

Pyloric atresia-junctional epidermolysis bullosa syndrome showing novel c.4505-4508insACTC mutations in integrin b4 gene (ITGB4)

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SUMMARY: Mutlu M, Kalay E, Dilber B, Aslan Y, Dilber E, Almaani N, McGrath JA. Pyloric atresia-junctional epidermolysis bullosa syndrome showing novel c.4505-4508insACTC mutations in integrin b4 gene (ITGB4). Turk J Pediatr 2015; 57: 385-387.

Epidermolysis bullosa (EB) is a group of inherited blistering skin diseases that vary widely in their pathogenesis and severity. It has been divided into distinct subtypes depending on the level of tissue separation in the dermal-epidermal basement membrane zone. There are four main categories of EB: simplex, junctional, dystrophic and Kindler syndrome. Junctional epidermolysis bullosa with pyloric atresia (JEB-PA) is a rare autosomal recessive form and characterized by severe mucocutaneous blisters and gastric outlet obstruction. Most of the mutations in JEB-PA are associated with the $\alpha 6\beta 4$ integrin genes (*ITGA6*, *ITGB4*). Herein, we present a female newborn with lethal JEB-PA caused by a novel beta4 integrin mutation.

Key words: epidermolysis bullosa, pyloric atresia, mutation, newborn.

Epidermolysis bullosa (EB) is an inherited, bullous disease, characterized by blisters followed with skin and mucosal erosions. Its severity ranges from mild to lethal. Depending on the level of tissue separation in the dermal-epidermal basement membrane zone EB is classified four major categories such as EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome¹. *Junctional epidermolysis bullosa with pyloric atresia* (JEB-PA) is a rare autosomal recessive form and characterized by severe mucocutaneous blisters and gastric outlet obstruction. Herein, a novel homozygous *ITGB4* mutation (c.4505-4508insACTC) leading to premature translation termination (p.Thr1504LeufsX69) was described in a newborn with JEB-PA.

Case Report

A one-day-old female infant was referred to our neonatal intensive care unit with symptoms of non-bilious vomiting, non-sucking and bullous

skin lesions. The infant was born from a gravida 3, para 1 (G3P1) mother by caesarian section at 37 weeks' gestation with a birth weight of 2850 g. Antenatal ultrasonography examination revealed polyhydramnios. Her parents were cousins. The family history of the mother revealed one previous in-utero exitus and one normal delivery. Both of those children had pyloric atresia and skin lesions. The baby born from the second pregnancy died due to sepsis in the first month of life.

Examination of our case revealed blistering skin lesions (Fig. 1A). Mucosal lesions were seen in the mouth. The upper abdomen was mildly distended. Abdominal X-ray showed a single prominent gastric air bubble and lack of air distally (Fig. 1B). Renal ultrasonography and hematological and biochemical analysis were normal. Heineke-Mikulicz pyloroplasty was performed for pyloric atresia on the fifth day of life. The patient's general condition deteriorated progressively during 58 days of

life, and she died due to septic shock.

Mutation Analysis

Genomic DNAs of family members were extracted from peripheral blood lymphocytes as described by Miller et al². Mutation screening on *ITGB4* (GenBank accession number: NM_000213.3) were carried out by Sanger sequencing. Co-segregation of c.4505-4508insACTC mutation with disease was tested by amplification refractory mutation system (ARMS). Primers sequence and amplification conditions are available on request.

As a result of mutation analyses, a homozygous four base-pair insertion (c.4505-4508insACTC) was found in *ITGB4*, which leads a frame shift and premature translation termination (p.Thr1504LeufsX69) that causes loss of last two Fibronectin type 3 domains of the protein in the patient. ARMS revealed that parents were heterozygous carrier for the mutation and her uncle was homozygous normal genotype (III: 1) (Fig. 2).

Discussion

Junctional epidermolysis bullosa with pyloric atresia is an autosomal recessive blistering disease including lethal and non-lethal variants. Most of the mutations in JEB-PA are associated with the $\alpha 6\beta 4$ integrin genes (*ITGA6*, *ITGB4*). In JEB, the tissue separation takes place within the lamina lucida¹. In this study, we have determined a novel homozygous 4-bp insertion mutation (c.4505-4508insACTC) in *ITGB4* in a newborn with lethal EB with pyloric atresia.

