A distinct cutaneous blistering phenotype with multi-system manifestations caused by a mutation in *CD151*, the 20th causative gene in epidermolysis bullosa

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Background

Basement membranes comprise of complex networks of interacting matrix macromolecules separating epithelia from the underlying mesenchymal tissues where they play physiologically important roles¹. Mutations in the cutaneous basement membrane zone genes can result in fragility of the skin, which as a result of minor trauma leads to blistering and erosions. The prototype of such heritable skin fragility disorders is epidermolysis bullosa (EB), currently known to be associated with mutations in as many as 19 distinct genes.

We have developed a next generation sequencing (NGS) panel covering 21 skin fragility-associated genes, including *CD151*, which was used to screen for pathogenic sequence variants in EB.

In this study, we report a patient with a homozygous splice site mutation in *CD151* resulting in deletion of exon 5 corresponding to a transmembrane domain, associated with multi-system involvement, including skin fragility with a Kindler syndrome-like phenotype.

Materials and Methods

Targeted next-generation sequencing

DNA from 92 probands with a clinical diagnosis of EB was extracted from blood, underwent target enrichment using TruSeq Custom Aplicon Kit (Illumina, Inc., San Diego, CA), and sequenced using a custom library containing 21 EB-related genes (*CD151*, *CDSN*, *CHST8*, *COL17A1*, *COL7A1*, *DSP*, *DST*, *EXPH5*, *FERMT1*, *ITGA3*, *ITGA6*, *ITGB4*, *JUP*, *KRT5*, *KRT14*, *LAMA3*, *LAMB3*, *LAMC2*, *PKP1*, *PLEC*, and *TGM5*) on a single MiSeq flow-cell (Illumina) for analysis.

RNA sequencing

Total RNA from whole skin was sequenced using the TruSeq Stranded Total RNA kit run on the Illumina NextSeq 500 platform.

Western Blot

Western blot of whole skin was performed using primary HPA011906 anti-CD151 (Sigma, MO, USA) and secondary antibody HPA011906 (Sigma Aldrich, MO, USA), along with beta-actin controls.

Immunofluorescence

Staining of skin samples using anti-CD151 (ab33315, Abcam, Cambridge, UK), and fluorescein secondary antibody (Invitrogen, Paisley, UK) was performed.

Transmission Electron Microscopy

Transmission electron microscopy was performed using previously reported techniques².

Results - Clinical Findings

A 33-year old proband from the panel of 92 patients with EB was referred for genetic diagnosis with an initial clinical diagnosis of Kindler syndrome.

Figure 1. Clinical features and pedigree of the proband.

Table 1. Clinical features of the proband

<u>Dermatologic</u>	<u>Gastrointestinal</u>
Blistering	Esophageal webs
Poikiloderma	Reflux
Nail dystrophy	Malnutrition
Alopecia	Constipation
<u>Renal</u>	<u>Genitourinary</u>
Nephropathy	Urinary incontinence
<u>Ocular</u>	<u>Oral</u>
Ectropion	Microstomia
Lacrimal duct stenosis	Edentulous

Results - Genetic findings

Targeted-next generation sequencing identified a homozygous donor splice site mutation in *CD151* (c.351+2T>C).

Figure 2. The splice site mutation was identified using genomic DNA in the NGS panel (a). RNA-seq showed skipping of exon 5 of *CD151* (b). Sanger sequencing of genomic and cDNA confirmed the mutation and exon 5 skipping (c).

Results - Protein and Ultrastructural Findings

This mutation was predicted to result in the loss of a transmembrane domain of CD151. The following studies were performed to determine the functional consequences of this mutation in patient's skin.

Figure 3. Structure of CD151 domains (a), immunostaining and Western blot identified no CD151 in patient's skin compared to control (b,c,d), and electron microscopy showed basal keratinocyte dysadhesion and intercellular disruption (e,f).

Discussion and Conclusion

As a consequence of a loss of functional CD151, compromised interactions between laminin 332 and both $\alpha3\beta1$ and $\alpha6\beta4$ integrin would result in perturbations in both skin and glomerular basement membranes. The form of EB in our patient is clinically consistent with these perturbations. Our patient has clinical similarities and several differences to three patients with renal disease and pretibial EB with a homozygous indel mutation in *CD151*³ which calls for identification of additional patients with CD151 deficiency to further elucidate genotype-phenotype correlations.

Our data provide strong justification for inclusion of *CD151* officially as the 20th gene in the classification of EB.

References

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