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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.02.762.

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Homozygous *ITGA3* Missense Mutation in Adults in a Family with Syndromic Epidermolysis Bullosa (ILNEB) without Pulmonary Involvement

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EDITOR

Epidermolysis bullosa (EB), the prototype of skin fragility disorders, manifests with blistering and erosions of the skin and mucous membranes (Has et al., 2020; Vahidnezhad et al., 2019a). The classic forms of EB are associated with 16 distinct genes expressed in the cutaneous basement membrane zone (Has et al., 2020). One such gene is *ITGA3* encoding α3 integrin subunit, which combines with β1 subunit to form α3β1 integrin. Mutations in the *ITGA3* gene have been reported in 10 cases with EB, and the characteristic feature in the majority of these patients is severe respiratory and renal involvement causing early

Abbreviation: EB, epidermolysis bullosa

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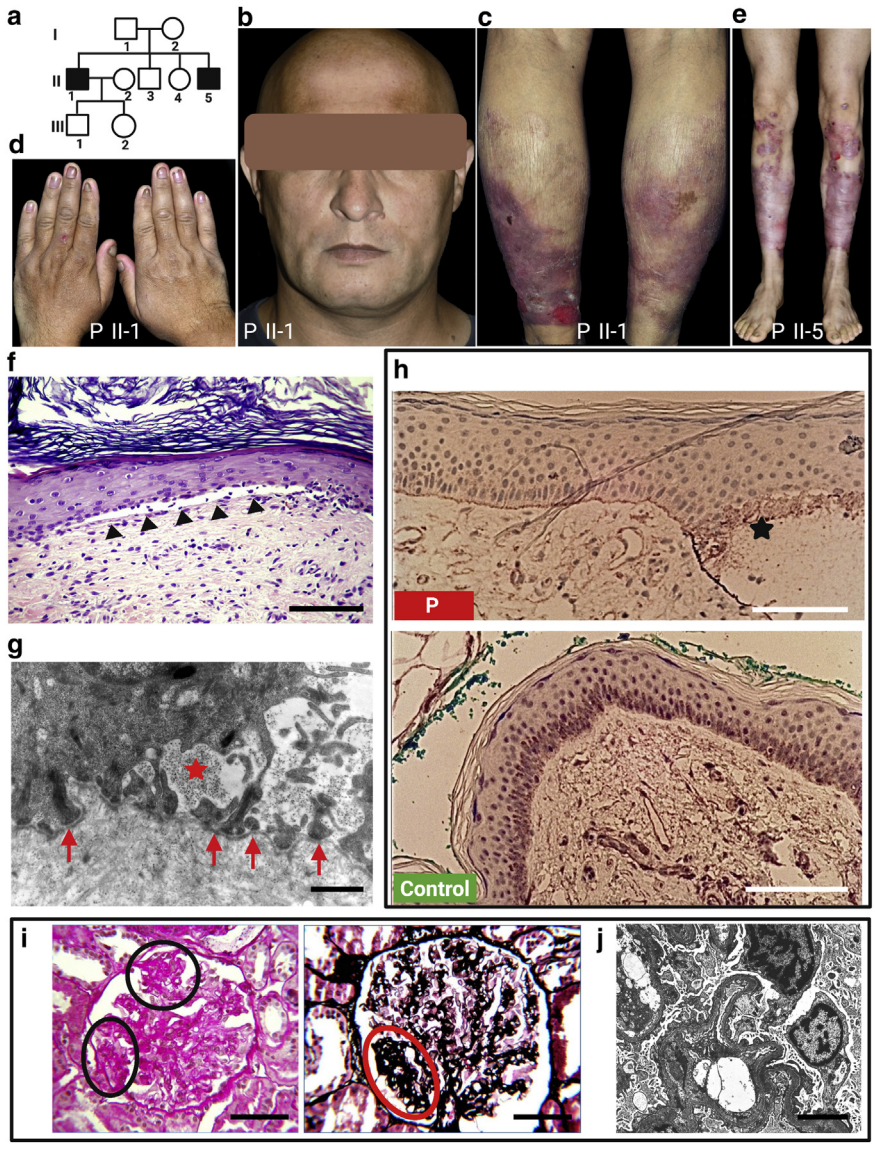

