Long-term Follow-up of a Spontaneously Improving Patient with Junctional Epidermolysis Bullosa Associated with ITGB4 c.3977-19T>A Splicing Mutation

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Junctional epidermolysis bullosa with pyloric atresia (JEB-PA, OMIM 226730) is a rare autosomal recessive genodermatosis characterized, as primary manifestations, by neonatal blistering of the skin and mucous membranes associated with foregut obstruction (1, 2). Aplasia cutis congenita, genitourinary tract abnormalities, nail dystrophy, enamel hypoplasia, corneal erosions, respiratory tract involvement and lack of prominent granulation tissue formation represent additional features of this disorder (3). In most cases, affected children die of systemic complications despite the surgical correction of the foregut obstruction (3). However, non-lethal forms of JEB-PA have also been reported (4, 5). JEB-PA is caused by mutations in either of 2 genes coding for α6β4 integrin (*ITGA6*, *ITGB4*) (2, 6). Lethal JEB-PA is generally caused by premature termination codons (PTC) on both alleles of the *ITGB4* gene or, rarely, of the *ITGA6* gene. Non-lethal JEB-PA is mainly caused missense or splice-site mutations in at least one of the alleles. However, the position in the domains of the β4 integrin may also affect the phenotype of JEB-PA (4).

We report here a case of JEB-PA, due to compound heterozygous mutations in the *ITGB4* gene, characterized by an extremely benign course of the skin disease and by the development of genitourinary abnormalities.

CASE REPORT

The proband was a girl born preterm by normal delivery at 31 weeks gestation (birth weight 1600 g, Apgar scores were 7 and 9 at 1 and 5 min, respectively) to a 32-year-old G1P0, after an uneventful pregnancy. The parents were non-consanguineous. In the first few days of life, the infant presented the hallmarks of the JEB-PA, including erosions and blisters of the skin of the head, arms and legs, and gastric outlet obstruction revealed at abdominal radiographs. Family history was negative for skin or intestinal disorders. At the age of 17 days the infant was referred to our Neonatal Department for treatment of the suspected pyloric atresia and the bullous skin lesions. On admission, the patient presented with blisters on the skin of both arms and legs and onychodystrophy (Fig. 1a). Kidney ultrasonography did not reveal any abnormalities. The patient underwent excision of a pyloric web and corrective gastroduodenostomy at day 19. Full enteral feeding was achieved on post-operative day 36. Starting from the post-operative period, the tendency to blister formation markedly decreased, with new lesions limited to areas where placement of adhesive tape was necessary. Patient's clinical follow-up confirmed the increased skin resistance to trauma with development of only a few blisters localized to hands and feet during summertime (Fig. 1b and c). By the age of 3 years the patient presented recurrent episodes of afebrile dysuria, macrohaematuria and proteinuria. The last admission was made at the age of 6 years because of a gastroenteritis followed by exacerbation of urological symptoms with proteinuria (maximum proteinuria/creatinine ratio=4.17, n.v. 0–0.15) and strangury. The patient then made periodic checks of the urine, which was

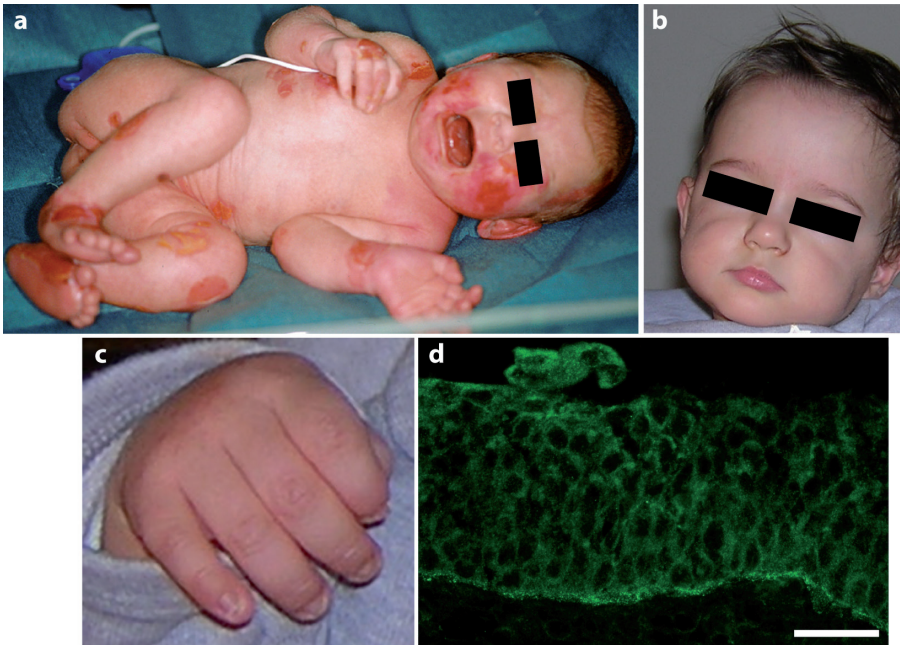

Fig. 1. (a) Widespread erosions and blisters of the skin in the neonatal period; the face and limbs are particularly affected. (b) At the age of 12 months the patient shows very good skin condition with no lesions on the face and (c) only mild onychodystrophy on the hands. (d) Immunofluorescence examination of a frozen bladder biopsy shows a very faint staining for β4 integrin of the urothelium still polarized at the junction with the lamina propria.

permanently normalized at around the age of 7 years. At present the patient, who is 8 years old, is almost completely free from skin lesions, and presents only a mild onychodystrophy, enamel hypoplasia and caries.

