Fetal and Pediatric Pathology

ISSN: 1551-3815 (Print) 1551-3823 (Online) Journal homepage: http://www.tandfonline.com/loi/ipdp20

Epidermolysis Bullosa with Pyloric Atresia and Aplasia Cutis in a Newborn Due to Homozygous Mutation in ITGB4

Gozdem Kayki, Davut Bozkaya, Fatih Ozaltin, Diclehan Orhan, Figen Kaymaz, Emine Korkmaz & Sule Yigit

To cite this article: Gozdem Kayki, Davut Bozkaya, Fatih Ozaltin, Diclehan Orhan, Figen Kaymaz, Emine Korkmaz & Sule Yigit (2017) Epidermolysis Bullosa with Pyloric Atresia and Aplasia Cutis in a Newborn Due to Homozygous Mutation in ITGB4, Fetal and Pediatric Pathology, 36:4, 332-339, DOI: 10.1080/15513815.2017.1324545

To link to this article: http://dx.doi.org/10.1080/15513815.2017.1324545

Published online: 30 May 2017.

FETAL AND PEDIATRIC PATHOLOGY
2017, VOL. 36, NO. 4, 332–339
https://doi.org/10.1080/15513815.2017.1324545

Taylor & Francis
Taylor & Francis Group

CASE REPORT

Epidermolysis Bullosa with Pyloric Atresia and Aplasia Cutis in a Newborn Due to Homozygous Mutation in ITGB4

Gozdem Kayki[a], Davut Bozkaya[b], Fatih Ozaltin[c], Diclehan Orhan[c], Figen Kaymaz[d], Emine Korkmaz[e], and Sule Yigit[b]

[a]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey; [b]Division of Neonatology, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey; [c]Department of Pediatric Nephrology, Nephrogenetics Laboratory, Center for Biobanking and Genomics, Hacettepe University, Ankara, Turkey; [d]Department of Histology, Hacettepe University Faculty of Medicine, Ankara, Turkey; [e]Nephrogenetics Laboratory, Hacettepe University Faculty of Medicine, Sihhiye, Ankara, Turkey

ABSTRACT

Background: Epidermolysis bullosa with pyloric atresia (EB-PA) is an autosomal recessive disorder due to mutations in ITGA6 and/or ITGB4, resulting in altered expression of $\alpha 6\beta 4$ integrin. EB-PA can also occur with aplasia cutis. *Case report*: We present a newborn with EB-PA and aplasia cutis, born of consanguineous parents, with a homozygous c.3793+1G>A mutation affecting ITGB4, previously described only in the heterozygous state with other mutations. *Conclusion*: The previously unreported homozygous c.3793+1G>A mutation affecting ITGB4 causes a severe form of junctional epidermolysis bullosa with pyloric atresia and aplasia cutis.

ARTICLE HISTORY
Received 28 January 2017
Revised 24 March 2017
Accepted 28 March 2017

KEYWORDS
Junctional epidermolysis bullosa; pyloric atresia; newborn; mutation in ITGB4

Introduction

Junctional epidermolysis bullosa with pyloric atresia (JEB-PA) (MIM #226730) is an autosomal recessive disease, called Carmi Syndrome previously. Mutations in *ITGA6* (MIM #147556) and *ITGB4* (MIM# 147557) cause an altered expression of $\alpha 6\beta 4$ integrin resulting in a structural defect of hemidesmosome [1]. The defective hemidesmosomes results in blisters and erosions on skin, gastrointestinal and urogenital tract abnormalities. Some patients with JEB-PA may have also aplasia cutis congenita (ACC). A small number of cases with JEB-PA and ACC have been published [2– 4]. We report a newborn with JEB-PA and ACC whose molecular analysis showed homozygous *ITGB4* mutation.

Case report

A full-term female infant was born by spontaneous vaginal delivery to a 20-year-old gravida 1, para 1 mother. Apgar score at 1 minute was 9 and birth weight was 2450 g. The parents were first-degree cousins. On prenatal ultrasonography gastric dilatation was described. The baby who was sent from a local hospital was admitted to the neonatal intensive care unit. On admission the vital signs were normal. On physical examination, skin was absent from right and left toes to middle of thighs (Fig. 1). She had multiple bullous lesions on both hands, near the umbilicus, and the face, along with dystrophic nails.