Figure 1. Family pedigree and clinical features of the patients with *ITGA3* mutations. (**a**) Note the two affected brothers (II-1 and II-5). (**b-d**) Clinical presentation of patient II-1, manifesting with blistering and erosions on the lower legs, loss of scalp hair and eyebrows, and dystrophic nails. (**e**) The younger patient (II-5) showed similar pretibial blisters and erosions with atrophic scars. (**f**) Histopathology of a skin biopsy revealed separation of the epidermis from the dermis at the level of cutaneous basement membrane (arrowheads, H&E stain). Bar = 100 μm. (**g**) Transmission electron microscopy showed blistering at the BMZ within the lamina lucida, and the lamina densa (arrows) is on the floor of the blister, consistent with the diagnosis of junctional EB. Bar = 1,000 nm. (**h**) Immunostaining of the patient's skin biopsy (upper panel; bar = 50 μm) revealed decreased and altered staining pattern for CD151, with linear granular staining along the dermal–epidermal junction, compared with that in the control skin (lower panel; bar = 75 μm) stained in parallel with the same antibody, which showed strong peripheral staining of the basal KCs at the basal layer of the epidermis. Note the blister in the patient's skin within the lamina lucida (asterisk). (**i**) Renal biopsy revealed focal segmental glomerulosclerosis with PAS staining (left panel) shows the segmental sclerosis of the glomerulus in circles. Bar = 50 μm. The glomerular sclerotic area stains positively with silver stain (right panel, Jones methenamine silver stain). (**j**) Transmission electron microscopy of renal biopsy showed the effacement of the visceral epithelial cell foot processes and microvillous transformation. Bar = 2,000 nm. The patients consented to the publication of their images. BMZ, basement membrane zone; EB, epidermolysis bullosa; KC, keratinocyte; P, patient; PAS, periodic acid–Schiff.

postnatal demise (Has et al., 2012). This subtype of EB is known as EB with renal and respiratory involvement or interstitial lung disease, nephrotic syndrome, and EB (Online Mendelian Inheritance in Man #614748). The pathogenic sequence variants are characteristically loss-of-function mutations, and EB with renal and respiratory involvement has been considered to be a severe lethal condition. In this study, we report two adult males aged 45 and 30 years with EB associated with a homozygous missense mutation in *ITGA3*. A written informed consent was obtained from both patients to participate in this study, and they gave their permission to the publication of their images.

The proband (Figure 1a, II-1) was a male aged 45 years with blisters and erosions that were reported from age 22 years on and limited to lower extremities (Figure 1c). He had dysmorphic facial features, distal onycholysis with nail dystrophy since birth, and loss of scalp hair and eyebrows since his teenage years, whereas eyelashes were present (Figure 1b and e). He had renal involvement with proteinuria (1.4–1.5 g/day) since he turned 26 years. The proband's younger brother (II-5), a male aged 30 years, had similar cutaneous lesions (Figure 1d), with bullae and erosions on the lower legs, which were reported from age 8 years on. The lesions healed with atrophic scars. He also has sparse hair, a loss of eyebrows since his teenage years, and distal onycholysis since birth. At age 7 years, he was diagnosed with hydronephrosis and pyeloureteral stenosis requiring surgery. He also had surgery for congenital nasolacrimal duct obstruction. Proteinuria (2.8–3.2 g/day) was documented at age 13 years leading to end-stage renal disease, and he was placed on hemodialysis at the age of 26 years. Renal biopsy documented focal segmental glomerulosclerosis, perihilar form (Figure 1i). Electron microscopy demonstrated that glomerular structures were partially retained and were hypertrophic, with villous podocytes. Effacement of the visceral epithelial cell foot processes and microvillous transformation as well as thickened glomerular basement membrane and swollen endothelial cells, which contained myelin-like structures, were also noted (Figure 1j).

Skin biopsy of the younger brother at age 17 years revealed evidence of separation of the epidermis from the dermis at the level of dermal–epidermal junction (Figure 1f).

