

## *ITGB4*-mutated Junctional Epidermolysis Bullosa without Pyloric Atresia Presenting with Severe Urinary Involvement and Late-onset Minimal Skin Fragility: Diagnostic and Therapeutic Challenges

Girolamo MATTIOLI<sup>1</sup>, Andrea DIOCIAIUTI<sup>2</sup>, Sabrina ROSSI<sup>3</sup>, Giovanna ZAMBRUNO<sup>4</sup>, Marcello CARLUCCI<sup>5</sup>, Elisa PISANESCHI<sup>6</sup> and May EL HACHEM<sup>2</sup>

<sup>1</sup>Pediatric Surgery Unit, IRCCS Gaslini, DINOGMI Department, University of Genoa, Genoa, <sup>2</sup>Dermatology Unit and Genodermatosis Unit, Genetics and Rare Diseases Research Division, <sup>3</sup>Pathology Unit, Department of Laboratories, <sup>4</sup>Genodermatosis Unit, Genetics and Rare Diseases Research Division and <sup>5</sup>Laboratory of Medical Genetics, Department of Laboratories, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio, 4, IT-00165 Rome, and <sup>6</sup>Pediatric Surgery Unit, IRCCS Gaslini, Genoa, Italy. E-mail: andrea.diociaiuti@opbg.net

Accepted Feb 15, 2022; Epub ahead of print xx

Acta Derm Venereol 2022; 102: adv00706. DOI: 10.2340/actadv.v102.935

Junctional epidermolysis bullosa with pyloric atresia (JEB-PA) is a fragility disorder of skin and mucous membranes characterized by congenital PA and blistering frequently associated with aplasia cutis (1). JEB-PA is caused by mutations in the *ITGB4* or *ITGA6* gene encoding for integrin  $\alpha 6\beta 4$  expressed in hemidesmosomes. Additional manifestations include nail dystrophies, tooth abnormalities, and ocular, oral, gastrointestinal and genitourinary involvement. The majority of cases are early lethal despite prompt treatment of PA (1–4). However, several patients without PA and with milder phenotypes have been described (1, 4, 5). We report here a child carrying previously undescribed compound heterozygous missense *ITGB4* mutations who presented severe obstructive uropathy and minimal, late-onset skin fragility.

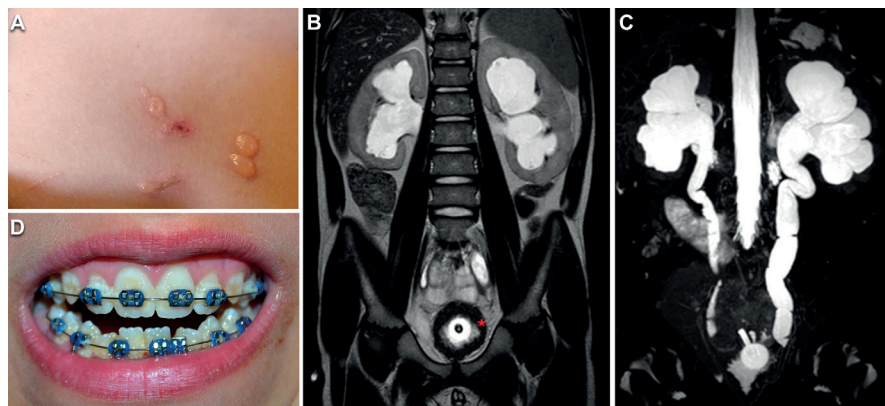
### CASE REPORT

The proband, a 10-year-old male, born to healthy non-consanguineous parents did not present muco-cutaneous lesions at birth. Episodes of urinary tract infections and dysuria started in infancy. Due to progressive worsening of urinary symptoms, he was addressed to us at age 5. Ultrasound examination revealed thickened bladder wall and bilateral ureteral dilation. Cystoscopy documented exuberant and disepithelialized bladder mucosa with severe urethral inflammation, involving both intravesical ureteral tracts. Magnetic resonance urography showed increased bladder wall thickness, and bilateral hydroureteronephrosis. Urodynamic studies demonstrated an overactive bladder. Due to urethral obstruction, cystostomy was performed at age 6. During hospitalization, a trauma-induced tense blister on the leg was noticed, leading to a suspicion of epidermolysis bullosa (EB) simplex. In following years, occasional blisters continued to develop at trauma sites (Fig. 1A). Further worsening

of obstructive uropathy with reduced right renal function (35%) led to positioning of a ureteral stent at age 7.5. Obstructive uropathy persisted at stent removal (Fig. 1B, C). Because of the involvement of urethra, bladder and ureters, a laparoscopic ileal neo-bladder creation with bilateral ureteral reimplantation, accompanied by Mitrofanoff appendicovesicostomy, was performed at age 9. The procedure has been well-tolerated and the child is currently in good general health; endoscopic and magnetic resonance imaging (MRI) follow-up showed a reduced upper urinary tract dilation, renal function recovery (48% right kidney, 52% left kidney), and persistent urethral inflammation. The patient also has enamel defects (Fig. 1D) and continues to present skin blistering after significant traumatism (Fig. S1).

