

Homozygous Nonsense Mutation and Additional Deletion of an Amino Acid in BPAG1e Causing Mild Localized Epidermolysis Bullosa Simplex

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The bullous pemphigoid antigen 1 (BPAG1) is a cytoskeletal linker protein connecting intermediate filaments to the cell membrane. Alternative splicing gives rise to multiple tissue isoforms with variable expression in the skin, nervous system and muscles, and a high functional complexity, which is poorly understood (1, 2). The biological relevance of BPAG1 isoforms is underscored by their association with human genetic and acquired disorders (3).

The 230-kDa epidermal isoform, BPAG1e consists of a coiled-coil rod domain flanked by globular NH₂-terminal head and COOH-terminal tail domains and was first identified as the autoantigen in bullous pemphigoid (1, 4). Recently 2 homozygous nonsense mutations, p.Gln1124* and p.Arg1249* in the BPAG1 gene, dystonin (*DST*) were reported in patients with a new subtype of autosomal recessive epidermolysis bullosa (EB) simplex (5–7). Both mutations are located within the coiled-coil rod domain of BPAG1e, which is involved in homodimerization. The amino terminus is important for the recruitment of BPAG1e into hemidesmosomes, while the C-terminus binds to keratin intermediate filaments (8). Loss of BPAG1e has been shown result in lack of hemidesmosomal inner plaques in the skin (5, 6).

We describe here a patient with very mild skin fragility associated with a previously unreported homozygous nonsense mutation and homozygous deletion of an amino acid in the coiled-coil rod domain of BPAG1e, and reveal the consequences of these mutations in the skin and epidermal keratinocytes.

MATERIALS AND METHODS (see Appendix S1¹)

CASE REPORT

The index case was a man who was first examined at the age of 19 years, when no blisters, but only plantar keratoderma was noted (Fig. 1A). He reported having had recurrent skin blistering since childhood, mainly following sporting activities, but was otherwise healthy. His parents were related and originated from Turkey. His father reported that he and his sister had skin blistering in childhood, which alleviated after puberty. Initially, we suspected autosomal dominant localized EB simplex, but mutations in the keratin 5 and 14 genes were excluded. After being involved in a fight, the patient presented again, with blisters on the hands (Fig. 1B), lower legs and feet, and a skin biopsy was obtained from a

fresh blister. Skin cleavage was noted in the basal epidermal layer, and immunoreactivity for BPAG1e was negative in both blistered and not blistered skin (Fig. 1E), indicating that skin fragility was due to loss of this protein. Hence, sequencing of *DST* identified an homozygous in-frame deletion in exon 17, c.2618_2620delAAG, which leads to deletion of a glutamic acid residue, p.Glu873del, and a homozygous transversion in exon 23, c.3805C>T, that converts glutamine to a premature termination codon, designated p.Gln1269* (Fig. 1C and D). The first variant is referenced in databases (rs770713340) with a minor allele count of 0.00003/4, while the latter has not been reported previously (ExAC, dbSNP, HGMD professional).

Next, we examined the consequences of these mutations in keratinocytes isolated from the patient's skin. Morphologically the patient's keratinocytes demonstrated good spreading capacity (Fig. S1A¹). The mutation did not lead to complete mRNA decay (not shown), but BPAG1e was not detectable in keratinocytes when using an antibody directed to the N-terminus (Fig. S1A¹). The mRNA levels of collagen XVII were reduced and those of focal adhesion components were upregulated (not shown). In cultured keratinocytes, immunoreactivity for integrin $\alpha 6$ was reduced and collagen XVII was reduced at the periphery of the cells. Immunoblot analysis revealed that full-length collagen XVII was increased (3.21-fold) in cell lysates, while the ectodomain was decreased (0.61-fold) in cell culture media. Integrin $\beta 1$ and fibronectin were enhanced (Fig. S1¹). In line with this, immunoblot analysis demonstrated an increase in vinculin (2.21-fold), kindlin-1 (2.22-fold) and 2 (2.44-fold) and fibronectin in the keratinocytes of the patient compared with the control cells (Fig. S1B¹). Moreover, the immunoreactivity, the protein and mRNA levels of keratin 5, 14 and 15 appeared to be increased in the keratinocytes of the patient compared with the control (Fig. S1¹). These results suggest that lack of BPAG1e impacts the interconnected molecular complexes, hemidesmosomes, focal adhesions and keratin intermediate filaments.

