

## CLINICAL REPORT

# Frequent Occurrence of Aplasia Cutis Congenita in Bullous Dermolysis of the Newborn

Andrea DIOCIAIUTI<sup>1</sup>, Daniele CASTIGLIA<sup>2</sup>, Simona GIANCRISTOFORO<sup>1</sup>, Liliana GUERRA<sup>2</sup>, Vittoria PROTO<sup>1</sup>, Andrea DOTTA<sup>3</sup>, Renata BOLDRINI<sup>4</sup>, Giovanna ZAMBRUNO<sup>2</sup> and May EL HACHEM<sup>1</sup>

<sup>1</sup>Dermatology Unit, <sup>2</sup>Neonatal Intensive Care Unit and <sup>4</sup>Department of Pathology, Bambino Gesù Children's Hospital-IRCCS, and <sup>3</sup>Laboratory of Molecular and Cell Biology, Istituto Dermatologico dell'Immacolata-IRCCS, Rome, Italy

**Bullous dermolysis of the newborn (BDN) is a subtype of dystrophic epidermolysis bullosa characterized by rapid improvement in skin fragility within the first months of life, associated with typical immunofluorescence and ultrastructural features. Inheritance can be autosomal dominant or recessive. We report here 4 cases of BDN, 2 of which presented with aplasia cutis congenita of the lower extremities. All patients improved rapidly and blister formation ceased by the third month of life in 3 cases. In these patients only residual milia, nail dystrophies and atrophic scarring at sites of aplasia cutis were visible by one year. Family history indicated dominant inheritance in 2 cases, confirmed by identification of *COL7A1* mutation. Molecular analysis also revealed recessive inheritance in the 2 sporadic cases. A literature search identified several patients with BDN born with skin defects localized to the lower extremities. In conclusion, these findings indicate that aplasia cutis congenita is not an infrequent manifestation of BDN. Key words: bullous dermolysis; newborn; dystrophic epidermolysis bullosa; aplasia cutis congenita; *COL7A1*; missense mutation.**

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Andrea Diociaiuti, Dermatology Unit, Bambino Gesù Children's Hospital-IRCCS, P.zza St. Onofrio 4, IT-00165 Rome, Italy. E-mail: andrea.diociaiuti@opbg.net

Bullous dermolysis of the newborn (BDN) is a rare subtype of dystrophic epidermolysis bullosa (DEB) clinically

characterized by rapid improvement in skin fragility that resolves within the first year of life in the majority of cases (1, 2). Like the other subtypes of DEB, BDN is caused by mutations in the *COL7A1* gene encoding type VII collagen (3), the major component of anchoring fibrils of the cutaneous basement membrane zone (BMZ). The disease can be inherited in an autosomal dominant or recessive mode (2–4). Diagnostic pathological features of BDN are the presence of type VII collagen granular deposits within the cytoplasm of basal keratinocytes and pathognomonic stellate bodies detected on ultrastructural examination (1, 2, 5, 6). BDN blisters are preferentially acral located and heal with no or minimal scarring. The oral mucosa is affected in a minority of patients, and nail dystrophy can occur (2).

We report here the clinical and molecular characterization of 4 cases of BDN, 2 of which presented with aplasia cutis congenita (ACC).

## CASE REPORTS

(for Materials and Methods see Appendix S1<sup>1</sup>)

**Case 1.** Female, first child of healthy non-consanguineous parents, born at term, with bilateral ACC extending from the knees to the feet (Fig. 1a) and blistering lesions on the abdomen and both hands. Several fingernails presented subungual haemorrhages and the nail plate of the left big toe was absent. The oral mucosa



Fig. 1. Case 1: (a) aplasia cutis congenita of both legs and feet at 13 days of age and (b) atrophic scarring at 3 months. Case 3: (c) vesicles, eroded and crusted lesions on digits and subungual haemorrhages at 1 month of age, (d) numerous milia are visible at the one-year follow-up visit.

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was also affected. The patient was in good general health. A perilesional skin biopsy was taken from the left knee at the age of 13 days. The skin fragility improved rapidly and at the 3-month follow-up visit the patient did not present any sign of active disease. Skin lesions had resolved with milia formation only, while healed ACC areas also showed atrophic scarring (Fig. 1b). Nails previously presenting with haemorrhages had been shed. At 3 years of age the patient continues to be free of disease, apart from the occasional formation of erosions and crusts limited to the atrophic skin of the knees.

