Various heritability in epidermolysis bullosa simplex caused by \textit{DST} mutations: a role for \textit{PLEC} as genetic modifier?

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**Importance**

Epidermolysis bullosa simplex (EBS) caused by DST mutations is a rare genodermatosis characterized by skin fragility and blistering. This gene encodes the epithelial isoform of dystonin, which constitutes a component of hemidesmosomes. To date, only four pathogenic DST mutations have been identified. The heritability was thought to be autosomal recessive, but the possibility of semi-dominant inheritance was considered because of anecdotal reports. Here we report a pedigree with a constellation of two novel DST and a PLEC mutations and propose a different way of viewing disease heritability associated with DST mutations.

**Observations**

The index patient and her three sons were affected by EBS, albeit one had blisters for a transitory period. DNA analysis revealed a novel homozygous nonsense mutation (c.4978delG; p.Val1660*) in exon 23 of DST in the index patient and heterozygous status for this mutation in her sons. Surprisingly, her middle son also had a heterozygous nonsense DST mutation (c.6298C>T; p.Gln2100*) on the other allele. Additionally, a heterozygous nonsense mutation (c.6292C>T p.Gln2098*) in exon 31 of the PLEC gene was identified in the index patient and two sons with recurrent blisters.

**Conclusions and relevance**

Our study illustrates an EB pedigree in which variable disease heritability for DST mutations was illustrated. These findings imply that EBS caused by DST mutations is a dominantly inherited condition of reduced penetrance; PLEC haploinsufficiency may be a genetic determinant for penetrance.
Epidermolysis bullosa simplex (EBS) is a heterogeneous group of intraepidermal blistering genodermatoses. Recently, several mutations in DST were reported in the literature (Figure 1A) 1-6. DST encodes, among other isoforms, the epithelial dystonin, also known as 230-kDa bullous pemphigoid antigen or BPAG1-e. This molecule is a structural component of the hemidesmosomal inner plaque and together with plectin provides anchorage of the keratin filaments to the hemidesmosomes. The clinical phenotype of the individuals reported in the literature manifests as generalized skin fragility and mild, predominantly acral skin blistering. A recently published case had an intermediate generalized phenotype with prurigo papules (Turcan et al, 2017). Joutel et al. described a patient that had additional neurological complaints such as: muscle weakness, headaches, numbness and collapse. It was, however, not possible to establish if these clinical symptoms were caused by the DST mutation given that the patient also had NOTCH3 gene pathology, a disorder associated with cerebral small-vessel arteriopathy (CADASIL syndrome, MIM125310) 7. Herein, we report a pedigree with a surprising combination of two novel nonsense DST mutations and a heterozygous PLEC mutation. This study provides a pathogenic insight into DST heritability.

Case report

The index patient was a 36-year-old female born from a non-consanguineous union (II:2, Figure 1B). Skin blistering began in the first year of life at sites of pressure or mechanical trauma. The sides and dorsae of her feet were the most commonly affected areas, followed by her wrists and lower legs (Figure 2A). Blisters healed without scaring, but sometimes with mild post-inflammatory hyperpigmentation. She also reported a lifelong history of thin and brittle toenails (Figure 2B). Hair, teeth and mucosal membranes were not affected. Her medical record revealed cardiac arrhythmia for which she repeatedly received a cardiac ablation. The youngest two sons (six (III:2) and five (III:3) years of age, respectively) developed recurrent skin blisters on their feet, lowers legs, buttocks area, and hands, occasionally up to several centimeters in diameter. The middle child (III:2) had the most severe skin phenotype with pruritus (Figure 2C), brittleness and dystrophy of his nails, most remarkably on his fingers (Figure 2D), and limited extent of failure to thrive. He had several episodes of fainting, which were ascribed to hypoglycemia of unknown origin. Detailed cardiologic and neurologic evaluation did not identify an underlying pathology. The eldest son (eight years of age (III:1)) had only a transient period of acral skin blistering in his early childhood years. Other family members had, reportedly, no recollection of (transient) skin blistering. The index patient and her sons suffered from recurrent herpes simplex infections on various locations, of which a relation to the EBS phenotype is uncertain.
Genetic analysis on the index patient’s DNA was performed through our diagnostic next generation sequencing gene panel test consisting of a comprehensive set of 33 genes associated with or mimicking EB. The test is based on targeted SureSelect enrichment (Agilent Technologies Inc., Santa Clara, CA USA) and subsequent sequencing on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA). We identified a homozygous nonsense mutation c. 4978delG (p.Val1660*); GenBank NM_001723.4 in exon 23 of DST-e (red arrow, Figure 1A). The mutation is located within the coiled-coil rod domain of BPAG1-e distally to previous reports. Sanger sequencing of genomic DNA confirmed the presence of this mutation in the index patient and the heterozygous status in all three sons (Figure 1B-C).