DISCUSSION

This study extends the spectrum of *DST* mutations associated with autosomal recessive EB. We found an interesting constellation of 2 homozygous variants, a nonsense mutation and an amino acid deletion, of which the first is most probably decisive. Like the other mutations reported so far, both variants are located in the coiled-coil domain, which is not present in other major isoforms of BPAG1. The father of our index case, who is an obligate heterozygous carrier, reported having had skin blistering, raising the question of a semi-dominant inheritance. However, family members were not available for genetic testing and clinical examination. The phenotype in our patient was quite unremarkable and clinically indistinguishable from localized EB with keratin 5/14 mutations. Because of different inheritance

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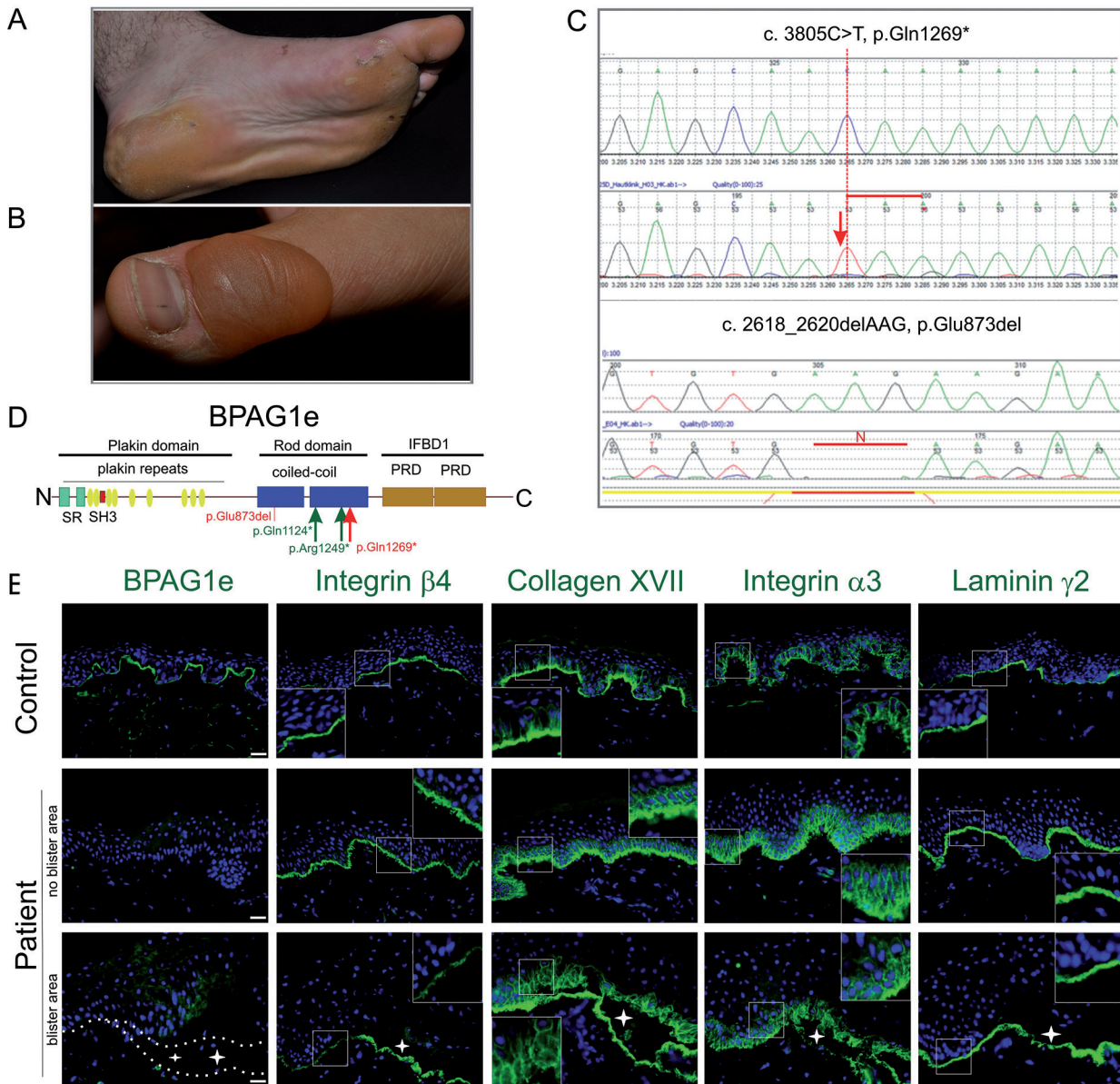


