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Splicing abnormality of integrin β4 gene (ITGB4) due to nucleotide substitutions far from splice site underlies pyloric atresia-junctional epidermolysis bullosa syndrome☆

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ABSTRACT

Background: Pyloric atresia-junctional epidermolysis bullosa syndrome (PA-JEB) is a rare subgroup of epidermolysis bullosa, which is inherited disorder characterized by skin fragile. PA-JEB is caused by mutation of ITGB4 or ITGA6, which encodes integrin β4 or α6, respectively.
Objective: To clarify the molecular basis of PA-JEB and to expand the mutational database, we carried out the mutational analysis of a 29-year-old Japanese PA-JEB patient.
Methods: Standard methods were used to prepare, PCR-amplify, and sequence DNA or mRNA in peripheral blood or skin samples, respectively.
Results: Sequence analysis revealed two novel mutations in ITGB4, c.264+2TtoA and c.1762−25TtoA. The paternal c.264+2TtoA resided within a splice site consensus region and generated two splice variants resulting in a premature termination codon (PTC). The maternal c.1762−25TtoA was a unique mutation because of its location, 25 bp away from the splice site, and resided in branch-point consensus sequence. This c.1762−25TtoA substitution resulted in generation of two abnormal transcripts each with a PTC. Genotype–phenotype correlation in this case was also unique because the proband showed a non-lethal phenotype regardless of both mutations resulted in only abnormal transcripts with a PTC.
Conclusion: The present case expands the mutational database and further elucidates the genotype–phenotype correlation for this rare disease, PA-JEB.

1. Introduction

Epidermolysis bullosa (EB) is an inherited skin disorder characterized by blister formation at the epidermal basement membrane zone in response to minor mechanical trauma [1]. Junctional EB, one of subgroup of EB, is characterized by blister formation within the lamina lucida of basement membrane and is mainly caused by mutations in any of six genes, LAMA3, LAMB3, LAMC2, COL17A1, ITGB4, or ITGA6, that encode basement membrane components playing crucial roles in adhering the epidermis to the underlying dermis [1,2]. Pyloric atresia-junctional EB syndrome (PA-JEB, OMIM #226730) is a subtype of junctional EB and is caused by mutations of ITGB4 or ITGA6, which encodes integrin β4 or α6, respectively [1,3]. Atypical cases of PA-JEB have also been reported; Inoue et al. [4] reported non-Herlitz JEB without PA caused by ITGB4 mutation, and Salvestrini et al. [5] described a case involving PA without skin disease caused by ITGB4 mutation. EB simplex, defined as tissue separation within basal keratinocyte, with PA was also reported to be caused by mutation of the plectin gene (PLEC1), which is the gene responsible for EB simplex associated with muscular dystrophy [6,7].

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A large number of PA-JEB-causing mutations reside in ITGB4. The database reported by Chung et al. [3] showed that 70 of PA-JEB-causing mutations were in ITGB4, but only 5 were in ITGA6. A generally accepted rule for the genotype–phenotype correlation is usually applicable to PA-JEB; specifically, a patient with a premature termination codon (PTC) mutation in each allele tends to show a relatively severe phenotype, and a patient harboring a missense mutation on either allele tends to show a less severe phenotype [8–10]. However, some cases represent exceptions to this rule; for example, non-lethal PA-JEB harboring PTC mutations in both alleles was reported [11]. Expanding the repertoire of PA-JEB-causing mutation is necessary to understanding precise genotype–phenotype correlation in this rare form of EB.

Here we showed the abnormal splices leading to PTC-containing ITGB4 transcripts resulted in a non-lethal PA-JEB phenotype, one mutation was in splicing consensus region and the other was an intronic nucleotide substitution far from a splice site.

2. Materials and methods

2.1. Patient characteristics

The proband was a 29-year-old Japanese male; he was the first child of healthy unrelated parents. Blisters and erosions were seen mainly on the extremities with pigmentation and mild scar formation (Fig. 1A). Hair, teeth, and oral mucous membrane were normal. All nails showed dystrophic change. He underwent tracheotomy during his school years. By careful history-taking from his parents, it became clear that he had undergone surgery for congenital pyloric atresia. A skin biopsy was taken from a blister on the left forearm. Immunofluorescence study of this tissue sample showed markedly reduced staining with the GoH3 monoclonal antibody against integrin α6 and the 3E1 monoclonal antibody against integrin β4 at the basement membrane zone (Fig. 1B). Other epidermal basement membrane components, including laminin 332, type VII, XVII, and IV collagens, and bullous pemphigoid antigen 1 (BPAG1), were expressed normally. Electron microscopic examination of this tissue sample revealed hemidesmosomes with thin attachment plaques on the blister roof and the lamina densa on the blister floor at epidermal basement membrane zone; these findings demonstrated that the blister formed at the lamina lucida (Fig. 1C).

