GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

A novel *COL7A1* mutation in a Korean patient with Hallopeau-Siemens recessive dystrophic epidermolysis bullosa

J. Park[1], H. Chae[1], M. Kim[1]*, Y. Kim[1], I.Y. Park[2]*, J.C. Shin[2] and Y.M. Park[3]

[1]Department of Laboratory Medicine, College of Medicine, Catholic University of Korea, Seoul, Korea
[2]Department of Obstetrics and Gynecology, College of Medicine, Catholic University of Korea, Seoul, Korea
[3]Department of Dermatology, College of Medicine, Catholic University of Korea, Seoul, Korea

*These authors contributed equally to this study.
Corresponding authors: M. Kim / I.Y. Park
E-mail: microkim@catholic.ac.kr / oooooobbbbb@catholic.ac.kr

Genet. Mol. Res. 12 (1): 678-682 (2013)
Received February 27, 2012
Accepted August 22, 2012
Published March 11, 2013
DOI http://dx.doi.org/10.4238/2013.March.11.15

ABSTRACT. Dystrophic epidermolysis bullosa (DEB) is an inherited skin fragility disorder that presents various clinical manifestations. DEB is characterized by separation of sublamina densa tissue and abnormalities in the anchoring fibrils that result from mutations in *COL7A1* and subsequent defects in type VII collagen. A 16-month-old boy was diagnosed with Hallopeau-Siemens recessive DEB on the basis of typical skin lesions composed of multiple blisters with moderately healed erosions, scarring on trauma-exposed body sites, including hands and feet, pseudosyndactyly and flexion contractures of the toes, and severely dystrophic nails on the right hand. Genomic DNA from the patient and parents were subjected to direct sequencing for the *COL7A1* gene. Two heterozygous mutations were detected in the affected child; one novel mutation designated c.4232delC in exon 38 and a single-base

substitution (c.6573+1G>C) in intron 81. Deletion of a single cytosine at codon 1411 within exon 38 had produced a frameshift mutation that created a stop codon at codon 1427 (p.Pro1411Leufs*17). This intronic base substitution had led to aberrant splicing and a premature termination codon. This is a novel mutation of *COL7A1* associated with DEB in a Korean patient, adding to the range of *COL7A1* mutations related to DEB.

Key words: Hallopeau-Siemens recessive dystrophic epidermolysis bullosa; *COL7A1*; Mutation analysis

INTRODUCTION

Dystrophic epidermolysis bullosa (DEB) is an inherited skin fragility disorder that presents various clinical manifestations. DEB is characterized by separation of sublamina densa tissue and abnormalities in the anchoring fibrils (AFs) that result from mutations in *COL7A1* and subsequent defects in type VII collagen. The clinical features of DEB cover a broad range of severity, from isolated nail dystrophy through relatively mild, localized blistering of the extremities, to generalized blistering and failure to thrive with premature demise. Milder variants of DEB are characterized by protracted skin involvement that does not influence the overall lifespan of the affected individual, whereas the most severe forms can cause mortality during the early postnatal period (Tosti et al., 2003). In addition to skin symptoms, a variety of extracutaneous manifestations are observed in different forms of DEB, such as corneal erosions, enamel hypoplasia, nail dystrophy, scarring alopecia, tracheal epithelial erosion, and muscular dystrophy (Smith et al., 1996). Autosomal dominant DEB presents milder symptoms than autosomal recessive DEB (RDEB) (Dang and Murrell, 2008). RDEB typically exhibits the most extreme degree of separation of the epidermis from the dermis in the plane below the lamina and loss of AFs (Holbrook et al., 1993).

Herein, we present a molecular genetic study of a child referred to our laboratory who was suspected to have RDEB. Despite the lack of a skin biopsy for histological examination, and based solely on symptoms and family history, we evaluated putative mutations in the *COL7A1* gene to confirm the diagnosis of RDEB.

SUBJECT AND METHODS

A 16-month-old boy was referred to our genetic laboratory center for molecular diagnosis of a skin disease. At birth, the newborn had reddened areas on the hands and toe tips, which began to blister after he was 1 week old. Shortly thereafter, scarring began to appear over the entire body, leading to disfigurement, and continued to progress. The patient was diagnosed with Hallopeau-Siemens RDEB based primarily on typical skin lesions composed of multiple blisters with moderately healed erosions, scarring on trauma-exposed body sites such as the hands and feet, pseudosyndactyly and flexion contractures of the toes, and severely dystrophic nails on the right hand (Figure 1). No other family members had a history of congenital abnormalities or skin disease, and the parents were not related. In this case, an RDEB inheritance pattern was assumed based on a negative previous family history and/or a characteristically severe phenotype in the affected child.