**Histopathological, immunofluorescence antigen mapping (IFM) and ultrastructural findings.** Histopathological examination of cystoureterectomy samples revealed extensive metanephric metaplasia and focal disepithelialization together with an abundant inflammatory infiltrate, haemorrhages and fibrosis of the chorion. IFM of ureter showed markedly reduced expression of  $\beta 4$  and  $\alpha 6$  integrin subunits along the urothelial-chorion junction compared with control (Fig. S2). IFM of rubbed skin revealed a slightly reduced expression of integrin  $\alpha 6\beta 4$  (Fig. S3). Ureter ultrastructural examination showed a reduced number of hypoplastic hemidesmosomes and thickened urothelial basement membrane, compared with control (Fig. S4). Skin ultrastructural examination documented cleavage within the lamina lucida of the basement membrane zone and mildly hypoplastic hemidesmosomes (Fig. S4).

**Molecular genetic testing.** Following informed consent, molecular testing with a customized NGS EB panel (NimbleGen SeqCap Target Enrichment Kit, Roche, on Illumina platform) identified in the proband the compound heterozygous missense sequence variants c.320G>C, p.Arg107Pro, and c.542C>T, p.Pro181Leu in exon 5 and 6 of *ITGB4* gene (NM\_000213.3), respectively. Sanger sequencing confirmed the paternal and maternal inheritance of variants c.320G>C and c.542C>T, respectively (Fig. S5). Neither variant has been reported nor annotated in GnomAD database of



**Fig. 1. Patient's clinical features and magnetic resonance imaging (MRI) findings.** (A) Clear blisters on the chest ensuing scratching of an insect bite and plaster application at age 8 years. (B, C) Functional MRI urography showing bilateral hydronephrosis due to ureterovesical junction obstruction. The right kidney appears hypoplastic compared with the left one, and both kidneys present a loss in corticomedullary differentiation. Note the increased bladder wall thickness (asterisk in (B)). Urographic evaluation shows a bilateral ureteral dilation due to stenosis at the ureterovesical junctions. At this stage, the split renal function at functional MRI evaluation was 32% on the right side and 68% on the left side. (D) Tooth appearance with enamel defects at age 10 years.

human variations. c.542C>T was considered likely pathogenic according to ACMG guidelines, as (i) it is a missense variant in a gene for which missense-variants are a known disease mechanism, (ii) it is novel, and (iii) multiple computational evidences support a deleterious effect on the gene product (<https://varsome.com/variant/hg19/itgb4%20c.542C%3ET?annotation-mode=germline>). c.320G>C was considered of uncertain significance as it is also a novel missense variant, but computational evidence does not support its pathogenicity ([https://varsome.com/variant/hg19/ITGB4\(NM\\_000213.5\)%3Ac.320G%3EC?annotation-mode=germline](https://varsome.com/variant/hg19/ITGB4(NM_000213.5)%3Ac.320G%3EC?annotation-mode=germline)). No other pathogenic variants were detected in EB-associated genes.

## DISCUSSION

The phenotype of our child is extremely peculiar as, in addition to lacking PA at birth, he did not manifest skin fragility until age 6, when rare blisters, exclusively localized to trauma-exposed sites, begun to appear. Although a few JEB-PA patients who presented mild skin fragility and started to develop skin blisters after birth have been reported (2, 5–7), the current case is characterized by the most delayed onset of skin fragility described to date. On the other hand, involvement of the urinary tract with recurrent infections and dysuria from the first year of life initially led to a misdiagnosis of Hinman syndrome (non-neurogenic neurogenic bladder). EB was suspected following appearance of trauma-induced blisters. The ultrastructural finding of skin lamina lucida cleavage was diagnostic for JEB, as confirmed by the reduced expression of integrin  $\alpha 6\beta 4$ . The diagnosis of JEB-PA due to compound heterozygous *ITGB4* missense mutations was then established by molecular genetic testing.

Urinary tract involvement has been reported in 21% of JEB-PA cases (8). Similar to our case, frequent urological manifestations in patients with *ITGB4* mutations comprise urethral stenosis, haemorrhagic cystitis, and vesicoureteral obstruction or reflux (3, 5–7, 9). Dysuria, urinary retention, haematuria and recurrent infections are common. Disease course is variable (5), but frequently chronic, and may lead to hydronephrosis and renal failure (4, 5, 9). The expression of integrin  $\alpha 6\beta 4$  in basal urothelial cells (10) correlates with the involvement of the lower urinary tract and ureters. Interestingly, the current patient presented more marked reduction in integrin  $\alpha 6\beta 4$  expression in the damaged urothelium than in keratinocytes. To our knowledge, this is the first report comparing the expression of integrin  $\alpha 6\beta 4$  in patient urothelium vs control, and patient urothelium vs skin.