CONTACT Gozdem Kayki gozdem.kayki@hacettepe.edu.tr Department of Pediatrics, Hacettepe University, Ankara 06xxx, Turkey.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ipdp.

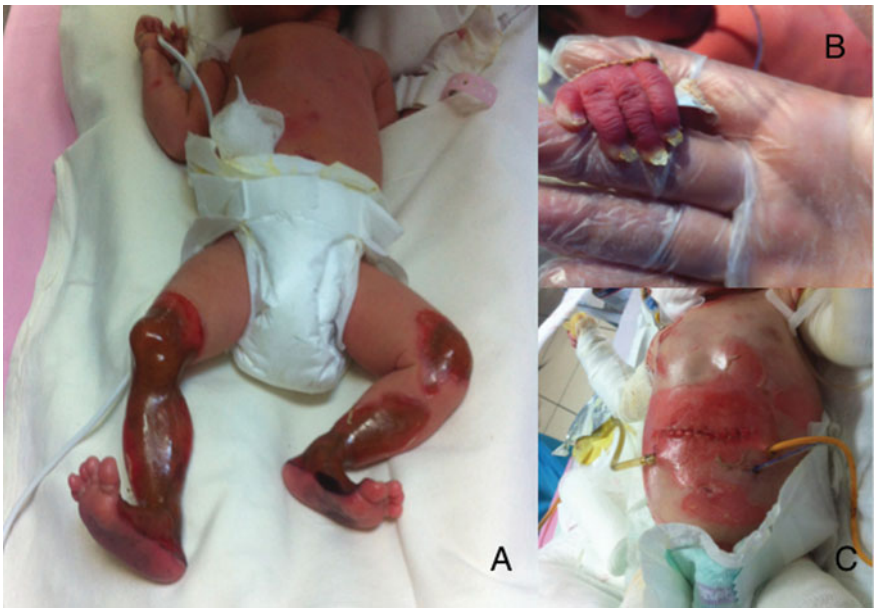

Figure 1. (A) aplasia cutis from right and left toes to middle of thighs; (B) dystrophic nails; (C) multiple bullous lesions on both hands, near the umbilicus after surgery.

Levels of blood urea nitrogen (27.7 mg/dL) and creatinine (1.38 mg/dL) were elevated because of the increasing loss of fluid from absence or damaged skin, so intravenous hydration was increased. Hydronephrosis in the left kidney was detected by abdominal ultrasonography. Echocardiography and cranial ultrasonography were normal. On the first day, total parenteral nutrition was started. Because of respiratory failure, the patient was placed on nasal continuous positive airway pressure (CPAP). Antibiotic therapy was administered due to infected skin lesions. On the 7th day of life pyloroplasty was performed. On postoperative 5th day, physical examination revealed gastric distention and on X-ray there was free air under the right side of diaphragm. She underwent a second surgery for an anastomotic leak. Gastrostomy and jejunostomy were also performed at the same time. On postoperative day 17, enteral nutrition was started, however, could not be increased due to emesis. During follow-up the patient received analgesic treatment because of increasing bullous lesions. She had also hypoalbuminemia, so protein in parenteral nutrition was increased and intravenous albumin was administered four times per day.

Sanger sequencing of all coding exons and adjacent intronic boundaries of *ITGB4* showed a homozygous donor splice site mutation (c.3793+1G>A). Both parents were heterozygous for the same mutation.

