

Biallelic Missense Mutations in the Integrin Alpha 3 Gene Causes Skin Fragility Without Structural Defects in Lungs and Kidneys

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Integrin $\alpha 3$ is a transmembrane adhesion receptor subunit that forms heterodimers with integrin $\beta 1$ (1). Integrin $\alpha 3\beta 1$ is widely expressed in the epithelia of the skin, lungs, and kidneys. In the skin, it is a major component of focal adhesions and plays an important role in the adhesion of basal keratinocytes and matrix by binding laminin-332 and laminin-511 (2). Integrin $\alpha 3$ also directly and indirectly influences the extracellular and adhesion proteins, including fibronectins. Integrin $\alpha 3\beta 1$ also plays a critical role in the organogenesis of the kidneys and lungs, as seen by the abnormal development of kidneys and lungs that leads to neonatal death in integrin $\alpha 3$ -deficient mice (2).

Biallelic loss-of-function mutations in the gene encoding integrin $\alpha 3$ (*ITGA3*) have recently been described as causing a rare multi-organ disorder characterized by interstitial lung disease, nephrotic syndrome, and epidermolysis bullosa (ILNEB) (3). A total of 11 patients with this syndrome have been reported in the literature to date (1, 3–9). Nearly all patients exhibited severe dysfunction and inflammation in the lungs and kidneys, with fatal outcomes in 6 cases, but cutaneous symptoms were mild or absent. We report here a rare, non-fatal case of ILNEB caused by novel compound heterozygous missense mutations in *ITGA3*, with primary clinical features of skin fragility and ectodermal dysplasia, without clinical involvement of internal organs.

CASE REPORT

An 18-year-old man presented with erythematous atrophic patches and recurrent trauma-induced blisters that healed with scarring on both extremities since infancy (Fig. 1). Deformed fingernails and toenails, dental dysplasia, and loss of scalp hair, eyelashes, and eyebrows were also observed (Fig. 1). The patient reported a history of lacrimal duct obstruction and recurrent respiratory infections in early childhood. He developed a subjective sense of urinary retention, but objective measurement of post-voiding residual urine volume was normal. He had no symptoms of anhidrosis, dyspnoea, oedema, or oliguria. He had no evidence of lung disease observed by chest X-ray and computed tomography scan. In addition, none of his family members reported similar symptoms of skin fragility.

Immunofluorescence mapping of a skin biopsy specimen revealed the presence of keratin 14 in the blister roof and both type IV collagen and type VII collagen in the blister floor (Fig. S1a). Transmission electron microscopy revealed hemidesmosomes with thin plaques on the blister roof and the lamina densa on the blister floor. These findings indicated cleavage within the lamina lucida, suggesting a diagnosis of junctional EB (JEB) (Fig. S1c).

Next generation sequencing identified biallelic missense mutations in *ITGA3* (c.485G>A, p.C162Y in exon 4 and c.1382G>A, p.R461Q in exon 9) that were predicted to be deleterious by *in silico* analysis (PolyPhen-2 and PROVEAN). Sanger sequencing confirmed these 2 mutations (Fig. S2). p.R461Q in *ITGA3* was reported as a single nucleotide polymorphism (rs772057144) and found in heterozygosity in 2 of 250,894 sequenced alleles according to gnomAD public database, but has not been reported to be associated with EB. p.C162Y turned out to be a novel mutation of *ITGA3*, as this variant was absent in 50 control chromosomes derived from healthy subjects and there were no previous reports

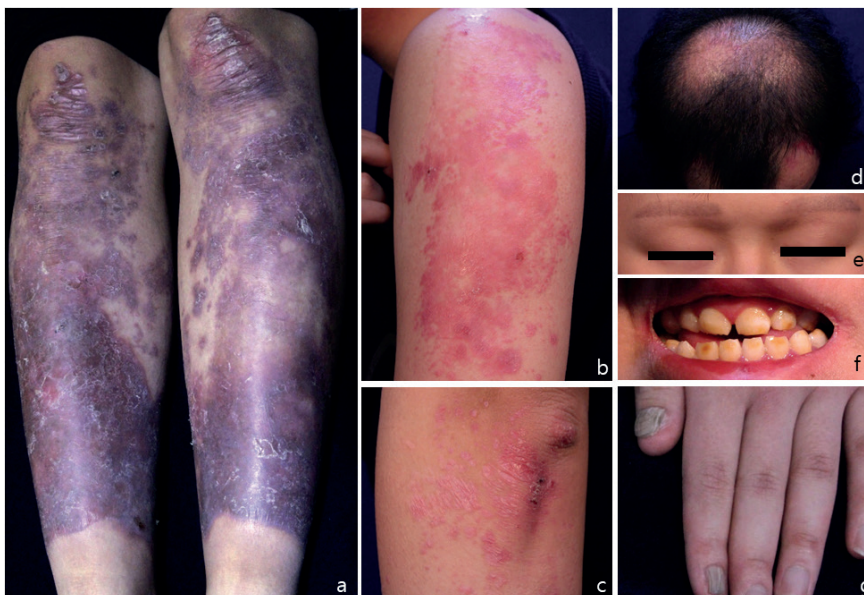


Fig. 1. Clinical manifestation of the patient. (a–c) Erythematous to dusky-red coloured atrophic patches with scarring on (a) the lower and (b, c) upper extremities. (d, e) Hypotrichosis on (d) the scalp, (e) eyebrows, and eyelashes. (f) Dental dysplasia. (g) Nail dystrophy.