2.2. Mutation search in genomic DNA

Genomic DNA was isolated from peripheral blood and subjected to mutation search in ITGB4 (GenBank Accession NG_007372.1). Previously described primer sets were used to PCR amplify all ITGB4 exon and the respective flanking regions [12]. PCR products were then subjected to direct sequencing. The mutations were verified by restriction enzyme digestion.

2.3. RT-PCR and mutation search in RNA

RNA extracted from skin sample taken from the proband was then used for mutational analysis because only one mutation in ITGB4 was identified *via* mutational analysis of genomic DNA, as mentioned below. An RNeasy Micro kit (Qiagen KK, Tokyo, Japan) was used to extract total RNA from the skin; the Super-Script™ First-Strand Synthesis System for RT-PCR (Invitrogen, Carsbad, CA,

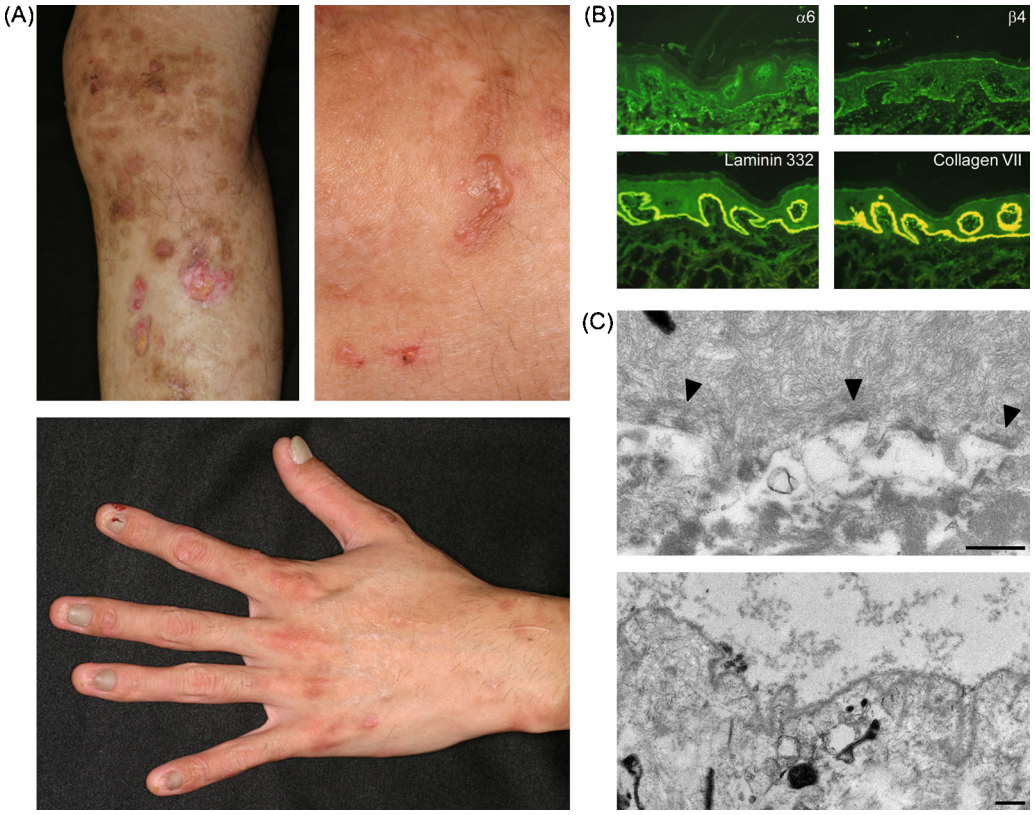

Fig. 1. Clinical, immunohistochemical, and ultrastructural features of the proband. (A) The proband presented with blisters and skin erosions with pigmentation and mild scar formation mainly on the extremities. Dystrophic changes on each nail were also seen. (B) Immunofluorescent examination revealed markedly reduced integrins α6 and β4 staining along the epidermal basement membrane zone of the patient skin. In contrast, laminin 332 and type VII collagen staining were apparently normal in patient skin. (C) The ultrastructural examination revealed immature hemidesmosomes (arrowheads) and lamina densa on the roof and floor, respectively, of the blister. Bars = 0.5 μm.