On the 33th day she died. Postmortem skin biopsy was performed. The skin biopsy sample was fixed in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4 for 4 hours and postfixed for 1 hour in 1% osmium tetroxide solution in 0.1 M phosphate buffer. After washing in phosphate buffer, they were dehydrated in a graded series of ethanols to absolute ethanol, treated with propylene oxide and embedded in Araldite/Epon812 (Cat No: 13940, EMS, Hatfield, PA, USA). After heat polymerization, sections were cut using a microtome. Semi-thin sections were stained with methylene blue–azure II and examined using a light microscope (Leica DM 6000B, Wetzlar, Germany) with a DC490 digital camera (Leica, Wetzlar, Germany). Ultra-thin sections (Leica ultra-cutR, Wetzlar, Germany) were double-stained with uranyl acetate and lead citrate (Leica EM AC20). These sections were examined in JEOL-JEM 1400 electron microscope and photographed by CCD camera (Gatan Inc., Pleasanton, CA, USA).

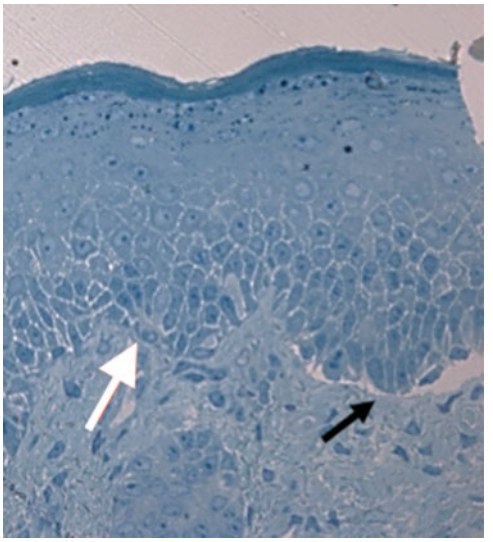

Figure 2. Light microscopic examination of a skin biopsy demonstrated dermal-epidermal separation (black arrow). Methylenblue - Azur II, x400. (White arrow shows basal membrane.)

Light microscopic examination of a skin biopsy demonstrated perilesional intact skin and cleavage at the dermal-epidermal junction (Fig. 2). Electron microscopic examination demonstrated perilesional intact dermal epidermal junction along with the area of epidermal dermal separation. In perilesional skin, hemidesmosomal plaques were reduced in size and number, keratin intermediate filaments association was disrupted (Fig. 3). Anchoring fibrils were present but markedly decreased. Lamina densa and lamina lucida of the basal lamina were continuous at the dermal epidermal junction. Beginning from the edge of the lesion electron microscopy revealed a split in the lamina lucida (Fig. 4), absence of hemidesmosomal plaques and anchoring fibrils (Fig. 5). Lamina densa was continuous beneath the cleavage. Immunofluorescence microscopy was not performed because EM and genetic studies were performed.

Discussion

Epidermolysis bullosa (EB) is a heterogeneous group of genodermatoses. Defect of cutaneous basal membrane attachment structures cause blisters and erosions of the skin. These lesions

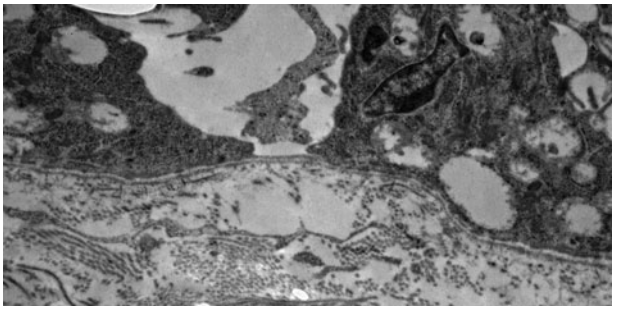

Figure 3. Electron micrograph showing perilesional intact skin. Hemidesmosomal plaques are reduced in size and number. Anchoring fibrils are present but markedly decreased. Lamina densa and lamina lucida of the basal lamina are continuous in the dermal epidermal junction. Uranyl acetate andlead citrate, x20000.

