Dear Editors,

Integrin α3 (ITGA3) gene mutations have recently been associated with a rare autosomal recessive, syndromic epidermolysis bullosa subtype comprising congenital nephrotic syndrome and interstitial lung disease (ILNEB syndrome, OMIM#614748) [1]. To the best of our knowledge, ten unrelated patients and a pair of siblings with ILNEB syndrome carrying mutations in the ITGA3 gene have been reported in the literature [1–8].

We report on a girl born to a 40-year-old mother (gravidia 5, para 2) of Sri Lankan descent at a gestation of 34 weeks and five days. The pregnancy was prone to complications. Ultrasound examination showed intrauterine growth retardation, isolated renal cysts, ascites and myocardial hypertrophy. Anhydramnios was diagnosed in the 30th week of pregnancy and premature labor led to imminent premature delivery. Three earlier pregnancies had ended in induced late abortions since the infants were diagnosed with renal malformation and pulmonary hypoplasia. A premature infant born at a gestation of 27 weeks deceased on the second day after delivery due to renal malformation and pulmonary hypoplasia. The parents were reportedly unrelated.

Immediately after birth, the girl required invasive ventilation due to respiratory distress and a small pneumothorax on the right side. Children's interstitial lung disease (chILD) was diagnosed by chest computed tomography (Figure 1) and caused continuous high-flow supplemental oxygen demand. Repeated chILD exacerbations required repeated intensive care admissions with invasive ventilation and methylprednisolone therapy. Since the age of 2.5 years continuous immunosuppressive therapy with mycophenolate mofetil and azithromycin has stabilized the course of chILD.

Moreover, multicystic dysplastic kidneys and oligoanuria caused the predominant clinical problems within the first months of life and a rapidly declining renal function required continuous peritoneal dialysis at the age of three months.

From the age of four months, the infant displayed increasing skin fragility, with blisters and erosions after mechanical manipulation especially at the extremities (Figure 2a, b). The lesions healed slowly, without scarring but with post inflammatory hypopigmentation (Figure 2a). The cutaneous alterations were compatible with a rather mild epidermolysis bullosa phenotype. There was no mucosal involvement. The scalp hair was dense; however, the eyebrows and eyelashes were rather fine and sparse. The nails were unremarkable. Throughout the clinical course, skin fragility persisted. However, neither an aggravation nor an improvement was observed.

After the infant’s parents had provided written informed consent, a biopsy was taken from the affected skin. Light microscopy showed subepidermal blisters without significant inflammation, consistent with epidermolysis. Immunofluorescence mapping revealed disruption of the dermal-epidermal junction with cleavage within the basement membrane (Figure 3a; upper and middle panels). Integrin α3

Figure 1 Chest computed tomography showing septal thickening and ground glass opacities predominant in the lower lobes of the lung.

Figure 2 Clinical picture. Crusty covered erosions on the left upper extremity and post inflammatory hypopigmentation on the trunk. Dressing on the lower abdomen in the context of peritoneal dialysis (a). Diffuse erythema with blisters and partly crusty covered erosions on the upper part of the left leg (b).
immunoreactivity in the skin was lacking (Figure 3a; lower panels). Subsequently, EDTA-treated blood samples were obtained from the patient and her parents for molecular genetic analyses. Genomic DNA was extracted from peripheral-blood leukocytes, and the coding region and exon-intron boundaries of ITGA3 were analyzed. The patient was found to be compound heterozygous for the ITGA3 mutations c.1922+1G>A in exon 14 and c.3021delG, p.Leu1008Ter in exon 24 (Figure 3b). Thus, ILNEB syndrome was diagnosed. The mutation c.1922+1G>A in intron 14 was inherited from the mother, but c.3021delG was not. Interestingly, the latter mutation was not identified by Sanger sequencing in DNA extracted from leukocytes from the father, but genetic testing from the sperm or from other tissues was not performed. The theoretical, estimated risk of recurrence if three affected children are born and there is no healthy child is 12.9 % [9]. The high recurrence of the disease in the family implies that the father is carrier of the mutation in a mosaic constellation that entirely includes the gonads.

Both ITGA3 variants have not been reported before in ILNEB patients, are absent in the Genome Aggregation (gnomAD) Database and are predicted to be pathogenic. The variant c.1922+1G>A affects the canonical splice site of exon 14 and should affect splicing, while c.3021delG causes a frame shift and a premature termination of translation at codon 1008. Integrin α3 is indispensable for structural assembly of basement membranes and is pivotal for adequate barrier functions in the kidney, lung, and skin [1, 10]. In our patient the mutation constellation lead to the absence of the integrin α3 subunit, as we could show by immunofluorescence staining of the skin and by immunoblot analysis of keratinocyte lysates (Figure 3a, c).