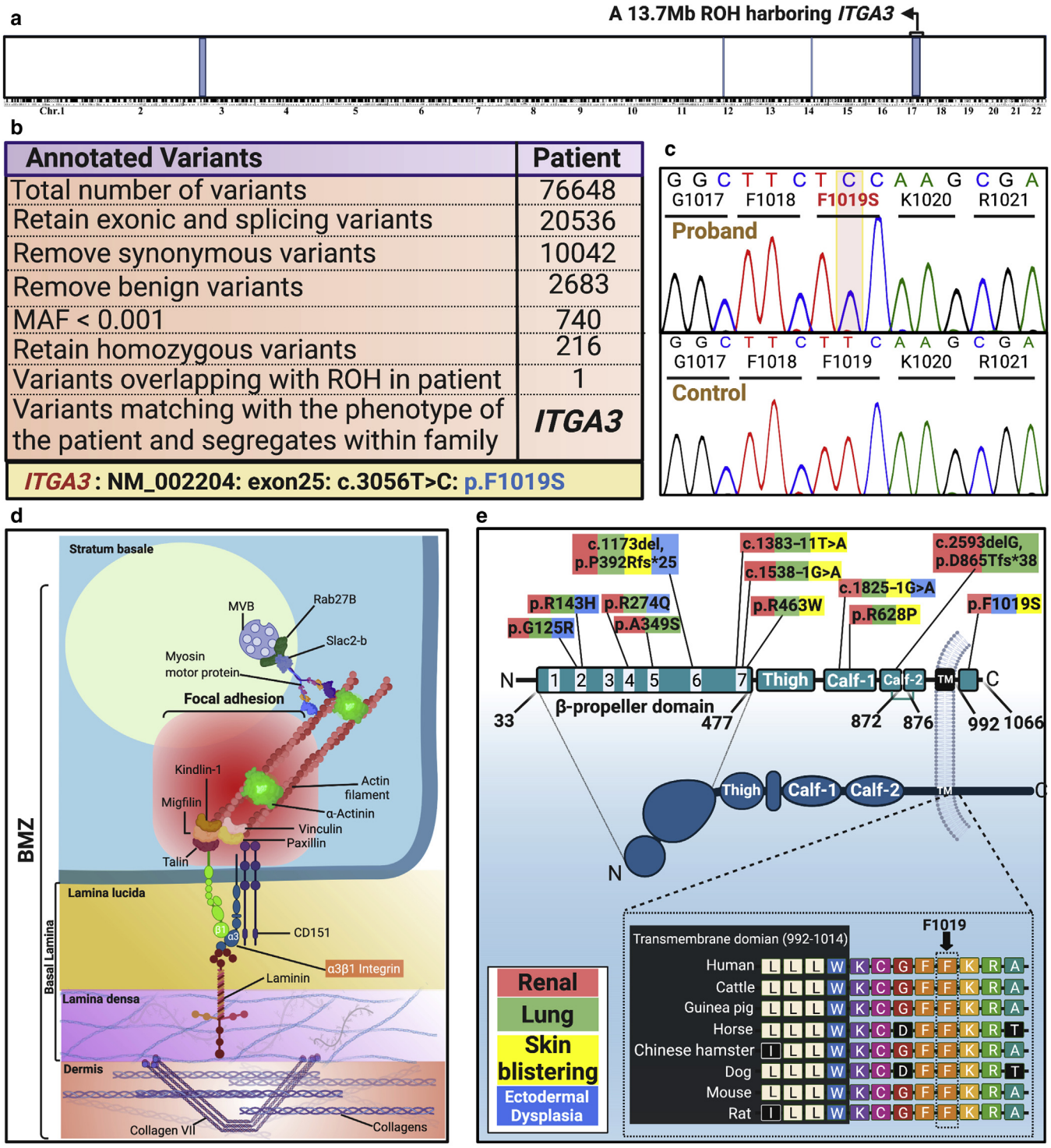

Figure 2. Identification of a homozygous *ITGA3* missense mutation in patients with a syndromic form of junctional EB and schematic representation of the adhesion molecules within the adherence junctions with the focus on α3β1 integrin. (**a**) Homozygosity mapping revealed four regions of homozygosity >4 Mb, one of them being 13.7 Mb and harboring the *ITGA3* gene locus. (**b**) Whole-exome sequencing of the genome of patient II-5 revealed 76,648 annotated variants. Bioinformatics filtering by steps indicated a reduction in the number of candidate variants from 76,648 to 216. Overlapping of these variants with the homozygosity mapping shown in (**a**) identified *ITGA3* as the candidate gene with the pathogenic sequence variant NM.002204:exon 25:c.3056T>C; p.Phe1019Ser. (**c**) This mutation was confirmed to be homozygous in both affected individuals by Sanger sequencing. (**d**) Schematic representation of the attachment complexes securing attachment of the basal cells of the epidermis to the underlying dermal–epidermal basement membrane. Note the central role of α3β1 integrin, which interacts with CD151 and the G domain of laminin 332. (**e**) Schematic presentation of the α3 integrin domain organization and the positions of *ITGA3* mutations identified in the junctional form of EB, with systemic manifestations in the kidneys, lung, and skin as well as with features of ectodermal dysplasia, as indicated by color coding. Note that all previously identified mutations reside within the extracellular domain, whereas the mutation identified in this study is the only one within the intracellular segment of the protein. Note that phenylalanine residue (i.e., F) 1019 is evolutionarily conserved between human and rat. BMZ, basement membrane zone; EB, epidermolysis bullosa; MAF, minor allele frequency; Mb, megabase; MVB, microvesicle body; ROH, runs of homozygosity.

Transmission electron microscopy revealed thin and fragmented tonofilaments with electron-dense patches. Hemidesmosomes were numerous, mostly of normal size (Figure 1g). There was focal widening within the lamina lucida, cleavage within the lower pole of keratinocytes, and widening of the spaces between the basal cells. The parents (deceased) or the children of the proband had no clinical findings suggestive of EB or renal disease. Consequently, the diagnosis of a syndromic form of EB with autosomal recessive inheritance was made.