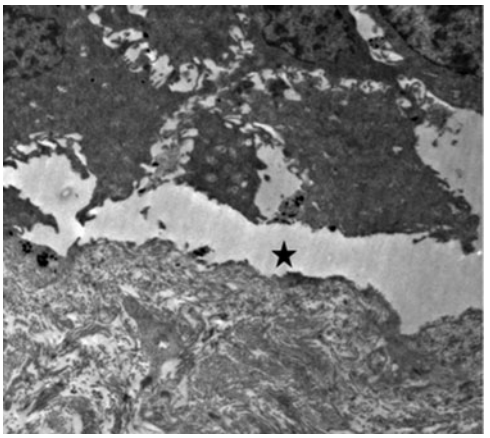

Figure 4. Electron microscopy revealed a split in the lamina lucida (star) and absence of anchoring fibrils and hemidesmosomal plaques. Uranyl acetate and leas citrate, x5000.

are characteristics of epidermolysis bullosa [2]. Epidermolysis bullosa has four major subgroups based on the ultrastructural level within which blisters develop. These groups are named: simplex (epidermolytic), junctional (lucidolytic), dystrophic (dermolytic) epidermolysis bullosa and Kindler syndrome (multiple layers) [5, 6]. Biopsy specimens must be confirmed by immunofluorescence mapping (IFM) and/or transmission electron microscopy (EM) in every patient for this classification. So far, data collection and analysis have been done on several thousand patients with EB worldwide, and more than 1000 mutations and more than 10 structural genes have now been published [6].

The frequency of recessive forms of EB is estimated at 1 in 300,000 [1]. Pyloric atresia frequency is 1 per 100,000 live births [1, 3]. So the relationship between these two rare conditions which are seen together are not coincidental. Familial pyloric atresia associated with epidermolysis bullosa was first described in 1968 by Swinburne and Kohler [3, 7, 8]. Pathophysiology was first described by Carmi so the disease has been also called "Carmi syndrome". Pyloric atresia in EB is a result of scarring. Dysfunctional or absent hemidesmosomes result in the separation of the epidermis or the intestinal mucosa, with resultant inflammation and fibrosis, leading to obstruction in areas like the pylorus [2].

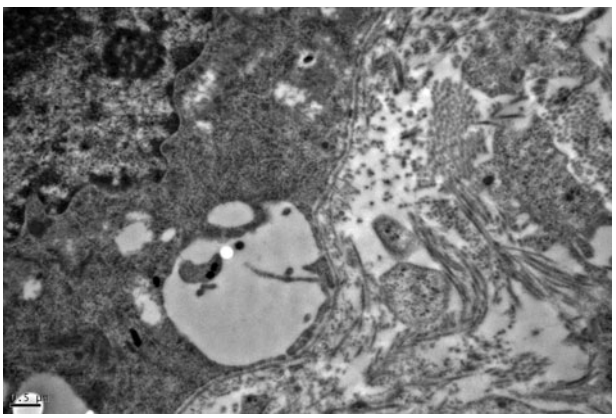

Figure 5. Electron micrograph showing perilesional intact skin. Hemidesmosomal plaques are reduced in size and number. Anchoring fibrils are present but markedly decreased. Uranyl acetate andlead citrate, x20000.

JEB-PA is autosomal recessively inherited. There is an altered expression of $\alpha 6\beta 4$ integrin. Mutations in *ITGA6* and *ITGB4* cause an altered expression of $\alpha 6\beta 4$ integrin resulting in a structural defect of hemidesmosome. Hemidesmosomes serve as anchoring sites for the intermediate filament system and have an important role in stabilizing the association of the dermis with the epidermis [9]. They provide keratinocytes adhesion to the basement membrane. The transmembrane components of hemidesmosomes include integrin $\alpha 6\beta 4$ and the bullous pemphigoid antigen 180. The $\alpha 6\beta 4$ integrin is found in both type 1 and 2 hemidesmosomes. Type 1 and 2 hemidesmosomes are seen in many epithelial tissues, including human skin and the gastrointestinal tract [10]. Therefore gastrointestinal, urinary, pulmonary, and eye disorders can be associated with EB-PA [3]. We also evaluated the patient for other malformations. Indeed she had no urinary tract malformation, but she suffered from respiratory insufficiency thus nasal CPAP had to be applied.