**Case 2.** Male, first child of healthy non-consanguineous parents, born at term with ACC of the anterior aspect of the left leg extending to the foot (Fig. S1a<sup>1</sup>) and blisters on the right knee. The patient was transferred to our hospital at the age of 7 days with a provisional diagnosis of epidermolysis bullosa (EB). A perilesional skin biopsy was performed on the left heel. In the following days the patient developed a few tiny erosions of the face and left hand and a large lesion on the hard palate (Fig. S1b<sup>1</sup>). At the 3-month follow-up visit the patient was in good general health, the area of aplasia cutis was completely healed with atrophic scarring, and occasional blisters were still developing only in the same area.

**Case 3.** Male, dizygotic twin born at term by Caesarean section from non-consanguineous parents. The patient presented a blistering lesion of the right hand. In the following days, tiny blisters developed on the extremities at friction sites. The infant was seen by us at the age of 1 month for confirmation of the diagnostic suspicion of EB. Family history revealed that the mother had manifested similar lesions on the knees and ankles from infancy, which ceased completely by 10 years of age; at the time of examination she presented only minimal dystrophy of the big toe nails. At one month of age the patient was in good general health and showed only a few, small erosions and crusts on the hands (Fig. 1c), feet, ankles, face and minimal subungual haemorrhages of the fingernails. No mucosal lesions were present. A biopsy was taken from perilesional skin of the foot. During follow-up the child, who is now aged 3 years, showed normal growth, but continued to develop blisters on the extremities (Fig. 1d), in particular during the summer months.

**Case 4.** Second male child of non-consanguineous parents, born preterm at 33 week gestation by Caesarean section with a weight of 2,170 g. At birth the patient presented blisters localized on the feet, hands, chin, abdomen and genital area and subungual haemorrhages of both finger- and toe-nails (Fig. S1c<sup>1</sup>), in the absence of mucosal lesions. A biopsy was obtained from the left foot in the second day of life. Family history revealed that the father, aged 31 years, and the older brother, aged 7 years, had also manifested blistering lesions during infancy. Both have only onychodystrophy at

present. The patient showed a rapid improvement in the skin fragility, accompanied by a reduction in blister formation and at the age of 3 months presented only numerous grouped milia (Fig. S1d<sup>1</sup>).

#### Laboratory investigations

Skin biopsies obtained from patients 1–4 were processed for immunofluorescence and transmission electron microscopy. Immunofluorescence antigen mapping revealed the presence of collagen VII-positive coarse granular deposits within the cytoplasm of basal and some suprabasal keratinocytes in all patients (Fig. S2a, b<sup>1</sup>). Cytoplasmic deposits were irregularly distributed along the epidermis and their number was variable, being less numerous in patient 3. In addition, the intensity of labelling for collagen VII along the cutaneous BMZ appeared variably reduced in all patient biopsies (Fig. S2a, b<sup>1</sup>), while expression of laminin-332,  $\alpha 6\beta 4$  integrin, and 180 kDa-bullous pemphigoid antigen was comparable to that in normal control skin. Finally, the skin biopsy from the father of patient 4 showed subtle cytoplasmic staining in some keratinocytes, while the type VII collagen labelling intensity along the BMZ appeared comparable to that in control skin (Fig. S2c, d<sup>1</sup>).

Electron microscopy examination demonstrated a cleavage below the lamina densa of the cutaneous BMZ in patients 1 (Fig. S3a<sup>1</sup>) and 4, and a reduced number of poorly formed anchoring fibrils in all cases (not shown). Perinuclear inclusions located within the cytoplasm of basal and some suprabasal keratinocytes were observed in all patients. The inclusions ranged in size from 3 to 13  $\mu\text{m}$ , were bounded by rough endoplasmic reticulum (RER), and showed a finely granular content with numerous dense bodies, sometimes presenting a cross-banded pattern, typical for stellate bodies (Fig. S3<sup>1</sup>). Inclusions were more numerous in the epidermis of patients 2 and 4 and less frequent in patients 1 and 3.

Based on immunofluorescence and ultrastructural findings, a diagnosis of BDN was made in all cases and, consequently, mutational screening of *COL7A1* gene was carried out leading to the identification of mutations in all patients. Sporadic BDN cases 1 and 2 were compound heterozygous for recessive mutations. Case 1 carried the c.497dupA frameshift and p.Gly2216Glu (c.6647G>A) glycine substitution, the latter being unpublished, but annotated in the International Dystrophic Epidermolysis Bullosa Patient Registry (8) in compound heterozygosity with a nonsense mutation (Fig. S4c<sup>1</sup>). In case 2 the c.4783-1G>A splice site mutation was found in combination with the missense mutation p.Pro1699Leu. Previously unreported glycine substitution mutations were found in dominant BDN families: the p.Gly2431Val (c.7292G>T) was identified in patient 3 and his mother and p.Gly1830Arg in patient 4, his brother and father (Fig. S4a, b<sup>1</sup>).