in the Human Gene Mutation Database. Furthermore, immunofluorescence microscopy revealed markedly decreased expression of integrin $\alpha 3$ in the patient's skin compared with normal skin, whereas other hemidesmosome proteins showed normal expression (Fig. S1b).

DISCUSSION

JEB related to *ITGA3* mutations is extremely rare. Only 9 mutations in *ITGA3* have been identified in 11 patients with ILNEB to date. Characteristically, these patients presented with severe respiratory distress and renal involvement that began within the first few months of life and was fatal. In contrast, skin manifestations were absent or mild. Of the 11 reported patients, 3 had no skin symptoms, and the other 8 patients showed mild and limited skin fragility (Table SI). In contrast to these reports, the current patient showed: (i) more extensive and pronounced skin fragility; (ii) prominent abnormalities in ectodermal tissues; and (iii) no apparent involvement of lungs and kidneys.

This variance in clinical phenotype in ILNEB seems to be closely related to the genotype and functional consequences of mutations at the protein level of integrin $\alpha 3$. A recent study demonstrated that integrin $\alpha 3$ -negative keratinocytes derived from patients with ILNEB alter their microenvironment by switching to a fibronectin-rich matrix with increased $\alpha 5$ and αV integrin receptors (10). These findings are thought to be compensatory mechanisms for integrin $\alpha 3$ deficiency in the skin (10) and may explain mild or absent skin fragility in lethal cases of ILNEB. Research has also suggested that involvement of internal organs was still severe because these compensations were not effective in functional rescue of the lungs and kidneys. Indeed, all 6 lethal cases reported to date have demonstrated complete absence of integrin $\alpha 3$ in their skin or internal organs. In contrast, in a recently reported non-lethal case of ILNEB limited to skin and mucosa, integrin $\alpha 3$ was reduced, but not completely absent (7). The current patient carrying compound heterozygous missense mutations in *ITGA3* also showed trace expression of integrin $\alpha 3$ in skin. Thus, we suspect that complete absence of integrin $\alpha 3$ may lead to severe internal organ involvement but mild skin fragility due to compensational alterations in integrin $\alpha 3$ -deficient keratinocytes, whereas trace expression of integrin $\alpha 3$ can lead to limited mucocutaneous involvement without severe internal organ involvement.

In the current patient, 2 *ITGA3* mutations were identified: c.485G>A, p.C162Y and c.1382G>A, p.R461Q. The splice site prediction program at the Berkeley Drosophila Genome Project (BDGP) (<http://www.fruitfly.org/about/index.html>) was used to predict cryptic splice sites and the influence on splice sites in the 1 of the 2 variants, c.1382G>A (p. R461Q). It predicts a splicing score of 87% for the wild-type sequences of c.1382G>A, but predicts complete loss of the donor site for the mutant

sequence of c.1382G>A, suggesting that p. R461Q variant might be the pathogenic splice mutation responsible for the phenotype in this patient.

The unique feature of the current patient was distinct abnormality in ectodermal tissues including hair, nails, and teeth. In previous reports, hypotrichosis, nail dystrophy, and dental abnormality were variably observed in 6/11 cases, 8/11 cases, and 1/11 cases, respectively. Integrin $\alpha 3\beta 1$ has been shown to play an important role in hair follicle morphogenesis (11) and enamel formation (12), and to be expressed in the nail matrix (13), which can explain the phenotypic abnormalities in ectodermal tissues in EB associated with *ITGA3* mutations.

The current patient is the second case of *ITGA3* mutation-associated JEB without apparent pulmonary and renal involvement. The current case, along with those in the literature, suggests that the clinical manifestations of ILNEB vary according to the functional consequences of *ITGA3* mutations. As suggested previously, the current case adds evidence that JEB with *ITGA3* mutations is more appropriate nomenclature than ILNEB. The *ITGA3* gene must be considered a candidate gene for JEB, even if there is no apparent internal organ involvement. The current case expands the clinical spectrum of JEB associated with *ITGA3* mutations and enlarges the spectrum of mutations in the *ITGA3* gene by adding 2 new mutations to the 8 mutations already reported.

The authors have no conflicts of interest to declare.

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