-e (gift Dr K. Owaribe) showed reduced expression at the epidermal basement membrane zone (EBMZ) compared to control (Figure 1e). Staining with mAb 279 (Cosmo Bio, Japan) against the C-terminus of BPAG1-e was negative in patient’s skin compared to control. Staining with 10F6 (Santa Cruz Biotechnology) against plectin showed increased expression at the EBMZ. Expression of integrin α6 and β4 subunits, type XVII collagen, laminin-332, and type VII collagen was normal (not shown). Immunoblot staining with mAb 1B10 (US Biologicals) against the N-terminus of BPAG1-e showed a truncated BPAG1-e product of an estimated 179 kDa weight in patient’s cells, consistent with the expected C-terminus truncation (Figure 1d). Quantification of BPAG1-e showed a decreased expression (~65%) in patient keratinocytes, compared with healthy control (methodology described by Gostyńska et al. (2015)). Transmission electron microscopy revealed blisters in the basal keratinocytes in close proximity to EBMZ (Figure 1f). Although the number of hemidesmosomes (HDs) was normal, their morphology was altered. The HD inner plaque was absent, resulting in reduced insertion of the extended intermediate filaments (IFs) into the HDs. The HD outer plaque was present but exhibited a ‘blurred’ (not sharply defined) aspect. The sub-basal dense plaque and other EBMZ components were normal. The medical ethical committee of the University Medical Center Groningen, the Netherlands, gave approval for studies on human material initially obtained for diagnostic means. Studies were performed according to the Declaration of Helsinki Principles. Written informed consent for publication and use of his photograph was obtained from the patient.

EBS resulting from DST mutations is rare. The homozygous nonsense mutation (c.6559 C>T, p. Gln2187*) is located within the last exon 24; the two previously reported mutations are within exon 23 (Figure 1a). The mutation is not present in the other tissue isoforms of DST, expressed in muscle and nerve tissue. Given the last exon location, we expect this mutation not to activate the nonsense-mediated mRNA decay mechanism. The consequence would be a C-terminus truncation of the BPAG1-e protein, which was confirmed by the immunoblot results (Figure 1d). The C-terminus binds specifically to IFs, and in conjunction with plectin, tethers them to hemidesmosomes. The mutation disclosed here is, thus, expected to critically affect BPAG1-e’s ability to bind IF proteins. Interestingly, the staining of plectin was brighter along the EMBZ. This resulted from an increased plectin expression which was quantified in keratinocytes to be 250% of control in a Western blot. This phenomenon
might constitute an upregulation of plectin 1a in basal cells resulting from a loss of C-terminus BPAG1-e functionality due to truncation.

Why our patient has an intermediate generalized phenotype with prurigo papules, in contrast to previous reports, is unknown. Considering the long disease history, a sister with, reportedly, similar clinical manifestation, exclusion of atopic constitution; normal IgE levels, eosinophils number, kidney function, liver enzyme assays, and good general health, we suspect EB as aetiology for pruritus. It is intriguing, however, that the C-terminus domain of BPAG1-e contains epitopes known to be involved in the pathophysiology of bullous pemphigoid (BP), a disorder inherently associated with pruritus. Rico et al. (1990) demonstrated an immunodominant locus against which most reactivity is seen in BP patients and suggested that this region may be relevant in the generation of an immune response (Figure 1a). This raises the question whether exposure to a C-terminally truncated BPAG1-e molecule might promote an inflammatory response against remaining epitopes and elicit pruritus in the host.

Of note, elevated levels of proinflammatory cytokines have been described in association with dystrophic and simplex EB; the authors suggested that EB might be considered a systemic inflammatory disorder rather than a skin-limited disease. Intense pruritus may, thus, be seen in the setting of mutations in other EB genes. Recently, upregulation of the TSLP protein, an IL-7 like cytokine associated with pruritus, has been noted in mice with KRT5/14 mutations. These data suggest the query whether disrupted keratin binding of BPAG1-e might also lead to upregulation of TSLP and, thus, pruritus in our patient.

In summary, we report a patient with EBS caused by a homozygous DST mutation that truncates the C-terminus of BPAG1-e. This represents, to our knowledge, a previously unreported intermediate generalized phenotype with prurigo papules associated with a DST mutation, underscoring a role for BPAG1-e pathology in skin integrity and, potentially, pruritus aetiology.

CONFLICT OF INTEREST
Authors state no conflict of interest.

ACKNOWLEDGMENTS
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**Figures**

(a) The previously reported DST mutations are indicated above the schematic protein structure with grey arrows (thin grey arrow mutations were considered as probably not decisive for the phenotype). The homozygous p. Gln2187* mutation in our patient is located in the intermediate filament binding domain (IFBD) and indicated with a red

![Figure 1. Schematic representation of the BPAG1-e protein, patient’s pedigree, mutation analysis and laboratory analysis of patient’s skin.](image-url)

(a) The previously reported DST mutations are indicated above the schematic protein structure with grey arrows (thin grey arrow mutations were considered as probably not decisive for the phenotype). The homozygous p. Gln2187* mutation in our patient is located in the intermediate filament binding domain (IFBD) and indicated with a red
arrow. (b) Pedigree of the family, affected index patient (arrow) and his reportedly affected sister indicated in black square/circle. (c) Sequence chromatogram showing the homozygous c.6559 C>T substitution resulting in a premature stop codon in the patient (Pt; Wt, wild-type). (d) Immunoblot staining with the monoclonal antibody 1B10 targeting the N-terminus of BPAG1-e demonstrates a reduced amount (~65%) of truncated protein product in patient’s cultured keratinocytes; its estimated molecular weight is 179 kDa, versus 230 kDa for the wild-type (Wt) BPAG1-e. Immunoblot staining with the monoclonal antibody 10F6 against plectin shows an increased expression (~250%) in patient’s cultured keratinocytes compared to control. (e) Immunofluorescence with the antibodies R815 (BPAG1-e rod domain) and 279 (BPAG1-e C-terminus) showed reduced and absent expression, respectively, in patient’s skin compared to control; expression of plectin with the 10F6 antibody (rod domain) was increased at the site of the epidermal basement membrane zone, but reduced in the basal epidermal layer in patient’s skin compared to control; note several microblisters in the basal layer of the epidermis (asterisks). Bar 50 μm. (f) Transmission electron microscopy showed cleavage in the basal keratinocyte; note remains of the plasma membrane (PM) on the blister floor and lack of insertion of the extended intermediate filaments (IFs) (upper image, bar = 1μm). Higher magnification shows hemidesmosomes (HDs) which lack inner plaque. HD outer plaque (HD op) had a ‘blurred’ aspect. Bar 200 nm.
Figure 2. Clinical and immunopathological findings.
(a) On the legs, markedly around the ankles, blisters and erosions with hemorrhagic crusts, lesions healed with hyperpigmentation; inset: detail of prurigo papules (b) Conglomerates of lesions on patient’s trunk. (c) Detail of tense blisters and residual lesions with desquamation on the lower leg. (d) Serous and hemorrhagic blisters on patient’s sole and toes. (e-i) Hematoxylin, eosin (scale bar = 50 μm) and immunohistochemical (scale bar = 100 μm) staining of a prurigo papule revealed epidermal acanthosis, orthohyperkeratosis, and hypergranulosis. In the superficial dermis a slight increase in small vessels is present surrounded by an infiltrate consisting of predominantly T lymphocytes (CD3) with some B lymphocytes (CD20), plasma cells (CD138), and mast cells (tryptase staining).
References