This mutation was not found in the Genome of the Netherlands Consortium 2014 \(^8\), 1000 genomes \(\text{http://www.internationalgenome.org/1000-genomes-browsers/}\), or the ExAc Browser databases \(\text{http://exac.broadinstitute.org/}\) and to our knowledge was not earlier described in the literature. In addition, we identified a heterozygous nonsense mutation c.6292C>T, p.Gln2098* in exon 31 of PLEC gene in the index patient and her youngest two sons (Figure 1B).

Immunofluorescence microscopy of index patient’s non-lesional skin with mAb R815 against the rod domain of BPAG1-e (gift Dr K. Owaribe) showed a normal expression at the EBMZ compared to control (Figure 1C). Staining with mAb 279 (Cosmo Bio, Japan) against the C-terminus of BPAG1-e was negative in index patient’s skin compared to control. Due to the phenotypic severity of the middle son, immunofluorescence studies were performed on his skin biopsy. Staining with mAb R815 showed slight reduction in expression, whereas staining with mAb 279 showed no expression. Subsequent immunoblot staining with mAb 1D2 against the N-terminus of BPAG1-e showed a truncated BPAG1-e product of an estimated 144 kDa weight (red strip, Figure 1D) in index patient’s cells (II:2), and two products of 144 kDa and 160 kDa (blue strip) in the middle son (III:2). Immunoblot staining with mAb 279 against the C-terminus of BPAG1-e showed no expression in both patients (Figure 1D). The application of our diagnostic next generation sequencing gene panel test in the middle son revealed an additional heterozygous DST nonsense mutation (c.6292C>T; p. Gln2100*) (thin blue arrow to indicate heterozygosity, Figure 1A). His brothers did not carry this mutation (Figure 1B), but his unaffected father was shown to carry this mutation heterozygously. Staining with mAb HD121 against the plectin’s rod domain (Dr K. Owaribe) in the index patient’s skin and middle son showed a normal expression along the EBMZ, while the faint panepidermal expression was lost compared to control (Figure 1C). Staining of integrin α6 and β4 subunits, type XVII collagen, as well as laminin-332 and type VII collagen was not altered (not shown).
Western blot with mAb HD121 showed no truncated plectin in patients II:2 and III:2 compared to control. The expression of plectin in cultured keratinocytes’ extract was, however, reduced in both patients (Figure 1D).

Transmission electron microscopy studies in the index patient’s skin noted hypoplastic hemidesmosomes with absent or inadequately formed inner plaques (Figure 1E, lower panel); additional findings were: reduced insertion of keratin filaments and very low basal cleavage with fragments of plasma membrane adhering to the basement membrane (Figure 1E, upper panel). Other components of the dermoeipidermal junction were normal.

**Discussion**

The current study provides interesting insight into the heritability of EBS due to \( DST \) mutations. The primary observation was that there is variation in the severity of the skin phenotype depending on the biallelic or heterozygous status for a particular pathogenic \( DST \) mutation. In previous pedigrees, a Kuwaiti father who was heterozygous for the nonsense mutation p.Gln1124* in \( DST \) had transient blistering in childhood. In an Iranian pedigree, two children and their grandfather had mild skin blistering, less severe than the mother (index patient) who was homozygous for the p.Arg1249* mutation in \( DST \). Dystrophy of all toenails has been reported only in one patient in association with EBS resulting from \( DST \) mutations. In our family, the index patient and her two youngest sons had different degrees of brittleness of their toenails and the middle child also showed prominent fingernail dystrophy. The cardiac pathology in the mother and the fainting episodes in her middle son were thought not to be related to their skin disorder. Noteworthy, in a previously described consanguineous Kuwaiti family, one individual had both skin fragility and dilated cardiomyopathy; an older sibling also had dilated cardiomyopathy (resulting in heart transplantation), but no skin fragility. The authors concluded the skin and heart abnormalities to be separate disorders. Given the heterogeneity in heritability in skin, a pathogenic role for \( DST \) mutations in cardiac tissue deserves further investigation.