Because the clinical features of our patients were very similar to those encountered in patients with mutations in the *CD151* gene (Vahidnezhad et al., 2018), immunostaining of a skin biopsy with an antibody recognizing CD151 was performed, and it revealed altered staining of this protein with a linear pattern along the basement membrane zone, compared with the peripheral immunoreactivity of the basal keratinocytes in control skin (Figure 1h). Omission of the primary antibody revealed no staining (not shown). However, RT-PCR amplification of exonic and flanking intronic sequences of *CD151* followed by Sanger sequencing failed to identify pathogenic mutations in this gene. Subsequently, DNA isolated from the peripheral blood of the proband was subjected to whole-exome sequencing, which identified 76,648 sequence variants (Figure 2b). No pathogenic variants were identified in *CD151*. Filtering of the variants by bioinformatics steps indicated in Figure 2b reduced the total number of candidate variants to 216. Considering the possibility that our patients have homozygous mutations owing to restricted ethnic background (Roma), homozygosity mapping of the proband's DNA was performed on the basis of the nucleotide sequence data derived from whole-exome sequencing (Vahidnezhad et al., 2019b). Four runs of homozygosity >4 megabases were found (Figure 2a). Superimposing the 216 candidate variants with the homozygosity map revealed that only one of them overlapped with a run of homozygosity, one of 13.7 megabases within chromosome 17 (Figure 2a). Examination of the sequence data revealed a homozygous *ITGA3* mutation NM_002204: exon 25:c.3056T>C:p.Phe1019Ser. This variant was confirmed to be homozygous in the two patients and heterozygous in the parents by Sanger sequencing (Figure 2c). This missense mutation was predicted by bioinformatics programs, including Sorting Intolerant From Tolerant, to be damaging with a Combined Annotation-Dependent Depletion score of 20.3. Its count in homozygous and heterozygous states in 172,424 healthy individuals (gnomAD r2.1) was zero and one, respectively, yielding an allele frequency of 0.0000058.

Immunostaining of the proband's skin revealed an altered pattern of CD151, which could be explained by perturbed protein–protein interactions between CD151 and α3β1 integrin, both being transmembrane proteins as part of focal adhesion complexes (Berditchevski et al., 2001) (Figure 2d). Loss of these interactions may render CD151 unstable and susceptible to degradation, supported by the observation that the Phe1019 residue is evolutionarily conserved in α3 integrin between human and rat (Figure 2e).