Aplasia cutis congenital (ACC) with EB-PA has been previously described [2–4]. There are few reports of JEB-PA-ACC in the literature (Table 1). Intrauterine mechanical trauma may cause ACC, but also the combination of ACC and EB-PA has suggested a common genetic basis of their pathogenesis [1, 2]. Literature shows that JEB-PA-ACC is most often lethal and suggests that ACC is a poor prognostic factor for JEB-PA patients. We think if the disease is severe, lesions can occur intrauterinally and it may cause ACC. Therefore JEP-PA with ACC may be the more severe end of the spectrum.

Histological examination of blistered skin demonstrates the dermo-epidermal cleavage at the level of the lamina lucida. Hemidesmosomes may be morphologically abnormal. Moreover, immunofluorescent analysis show altered expression of $\alpha 6\beta 4$ [11]. In this case, the findings of light and electron microscopy findings supported the diagnoses.

Mutation analysis showed a homozygous mutation in *ITGB4*. Mutations in *ITGA6* are less frequent than those in *ITGB4* [10]. There are more than 80 mutations that cause EB-PA in the literature. Some of them are lethal and others are nonlethal. Mutation analysis and immunofluorescence examination are useful for diagnosis and prognosis. In lethal cases $\alpha 6\beta 4$ integrin expression is absent [12]. In our case, c.3793+1G>A mutation has been identified. This mutation was first demonstrated in 1997 by Pulkkinen et al. [12, 13]. The authors have shown that the mutation causes frame-shift and premature termination codon of translation and reduction of mRNA levels and lead to a lethal form of the disease. Heterozygosity of this splice site mutation was defined in 1998 by Mellerio et al. [14]. In their case, one of the patients with EB-PA was a compound heterozygote for a splice site mutation (3793 1G-to-A) and a non-sense mutation (W1478X) in *ITGB4*. The patient had blisters and erosions on his limbs, dystrophic nails and pyloric atresia. He underwent a corrective gastroduodenostomy on the 6^{th} day. Renal symptoms (hematuria and dysuria) started when he was 3 years old. Bladder wall hemorrhage, blistering, and bilateral ureteric reflux developed. When he was 5 years old, a vesicostomy was performed. He had small blisters in his skin, nail dystrophy, enamel hypoplasia and abnormal dentition. He had a potentially lethal mutation but it illustrate that heterozygosity of the same splice mutation causes milder phenotype than patients with homozygous mutations.

EB-PA-ACC is known to be a non-treatable condition and its prognosis is poor. However, identification of underlying genetic abnormality is critical to give appropriate genetic counseling and prenatal or preimplantation genetic diagnosis. In lethal cases even if intestinal obstructions are corrected successfully, patients suffer from septicemia, electrolyte imbalance, protein loses, and hypoalbuminemia related to loss of skin barrier [15]. Intestinal surgery was not beneficial to our patient because of the complications didn't add any beneficial effect in our patient who suffered from complications. As it was not possible to enterally feed this baby,

Table 1. Literature review of JEB-PA patients with ACC.