Fig. 1. Clinical phenotype and molecular findings. (A) Plantar keratoderma, (B) skin blisters on mechanically stressed areas. (C) Sanger sequencing reveals a homozygous nonsense mutation and deletion in the *DST* gene coding for BPAG1e. (D) Schematic illustration of BPAG1e shows that mutations reported so far are all in the coiled-coil domain (<https://www.nextprot.org/>). *Green arrows* indicate known mutations and *red arrows* indicate mutations in the current study. (E) Immunofluorescence staining reveals that BPAG1e is completely undetectable in the not blistered and blistered area of the patient's skin (Patient) in contrast to the linear labelling at the dermal-epidermal junction in normal skin (Control). Intensities of the staining for collagen XVII, integrin $\alpha 3$ and laminin $\gamma 2$ are slightly increased in the patient compared with the control. The insets show 2-fold magnifications of the marked areas. Scale bars=50 μm . Blister floor and roof are indicated by *discontinuous lines* and blisters are indicated by *asterisks*. Nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI).

patterns of these entities, molecular testing is crucial for diagnosis and counselling.

In keratinocytes, depletion of BPAG1e resulted in subtle defects in cell polarity and no apparent changes in migration speed or adhesion (12). However, human keratinocytes, carrying homozygous nonsense *DST* mutations exhibited reduced adhesion, but increased spreading and migration, as well as abnormal protein levels of integrins $\beta 1$ and $\beta 4$, and keratin 14 (13). Our results support and extend these findings, showing increased levels of $\beta 1$ integrin associated focal adhesion

proteins vinculin, kindlin-1 and -2 and strong increases in keratin 5 and 15. The increase in full-length collagen XVII in cell lysates and decrease in the shed ectodomain in media may reflect decreased incorporation of collagen XVII into the membrane and accumulation in the cells, which may result in downregulation of its mRNA levels.

The apparent discrepancies between the results observed in skin sections and cells may be explained by at least 2 arguments. First, precise quantification of the staining intensity was not performed in skin, mainly because of the limited amount of sample available.

For abundant proteins, such as keratins, an increase is difficult to appreciate with this method. Secondly, cultured keratinocytes do not assemble bona fide hemidesmosomes, although hemidesmosome protein clusters (stable anchoring contacts) are found along the substrate-attached surface of the cells (14). The faster turnover and increased dynamic of these structures in cells compared with tissue may unravel discrete differences.

It remains astonishing that complete loss of this major component of the inner plaques of the hemidesmosomes leads only to mild skin fragility. The present findings suggest a possible role of the modulation of the focal adhesion proteins, in the context of the tight regulation dynamics of hemidesmosome complexes and focal adhesions in keratinocytes undergoing migration (14, 15). It is likely that the mild blistering associated with BPAG1e deficiency is also due to the keratin compensatory function, especially of keratin 15.

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