A perilesional skin biopsy was obtained a few days after birth. Ultrastructural analysis showed areas of cleavage within the lamina lucida of the cutaneous basement membrane zone, as well as a reduced number of hypoplastic hemidesmosomes. Immunofluorescence analysis revealed a slightly reduced level of integrin α6 expression and a marked reduction in β4 integrin expression (Fig. S1; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1381) (7), whereas immunoreactivity against other basement membrane components was normal (data not shown).

During admissions for cystitis, ultrasound examination of the bladder wall showed irregularly thickening with an oedematous mucosa, while the ureters were not dilated. Cystoscopy led to the diagnosis of chronic cystitis. Ultrastructural examination of a bladder bioptic fragment showed detachments at the level of the junction between the urothelium and the lamina propria, with a cleavage plane above the lamina densa and a mixed inflammatory infiltrate within the chorion (not shown). Immunofluorescence examination of a frozen fragment showed an extremely faint expression of β4 integrin polarized at the junction between urothelium and lamina propria (Fig. 1d). These findings are in keeping with cystitis secondary to EB bladder involvement.

After obtaining the parents' informed consent, mutational screening of ITGB4 gene was carried out according to a previously described amplification strategy (8). Sequencing of PCR products disclosed the compound heterozygous mutations c.3338_3354del (exon 28) and c.3977-19T>A (intron 31), which were confirmed to be inherited from the father and the mother, respectively. Designation is based on cDNA sequence (GenBank accession no. NM_001005731) with the A of the ATG translation initiation codon as first nucleotide. Mutation c.3338_3354del is a novel 17-bp deletion predicted to form a downstream PTC that interrupts translation of the integrin β4 polypeptide within the first fibronectin type 3 domain (amino acid residue 1135). Mutation c.3977-19T>A was previously reported as c.3986-19T>A by Chavanas et al. (9) (Fig. S2a; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1381) and shown to affect the branch point sequence of intron 31, resulting in aberrant splicing of ITGB4 pre-mRNA. However, this deleterious effect was leaky and influenced by environmental factors, leading to a certain amount of normal transcripts, as in our patient's keratinocytes (Fig. S2b; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1381).

DISCUSSION

A few cases of JEB-PA showing spontaneous amelioration of the skin condition have been reported in the literature (5). Among those with molecular diagnosis, Nakano et al. (10) published 3 JEB-PA patients who showed a tendency to spontaneous skin improvement. In another patient (11), skin blistering presented during the neonatal period in trauma-exposed sites, but then the lesions became occasional and limited to the knees and feet. Chavanas et al. (9) reported the follow-up of a patient born with extensive skin blistering, pyloric atresia and ureterovesical occlusion, and who subsequently developed good resistance to trauma (12). In these examples, missense, splicing and PTC-causing mutations in either homozygous or compound heterozygous status were shown to be associated with residual α6β4 integrin expression in the patients' skin (10, 11). Protein expression and phenotypic improvement correlated with the position of the missense mutation in the functional domains of the β4 integrin protein or with the rescue of functional splicing of the ITGB4 mRNA. The latter mechanism was described by Chavanas et al. (9) in the above-mentioned patient who was compound heterozygous for the same branch point mutation described here (c.3977-19T>A in intron 31) and a second splicing mutation affecting the donor splice site of intron 30 (c.3793+1G>A), which at the homozygous state was known to result in a lethal phenotype. Using a minigene approach, these authors demonstrated that functional splicing of exon 32 can be restored *in vitro* by seeding proband's keratinocytes on feeders of irradiated fibroblasts. They showed that culture conditions modulate the rate of illegitimate splicing driven by the branch point c.3977-19T>A mutation, thus influencing the levels of the normal transcripts. A cDNA fragment of normal size could also be detected in our patient's keratinocytes cultured on a fibroblast feeder-layer (Fig. S2b). This band probably corresponds to transcripts from the allele linked to the c.3977-19T>A mutation, as the c.3338_3354del PTC on the other allele is predicted to result in a marked instability of the mutant transcripts.

Our patient, followed-up until the age of 8 years, presented a remarkable, rapid and spontaneous improvement in the cutaneous condition in the first month of life, which was stable in subsequent years, while recurrent episodes of cystitis manifested between the third and the sixth year of life. The weak β4 expression in the urothelium, together with ultrastructural cleavage at the level of the junction between the urothelium and the lamina propria, allowed to confirm cystitis as a secondary manifestation of JEB. Currently, the child has no urological sequelae, but the future course is not predictable because of the young age.

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The authors declare no conflicts of interest.

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