Reference	Case no	GA/sex	Consanguineous parents	Clinical features	Mutations	Exitus at age of
Carmi et al. [4]	1	29 weeks/F	Yes	aplasia cutis congenita	—	36 hours
	1 (sibs)	30 weeks/M	Yes	pyloric atresia, aplasia cutis congenital, ear abnormalities, hypoplastic fingernails	—	3 days
Cowton et al. [17]	2	38 weeks/F	—	pyloric atresia, blisters, aplasia cutis congenita	—	43 hours
Achiron et al. [18]	3	35 weeks/F	—	pyloric atresia, blisters, aplasia cutis congenital, renal abnormalities	—	6 days
		37 weeks/M				
Shaw et al. [19]	4	28 weeks/M	—	pyloric atresia, blisters, aplasia cutis congenita	—	16 days
Maman et al. [2]	5	29 weeks/F	—	Only first case without pyloric atresia. All other patients have pyloric atresia, blisters, aplasia cutis congenital.	—	Three were fetuses. All other patients died 3 to 7 days after birth.
		30 weeks/M				
		36 weeks/F				
		27 weeks/F				
		34 weeks/F				
		30 weeks/M				
		37 weeks/M				
		23 weeks/M				
Nawaz et al. [20]	6	35 weeks/M	Yes	pyloric atresia, blisters, aplasia cutis congenita	—	27 days
Nakamura et al. [21]	7	39 weeks/M	No	pyloric atresia and polyhydramnios antenatally, blisters, aplasia cutis congenita	PLEC1 (heterozygous c.913C >T and c.1344C >T)	At age of 16 months, still required intensive care
	7 (sibs)	-/M	No	pyloric atresia, blisters, aplasia cutis congenita	PLEC1	4 months
	8	36 weeks/F	No	pyloric atresia and polyhydramnios antenatally, blisters, aplasia cutis congenita	PLEC1 (homozygous c.3565C > T and heterozygous c.7612C > T)	31 days
Stoevesandt et al. [22]	9	26 weeks/F	No	pyloric atresia, blisters, aplasia cutis congenita	ITGB4 (heterozygous c.600dupC and c.2533C > T)	18 days
Merrow et al. [23]	10	35 weeks/-	—	pyloric atresia, aplasia cutis congenita	PLEC1 (heterozygous)	18 days
This case	11	40 weeks/F	Yes	pyloric atresia, aplasia cutis congenital, blisters, dysplastic fingernails	ITGB4 (homozygous c.3793+1G > A)	33 days

GA: gestational age, F: female, M: male, -:unknown, sibs: siblings.

parenteral nutrition was required. Moreover, she had infected skin lesions and abdominal leak from the anastomosis. These co-morbid conditions resulted in a death.

There are a few patients who survive the neonatal period. In a study by Nakano et al, seven patients were investigated [10]. Four of them died during the first months of life and they were classified as lethal variants. Other three of them were alive and classified as nonlethal variants. In nonlethal cases skin lesions tends to improve with years [16].

This is the first case of a homozygous c.3793+1G>A mutation affecting ITGB6, previously described only in the heterozygous state with other mutations. This confirms the severity of this mutation.

Declaration of interest

The authors have no conflicts of interest relevant to this article.

ORCID

Fatih Ozaltin http://orcid.org/0000-0003-1194-0164

References

[1] Chung HJ, Uitto J. Epidermolysis bullosa with pyloric atresia. Dermatol Clin. 2010;28(1):43–54.
[2] Maman E, Maor E, Kachko L, Carmi R. Epidermolysis bullosa, pyloric atresia, aplasia cutis congenita: histopathological delineation of an autosomal recessive disease. Am J Med Genet. 1998;78:127–133.
[3] Birnbaum RY, Landau D, Elbedour K, Ofir R, Birk OS, Carmi R. Deletion of the first pair of fibronectin type III repeats of the integrin beta-4 gene is associated with epidermolysis bullosa, pyloric atresia and aplasia cutis congenita in the original Carmi syndrome patients. Am J Med Genet A. 2008;146A(8):1063–6.
[4] Carmi R, Sofer S, Karplus M, Ben-Yakar Y, Mahler D, Zirkin H, et al. Aplasia cutis congenita in two Sibs discordant for pyloric atresia. Am J Med Genet. 1982;11:319–328.
[5] Fine JD, Eady RAJ, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A, et al. The classification of inherited epidermolysis bullosa (EB): report of the third international consensus meeting on diagnosis and classification of EB. J Am Acad Dermatol. 2008;58(6):931–950.
[6] Fine JD. Inherited Epidermolysis bullosa: recent basic and clinical advances. Curr Opin Pediatr. 2010;22(4):453–8.
[7] Pulkkinen L, Uitto J. Mutation analysis and molecular genetics of epidermolysis bullosa. Matrix Biology. 1999;18:29–42.
[8] Swinburne LM, Kohler HG. Familial pyloric atresia associated with epidermolysis bullosa. J Pediatr. 1979;94(1):162.
[9] Geuijen CAW, Sonennberg A. Dynamics of the $\alpha 6\beta 4$ Integrin in Keratinocytes. Mol Biol Cell. 2002;13(11):3845–58.
[10] Nakano A, Pulkkinen L, Murrell D, Rico J, Lucky AW, Garzon M, et al. Epidermolysis Bullosa with congenital pyloric atresia: novel mutations in the $\beta 4$ integrin gene (ITGAB4) and genotype/phenotype correlations. Ped Res. 2001;49(5):618–626.
[11] Vidal F, Aberdam D, Miguel C, Christiano AM, Pulkinnen L, Uitto J, Ortonne JM, Meneguzzi G. Integrin $\beta 4$ mutations associated with junctional epidermolysis bullosa with pyloric atresia. Nat Genet. 1995;10:229–234.
[12] Pulkkinen L, Rouan F, Bruckner-Tuderman L, Wallerstein R, Garzon M, Brown T, et al. Novel ITGAB4 mutations in lethal and nonlethal variations of epidermolysis bullosa with pyloric atresia: missense versus nonsense. Am J Hum Genet. 1998;63:1376–1387.
[13] Pulkkinen L, Kurtz K, Xu Y, Bruckner-Tuderman L, Uitto J. Genomic organization of the integrin beta 4 gene (ITGB4): a homozygous splice-site mutation in a patient with junctional epidermolysis bullosa associated with pyloric atresia. Lab Invest. 1997;76(6):823–33.