The \( DST \) gene, through alternative splicing, encodes three major isoforms that perform complex functions in different organs, such as the epidermis (BPAG1-e), skeletal and cardiac muscle (BPAG1-b), and nervous system (BPAG1-a). A fourth neuronal isoform (BPAG1-n) may also exist; its expression in vivo is, however, not definite. The nonsense mutation c.4978delG (p.Val1660*) is located in the region of the \( DST \) gene that encodes the coiled-coil domain, which is exclusively expressed in the BPAG1-e and BPAG1-n isoforms. Thus, the mutation is expected to disrupt BPAG1-n. The clinical implication of this is uncertain since Leung et al showed no significant BPAG1-n mRNA
expression in brain and heart tissue. In the brain, trace amounts were detected only after an increased number of PCR cycles.

The immunoblot studies confirmed presence of truncated protein. This was confirmed by positive staining with mAb R815 against the coiled-coil rod domain and negative staining with mAb 279 against the C-terminus. The protein products were of the expected size, although reduced in expression. This reduction could possibly be due to nonsense mediated RNA decay, or that the protein formed is less stable and thus faster degraded. The compound heterozygous status for the DST mutation in the middle son and the absence of the second heterozygous DST mutation (c.6298C>T; p.Gln2100*) in the other brother explains the variation in the severity of the clinical phenotype. The location of this mutation was outside the coiled-coil rod domain of BPAG1-e. In our recent article (Turcan et al, 2017) mutations at the C-terminal outside the coiled-coil domain were seen in association with pruritus, a feature also noted in the middle son in this study.

The heterozygous mutation c.6292C>T, p.Gln2098* in exon 31 of PLEC gene found in the index patient and her two sons seems to segregate with phenotype severity. PLEC encodes plectin, a versatile linker protein that has several isoforms expressed in various tissues including skin, muscle, heart, and nervous tissue, of which plectin 1a is expressed along the EBMZ and plectin 1c dominantly in the epidermis. Mutations in PLEC cause recessive subtypes of EBS: EBS skin only, EBS with muscular dystrophy (EBS-MD) and EBS with pylorus atresia (EBS-PA), and one autosomal dominant subtype, called EBS-Ogna, resulting from a point mutation in the rod domain of plectin. Homozygous truncation mutations in exon 31 of PLEC, which encodes the rod domain of plectin, are known to cause EBS-MD. In relation to our study, the question arises whether the change in the amount of plectin might have a modifying role in conjunction with the DST mutation. Notably, the immunoblot staining against the rod domain of plectin was reduced in both the index patient and the middle son (Figures 1C and 1D). Generally, heterozygous carriers of truncating PLEC mutations were reported as being healthy. However, the possibility that mild phenotypes might have been overlooked, is not excluded. In vitro studies have established that presence of the plectin rod domain highly improved hemidesmosome stability. We speculate that a reduced amount of plectin with rod domain in conjunction with truncated BPAG1-e protein products may further impair hemidesmosomal inner dense plaque formation and keratin anchorage and thus aggravate the phenotype. Taken together with the phenotype segregation, we hypothesize that PLEC may function as a modifier gene for DST.
Conclusions:

In summary, we report two novel nonsense DST mutations responsible for basal EBS in a pedigree consisting of four affected individuals, all expressing a different combination of DST/PLEC mutations. The index patient exhibited a classic biallelic DST involvement and PLEC heterozygosity, the oldest son only a monoallelic DST involvement (phenotype with transient blisters), the middle son biallelic DST dysfunction (compound heterozygote) and PLEC heterozygosity, the youngest son monoallelic DST dysfunction and PLEC heterozygosity (mild, but permanent phenotype). The current study clinically shows various disease heritability for EBS caused by DST mutations and expands the phenotypic spectrum including hand nail dystrophy as a potential clinical feature. PLEC haploinsufficiency may have been the additional genetic factor necessary to reach the threshold level above which persistent disease phenotype is achieved.