Management of urinary tract manifestations is challenging, due to the inherent fragility of the urinary tract mucosa and frequent development of stenotic scarring (5, 11, 12). In the current case, delayed diagnosis and repeated instrumental work-up might have contributed to the severe obstructive uropathy, which eventually required bladder reconstruction.

The peculiar phenotype of the current patient was associated with 2 previously unreported missense mutations located in the extracellular domain of *ITGB4*. Splice-site

and missense mutations on at least 1 *ITGB4* allele are frequently associated with non-lethal JEB-PA phenotypes (5, 8, 9). However, some homozygous or compound heterozygous missense mutations resulted in lethal phenotypes, depending on the position and function of the involved amino acid (4). In addition, a recent literature review highlighted that *ITGB4* missense variants may frequently present with mild skin involvement but severe uropathy (9).

In conclusion, the case reported here further emphasizes the clinical variability of JEB-PA, showing that urinary tract involvement can represent the only disease manifestation for years. Thus, EB should be considered in children with unexplained obstructive uropathy, even in the absence of skin lesions.

*The authors have no conflicts of interest to declare.*

## REFERENCES

- Has C, Bauer JW, Bodemer C, Bolling MC, Bruckner-Tuderman L, et al. Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility. *Br J Dermatol* 2020; 183: 614–627.
- Pulkkinen L, Rouan F, Bruckner-Tuderman L, Wallerstein R, Garzon M, et al. Novel *ITGB4* mutations in lethal and nonlethal variants of epidermolysis bullosa with pyloric atresia: missense versus nonsense. *Am J Hum Genet* 1998; 63: 1376–1387.
- Diociaiuti A, Castiglia D, Morini F, Boldrini R, Fortugno P, Zambruno G, et al. Long-term follow-up of a spontaneously improving patient with junctional epidermolysis bullosa associated with *ITGB4* c.3977-19T>A splicing mutation. *Acta Derm Venereol* 2013; 93: 116–118.
- Dang N, Klingberg S, Rubin AI, Edwards M, Borelli S, Relic J, et al. Differential expression of pyloric atresia in junctional epidermolysis bullosa with *ITGB4* mutations suggests that pyloric atresia is due to factors other than the mutations and not predictive of a poor outcome: three novel mutations and a review of the literature. *Acta Derm Venereol* 2008; 88: 438–448.
- Schumann H, Kiritsi D, Pigors M, Hausser I, Kohlhasse J, Peters J, et al. Phenotypic spectrum of epidermolysis bullosa associated with  $\alpha 6\beta 4$  integrin mutations. *Br J Dermatol* 2013; 169: 115–124.
- Salvestrini C, McGrath JA, Ozoemena L, Husain K, Buhamrah E, et al. Desquamative enteropathy and pyloric atresia without skin disease caused by a novel intracellular beta4 integrin mutation. *J Pediatr Gastroenterol Nutr* 2008; 47: 585–591.
- Lee M, Chen Q, Wang H, Zhang J, Lin Z, Yang Y. *ITGB4*-associated junctional epidermolysis bullosa without pyloric atresia but profound genito-urinary involvement. *Acta Derm Venereol* 2015; 95: 112–113.
- Mylonas KS, Hayes M, Ko LN, Griggs CL, Kroshinsky D, Masiakos PT. Clinical outcomes and molecular profile of patients with Carmi syndrome: a systematic review and evidence quality assessment. *J Pediatr Surg* 2019; 54: 1351–1358.
- Ellis C, Eason C, Snyder A, Siegel M, Pai GS, Ryan E, et al. Novel missense p.R252L mutation of *ITGB4* compounded with known 3793+1G>A mutation associated with nonlethal epidermolysis bullosa-pyloric atresia with obstructive uropathy. *JAAD Case Rep* 2021; 11: 63–68.
- Liebert M, Washington R, Wedemeyer G, Carey TE, Grossman HB. Loss of co-localization of alpha 6 beta 4 integrin and collagen VII in bladder cancer. *Am J Pathol* 1994; 144: 787–795.
- Burgu B, Duffy PG, Wilcox DT. Single-centre experience of genitourinary complications of epidermolysis bullosa. *J Pediatr Urol* 2006; 2: 583–586.
- Chan SM, Dillon MJ, Duffy PG, Atherton DJ. Nephro-urological complications of epidermolysis bullosa in paediatric patients. *Br J Dermatol* 2007; 156: 143–147.