### Literature review

A literature review revealed 41 additional cases of BDN (9–29) and 4 molecularly characterized cases that were not included in the analysis because of insufficient clinical description (24, 30) (Table S1<sup>1</sup>). Interestingly, 11 out of 44 patients (25%) presented skin defects at birth.

Denuded skin areas were localized to lower extremities in all cases, more frequently bilaterally (6 cases) and healed with atrophic scarring and in one case with pseudosyndactyly of first and second toes (20). Congenital skin defects were reported in 8 out of 13 BDN patients in whom the disease was recessively inherited, as documented by mutation identification or supported by family history. On the other hand, only one out of 16 dominantly inherited BDN cases presented pretibial skin denudation at birth. Thus, congenital skin defects appear significantly more frequent in recessive vs. dominant BDN (odds ratio (OR) 24,  $p=0.004$ ). Mucosal involvement was always limited to the oral cavity and appeared more frequent in recessively inherited BDN forms (7 out of 8 cases for whom this finding was reported), compared with the dominant ones (3 out of 9 cases with informative data) (OR 14,  $p=0.049$ ).

### DISCUSSION

Including this report of BDN, 49 cases have been described and the pathogenetic mutations have been identified in approximately half of them (Table S1<sup>1</sup>). In 3 of our 4 patients skin fragility resolved by the third month of age, in line with literature data reporting recovery within the first year of life in the majority of cases (1, 5) (Table S1<sup>1</sup>). Healed lesions presented numerous grouped milia in 3 of 4 patients, further confirming the frequent occurrence of milia in BDN (Table S1<sup>1</sup>). Nail involvement was also present in 3 patients, manifesting mainly as subungual haemorrhages. These lesions are known to occur in DEB (31), but to our knowledge have not been previously described in BDN.

Of note, 2 of our 4 patients were born with ACC. In both cases congenital skin absence was localized to the anterior aspect of the lower legs and healed leaving a sharply demarcated atrophic scar. Interestingly, minimal skin fragility persisted within the atrophic areas when blistering formation on the rest of the body had completely ceased. ACC of lower legs associated with blistering of the skin and mouth and with nail abnormalities was originally described by Bart et al. in 1966 (32), and was shown to correspond with dominant DEB (33, 34). ACC has subsequently been found to be associated with all forms of EB and show the same ultrastructural changes, supporting that ACC results from *in utero* blistering (35). A recent retrospective study of 123 DEB patients reported the occurrence of ACC in 22 of them (17.8%) (36), confirming its preferential localization to the anterior aspect of lower extremities. In their original report

on BDN, Hashimoto et al. (1) described a newborn with a transient blistering skin disease and pathognomonic ultrastructural features, in the absence of congenital skin defects. However, our literature review revealed that 9 of 40 BDN cases presented at birth skin defects localized to the lower extremities, suggestive for ACC. Interestingly, congenital skin defects appear to be more frequent in recessively inherited BDN compared with the dominant form (OR 24,  $p=0.004$ ). In addition, mucosal lesions were also more frequent in recessive vs. dominant cases.

Chiaverini et al. (36) noted that ACC presenting in mild DEB forms is frequently associated with *COL7A1* missense mutations, which fall in proximity of triple helix interruptions and could result in a less stable, thermolabile, collagen VII and thus contribute to blister formation *in utero*. Indeed, our BDN patients with ACC carry missense mutations near the 8<sup>th</sup> (p.Pro1699Leu) and next to the 13<sup>th</sup> (p.Gly2216Glu) triple helix interruption.

In our recessive BDN cases mutations c.4783-1G>A and c.497dupA, recurrent in the Italian population, combine with p.Pro1699Leu and p.Gly2216Glu, respectively, and the dominant cases bear glycine substitutions in the triple helix domain (Fig. S4<sup>1</sup>). Thus, the vast majority of molecularly characterized BDN cases (14 out of 19) (Table S1<sup>1</sup>), including our patients, have in common a mutant *COL7A1* allele coding for full-length polypeptides with an amino acid change in the triple helix domain of collagen VII. This mutation type causes misfolding of the collagenous domain and RER-retention of mutant molecules, leading to delayed/reduced collagen VII secretion. In BDN such pathomechanism is gradually overcome, protein secretion is restored and anchoring fibril function recovered. Why this occurs is still a mystery; however, it might be hypothesized that environmental factors and/or expression levels of developmentally regulated genetic modifier(s) may play a role.

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