[14] Mellerio JE, Pulkkinen L, McMillan JR, Lake BD, Horn HM, Tidman MJ, et al. Pyloric atresia-junctional epidermolysis bullosa syndrome: mutations in the integrin $\beta 4$ gene (ITGB4) in two unrelated patients with mild disease. Br J Derma. 1998;139:862–871.
[15] Diociaiuti A, Castiglia D, Morini F, Boldrini R, Fortugno P, Zambruno G, El-Hachem M. Long-term follow-up a spontaneously improving patient with junctional epidermolysis bullosa associated with ITGB4 c.3977-19T>A splicing mutation. Acta Derm Venereol. 2013;93:116–118.
[16] Dang N, Klinberg 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.
[17] Cowton JAL, Beattie TJ, Gibson AAM, Mackie R, Skerrow CJ, Cockburn F. Epidermolysis bullosa in association with aplasia cutis congenital and pyloric atresia. Acta Pediatr Scand. 1982;71:155–160.
[18] Achiron R, Hamiel-Pinchas O, Engelberg S, Barkai G, Reichman B, Mashiach S. Aplasia cutis congenital associated with epidermolysis bullosa and pyloric atresia: the diagnostic role of prenatal ultrasonography. Prenatal Diagnosis. 1992;12:765–771.
[19] Shaw DW, Fine JD, Piacquadio DJ, Greenberg MJ, Wang-Rodriguez J, Eichenfield LF. Gastric outlet obstruction and epidermolysis bullosa. J Am Acad Dermatol. 1997;36:304–310.
[20] Nawaz A., Matta H., Jacobsz A., Al-Salem A. Congenital pyloric atresia and junctional epidermolysis bullosa: a report of two cases. Pediatr Surg Int. 2000;16:206–208.
[21] Nakamura H, Sawamura D, Goto M, Nakamura H, McMillan JR, Park S, Kono S, Hasegawa S, Paku S, Nakamura T, Ogiso Y, Shimizu H. Epidermolysis bullosa simplex associated with pyloric atresia is a novel clinical subtype caused by mutations in the plectin gene (*PLEC1*). J Mol Diagnos. 2005;7:28–35.
[22] Stoevesandt J, Borozdin W, Girschick G, Hamm H, Höcht B, Kohlhase J, Volz A, Wiewrodt B, Wirbelauer J. Lethal junctional epidermolysis bullosa with pyloric atresia due to compound heterozygosity for two novel mutations in the integrin $\beta 4$ Gene. Klin Padiatr. 2012;224:8–11.
[23] Merrow AC, Frischer JS, Lucky AW. Pyloric atresia with epidermolysis bullosa: fetal MRI diagnosis with postnatal correlation. Pediatr Radiol. 2013;43:1656–1661.