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Figures

A. Schematic representation of the BPAG1-e protein, clinical pedigree, mutation analysis and laboratory studies on the index patient’s skin.

B. Genetic analysis was performed in individuals I:2, II:2, II:3, III:1, III:2, III:3; m/wt denotes mutation/wildtype, m/m bi-allelic mutation, wt/wt wildtype/wildtype and underscores the genotype of the particular family member; DST p.Val1660* (red), DST p.Gln2100* (blue), PLEC p.Gln2098* (green); black circle/square denotes most severely affected individual, dark grey denotes mild persistent phenotype, light grey represents mild transient phenotype.

C. Immunostaining of index patients’ skin against BPAG1-e shows normal expression with mAb R815 and absent staining with mAb 279 compared to control; staining for the rod domain of plectin with mAb HD121 shows normal expression at the epidermal basement membrane zone and absent staining panepidermal in both patients’ skin compared to control.

D. Immunoblot with the monoclonal antibody 1D2 targeting the N-terminus of BPAG1-e demonstrates truncated protein products in patients’ cultured keratinocytes; their estimated molecular weight is 144 kDa and 160 kDa, versus 230 kDa for the wild-type (Wt) BPAG1-e. Immunoblot with the monoclonal antibody HD121 against plectin shows a reduced expression in both patients’ cultured keratinocytes compared to control.

E. Transmission electron microscopy in index patient’s skin reveals a very low intraepidermal cleavage plane (*, asterisk) with remnants of plasma membrane on the blister floor (black arrow). Higher magnification shows hemidesmosomes with absent inner plaques.
Figure 1. Schematic representation of the BPAG1-e protein, clinical pedigree, mutation analysis and laboratory studies on the index patient’s skin.

A, All reported DST mutations to date are indicated with black arrows (grey arrow mutations were deemed as not pathogenic by the authors of the respective study); the homozygous nonsense mutation p.Val1660* in this report is indicated by the red arrow. The heterozygous nonsense mutation p.Gln2100* is indicated by the thin blue arrow. B, Genetic analysis was performed in individuals I:2, II:2, II:3, III:1, III:2, III:3; m/wt denotes mutation/wildtype, m/m bi-allelic mutation, wt/wt wildtype/wildtype and underscores the genotype of the particular family member; DST p.Val1660* (red), DST p.Gln2100* (blue), PLEC p.Gln2098* (green); black circle/square denotes most severely affected individuals, dark grey denotes mild persistent phenotype, light grey represents mild transient phenotype. C, Immunostaining of index patients’ skin against BPAG1-e shows normal expression with mAb R815 and absent staining with mAb 279 compared to control; staining for the rod domain of plectin with mAb HD121 shows normal expression at the epidermal basement membrane zone and absent staining panepidermal in both patients’ skin compared to control. D, Immunoblot with the monoclonal antibody 1D2 targeting the N-terminus of BPAG1-e demonstrates truncated protein products in patients’ cultured keratinocytes; their estimated molecular weight is 144 kDa and 160 kDa, versus 230 kDa for the wild-type (Wt) BPAG1-e. Immunoblot with the monoclonal antibody HD121 against plectin shows a reduced expression in both patients’ cultured keratinocytes compared to control. E, Transmission electron microscopy in index patient’s skin reveals a very low intraepidermal cleavage plane (*, asterisk) with remnants of plasma membrane on the blister floor (black arrow). Higher magnification shows hemidesmosomes with absent inner plaques.
Figure 2. Clinical phenotype of the index patient and the middle son

A, Blisters, superficial erosions and crusting on the lower legs and ankles (II:2). B, Brittle and dystrophic toenails (II:2). C, Blisters, erosions and crusts on the knees, lower legs and feet (III:2). D, marked dystrophy of finger nails (III:2).
References