ITGB4 encodes a transmembrane protein $\beta 4$ integrin. The extracellular amino site of $\beta 4$ integrin contains four cysteine rich

motifs and the intracellular carboxyl site of the protein consists of two pairs of fibronectin type III (FNIII) repeats spaced by a connecting segment and a calx- β motif³. Integrin $\beta 4$ associated with only integrin $\alpha 6$ to form a laminin receptor $\alpha 6\beta 4$ integrin which is predominantly expressed in stratified squamous and transitional epithelial cells, such as keratinocytes, endothelial cells and epithelial cells lining of the gastrointestinal, respiratory and genitourinary tracts⁴. The $\beta 4$ integrin participate in the assembly and stabilization of the specialized structure called hemidesmosomes⁵. Hemidesmosomes are adhesion structures that mediate the link between the intermediate filament cytoskeleton of epithelial cells and the underlying basement membrane. The intracellular site of the $\beta 4$ subunit of $\alpha 6\beta 4$ integrin is playing crucial role for interaction with other component of the hemidesmosomes such as plectin, bullous pemphigoid antigen 180 (BP-180) and bullous pemphigoid antigen 230 (BP-230)⁵⁻⁸. Interaction of $\beta 4$ integrin with BP180 and BP-230 is carried out by last pair of FNIII domains⁵⁻⁷. In addition to protein-protein interaction, last pair of FNIII domains also contributes the signal transduction upon MAP kinase ERK and Jnk signaling pathways (FER EKLE)⁹. The

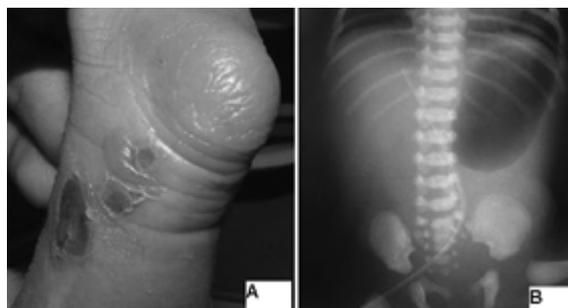


Fig. 1. A. Clinic appearance of the epidermolysis bullosa B. Radiologic appearance of the pyloric atresia

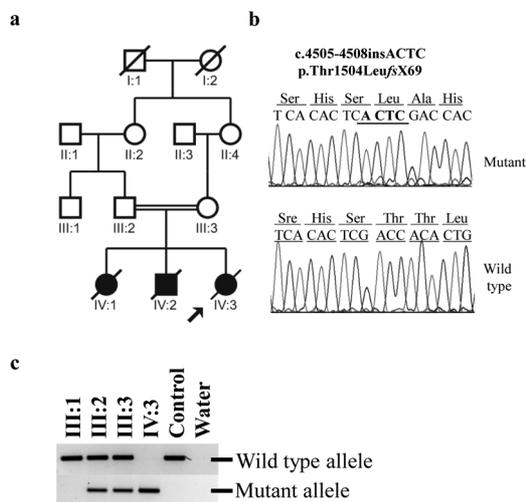


Fig. 2. Family pedigree and mutation analysis of *ITGB4*. a. Family pedigree of the patient. Arrow indicates the index patient. b. Sequence chromatogram showing the homozygous c.4505-4508insACTC mutation (upper panel) and wild-type sequence (lower panel). c. Segregation of c.4505-4508insACTC with disease in family is shown by ARMS. The wild-type allele is shown in the upper panel and the mutant allele in the lower panel.

four-base insertion mutation of *ITGB4* which we presented here causes the frame shift and premature translation termination codon (p.Thr1504LeufsX69) resulted with deletion of last two FNIII domains of $\beta 4$ integrin which are crucial for hemidesmosome assembly and signaling transduction. Consistent with our finding deletion of the last two FNIII domain of the $\beta 4$ integrin was associated with JEB-PA in several patients¹⁰⁻¹².

In summary, the present study's findings show that lethal JEB-PA was caused by a novel $\beta 4$ integrin mutation.

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