Interestingly, all previously published *ITGA3* mutations reside within the large extracellular segment of the α3 integrin polypeptide, which interacts not only with CD151 but also with the G domain of the α3 polypeptide subunit of laminin 332 (Figure 2d). Thus, mutations in either *CD151* or *ITGA3* can result in a similar blistering phenotype in the spectrum of EB.

Although most patients with interstitial lung disease, nephrotic syndrome, and EB die in early childhood, *ITGA3* mutations were previously reported in two siblings aged 13 and 9 years with skin manifestations and pulmonary involvement but without nephrotic impairment (Colombo et al., 2016). These patients were compound heterozygous for missense variants, p.Gly125Arg and p.Arg274Gln. In addition, delayed presentation of respiratory and renal symptoms and prolonged survival were reported in a patient who died at age 9 years (Tarur et al., 2020). This patient had a homozygous splice site mutation *ITGA3*: c.1825-1G>A; p.Val609-Serfs*31. Finally, a male patient in his late teens with cutaneous findings similar to those of our patients but without renal and respiratory involvement was recently reported (Cohen-Barak et al., 2019). Whole-exome sequencing identified a homozygous missense mutation p.Arg274Gln in *ITGA3*. In the case of our patients, at ages 45 and 30 years, no evidence of lung disease was noted by routine X-rays, and there was no clinical evidence of pulmonary involvement. Thus, the absence of pulmonary involvement may explain the survival of our patients beyond the early postnatal period and subsequent development into adulthood.

Data availability statement

Datasets related to this article can be found at https://www.ncbi.nlm.nih.gov/sra/, hosted at National Library of Medicine Sequence Read Archive with submission number SUB9249514.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: AK, HV, JU; Data Curation: PD, EK, RG; Formal Analysis: AHS, LY; Funding Acquisition: AK, JU; Investigation: AK, ALK, NN, SK, AHS, LY

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Frequent *FGFR3* and *Ras* Gene Mutations in Skin Tags or Acrochordons

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TO THE EDITOR

The skin is subject to age-dependent development of benign tumors, the most common of which are seborrheic keratoses (SKs) and skin tags (STs). SKs are flat or dome-shaped nodules that develop predominantly on sun-exposed skin, whereas STs, also called acrochordons, are polypoid skin lesions that develop predominantly on the neck and axilla. Although polypoid or pedunculated protrusions sometimes develop from a part of SKs, SKs and STs are considered distinct tumors.

SK is caused by the clonal expansion of mutant keratinocytes possessing a somatic mutation in genes associated with the cell proliferation signaling pathway, that is, *FGFR3* and associated genes (Heidenreich et al., 2017). Such mutation has also been identified in dermatosis papulosa nigra, solar lentigines, and benign lichenoid keratosis and/or lichen planus-like keratosis (Hafner, 2020). Despite being common, the pathogenesis of STs remains elusive. STs or acrochordons may include several histologically different tumors, including a small skin protrusion, typically developed multiple lesions on the neck and axilla and a much larger pedunculated tumor called soft fibromas. In this study, we genetically analyzed a major subtype of ST characterized by developing multiple lesions on the neck and axilla and having a thin stalk and single spherical or disc-shaped head (Figure 1a and b). We performed genetic analyses after obtaining written informed consent in accordance with the guidelines of the institutional review board of Keio University School of Medicine (Tokyo, Japan).

We performed whole-exome sequencing of eight STs and target-exome sequencing of four STs from a woman aged 54 years (participant ST1) who started to develop numerous STs on the neck and axilla from her 20s (see Supplementary Materials and Methods section and Supplementary Table S1). Because she had a family history of multiple STs, suggesting autosomal dominant inheritance, we expected a disease mechanism such as that seen in familial tumor syndromes, that is, a combination of congenital heterozygous mutation in a particular gene and lesion-specific second-hit genetic changes (Knudson, 2001). Unexpectedly, whole-exome sequencing revealed ~20 acquired mutations with various mosaic ratios in each ST. No common mutations were detected, except for a single mutation in *FGFR3*, *HRAS*, or *KRAS* in each ST, all of which are recurrent causative mutations of SKs

Abbreviations: EN, epidermal nevi; SK, seborrheic keratosis; ST, skin tag

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