Despite intensive care, the child died at four years and four months of age from septic shock as a result of a pulmonary infection with Pseudomonas aeruginosa.

In our patient, the renal and respiratory features predominated, with the lung involvement ultimately accounting for the lethal course of the disease. However, it was the investigation of the skin fragility that led to identification of the molecular defect. In patients with congenital interstitial lung disease, renal failure and cutaneous alterations ranging from mild skin fragility to blistering and skin erosions consistent with an epidermolysis bullosa phenotype, ILNEB syndrome should be taken into consideration and appropriate diagnostics initiated. Interdisciplinary care is indispensable for these severely ill patients, most of whom die in early infancy [11].

Acknowledgements

Open access funding enabled and organized by Projekt DEAL.
Conflict of interest
None.

Wiebke Sondermann¹, Rainer Büscher², Henrike Forster², Florian Stehling¹, Judith Fischer⁴, Yinghong He⁵, Cristina Has⁶

(1) Department of Dermatology, Venereology and Allergology, University Hospital Essen, Essen, Germany
(2) Department of Pediatrics II, Pediatric Nephrology, University Hospital Essen, Essen, Germany
(3) Department of Pediatrics III, Pediatric Pulmonology, University Hospital Essen, Essen, Germany
(4) Institute of Human Genetics, Medical Center – University of Freiburg, Freiburg im Breisgau, Germany
(5) Department of Dermatology, University of Lübeck, Lübeck, Germany
(6) Department of Dermatology, Medical Center, University of Freiburg, Freiburg im Breisgau, Germany

Correspondence to
Cristina Has, MD
Department of Dermatology
Medical Center, University of Freiburg
Hauptstrasse 7
79104 Freiburg im Breisgau, Germany
E-Mail: cristina.has@uniklinik-freiburg.de

References
1 Has C, Spartà G, Kiritsi D et al. Integrin α3 mutations with kidney, lung, and skin disease. N Engl J Med 2012; 366: 1508–14.
2 Colombo EA, Spaccini L, Volpi L et al. Viable phenotype of ILNEB syndrome without nephrotic impairment in siblings heterozygous for unreported integrin alpha3 mutations. Orphanet J Rare Dis 2016; 11: 136.
3 Nicolaou N, Margadant C, Kevelam SH et al. Gain of glycosylation in integrin α3 causes lung disease and nephrotic syndrome. J Clin Invest 2012; 122: 4375–87.
4 Yalcin EG, He Y, Orhan D et al. Crucial role of posttranslational modifications of integrin α3 in interstitial lung disease and nephrotic syndrome. Hum Mol Genet 2015; 24: 3679–88.
5 He Y, Balasubramanian M, Humphreys N et al. Intronic ITGA3 mutation impacts splicing regulation and causes interstitial lung disease, nephrotic syndrome, and epidermolysis bullosa. J Invest Dermatol 2016; 136: 1056–9.
6 Tarur SU, Srinivasan S, Seeralar A. Delayed presentation of respiratory symptoms and prolonged survival in homozygous α3 integrin deficiency. Indian Pediatr 2020; 57: 268–9.
7 Cohen-Barak E, Danial-Farran N, Khayat M et al. A nonjunctional, nonsyndromic case of junctional epidermolysis bullosa with renal and respiratory involvement. JAMA Dermatol 2019; 155: 498–500.
8 Lovric S, Fang H, Vega-Warner V et al. Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2014; 9: 1109–16.
9 Campbell IM, Stewart JR, James RA et al. Parent of origin, mosaicism, and recurrence risk: probabilistic modeling explains the broken symmetry of transmission genetics. Am J Hum Genet 2014; 95: 345–59.
10 He Y, Thriene K, Boerries M et al. Constitutional absence of epithelial integrin α3 impacts the composition of the cellular microenvironment of ILNEB keratinocytes. Matrix Biol 2018; 74: 62–76.
11 Prodinger C, Laimer M, Bauer JW, Hintner H. EB (epidermolysis bullosa)-House Austria: Pioneering work for the care of patients with rare diseases. J Dtsch Dermatol Ges 2020; 18(11): 1229–35.
12 DiPersio CM, Shah S, Hynes RO. Alpha 3A beta 1 integrin localizes to focal contacts in response to diverse extracellular matrix proteins. J Cell Sci 1995; 108 (Pt 6): 2321–36.