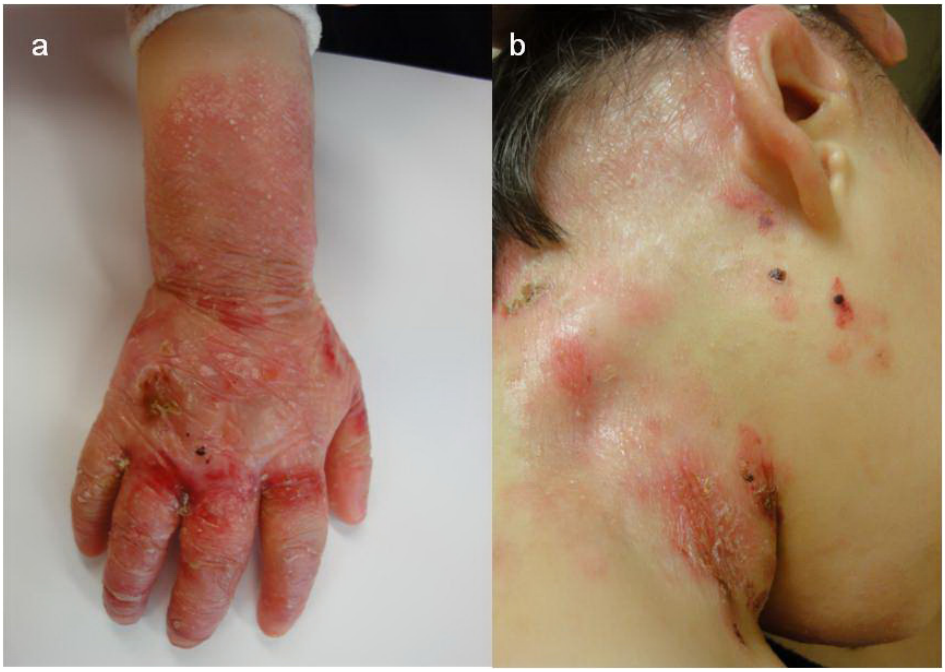

Figure 1. Moderately to severely healed erosions and blisters with scarring and severely dystrophic nails on the right hand (**a**). Moderately healed erosions with scarring on the nape of neck and the occipital region of his head (**b**).

Genomic DNA was extracted from the peripheral blood of the patient and his parents using the QIAamp DNA Mini Kit (Qiagen, Germany). Polymerase chain reaction (PCR) was carried out using previously published primer sets for all 118 exons of *COL7A1* and their flanking intronic sequences according to a previous report (Christiano et al., 1997). The PCR amplicons were bi-directionally sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The chromatograms were analyzed with the Sequencher version 4.9 software (Gene Codes, USA). Mutations were confirmed by sequencing two or more independent PCRs.

RESULTS

Two heterozygous mutations were detected in the affected child: a single-base deletion (c.4232delC) in exon 38 and a single-base substitution (c.6573+1G>C) in intron 81 (Figure 2). The deletion of a single cytosine at codon 1411 within exon 38 had produced a frameshift mutation that created a stop codon at codon 1427 (p.Pro1411Leufs*17). The intronic base substitution had led to aberrant splicing and a premature termination codon (PTC) (Tamai et al., 1997). The parents were heterozygous for each mutation and were considered obligate carriers; c.4232delC was the paternal mutation and c.6573+1G>C was the maternal mutation.

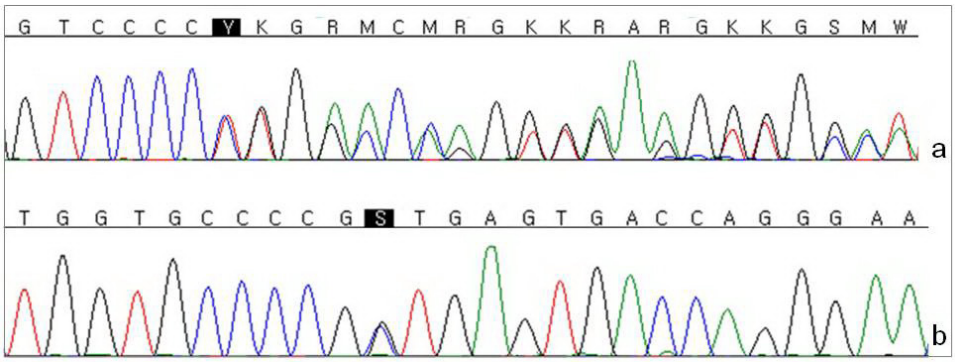

Figure 2. This 1-bp deletion of the exon 38 (c.4232delC) produces a frameshift mutation at codon 1411 creating a stop codon at codon 1427 (p.Pro1411Leufs*17), which may result in a truncated gene product (**a**). Partial sequence of intron 81 at *COL7A1* revealed a heterozygous single-base substitution (c.6573+1G>C) leading to a premature termination codon in the affected child (**b**).

DISCUSSION

RDEB has been classified according to different clinical features into the severe Hallopeau-Siemens type (RDEB-HS), with generalized lesions and scarring of the hands and feet that lead to fusion of the digits and severe mucosal involvement, and the milder non-Hallopeau-Siemens RDEB (RDEB-nHS), which can be localized or generalized and displays very mild or no pseudosyndactyly and presents less frequent extracutaneous involvement (Fine et al., 2000). RDEB-HS is generally caused by PTC mutations resulting from nonsense, frameshift, or splice-site mutations on both *COL7A1* alleles, which result in either nonsense-mediated decay of the mRNA or in truncated polypeptides that are degraded within the cell and are unable to assemble into functional AFs (Jarvikallio et al., 1997). The milder RDEB-nHS is often caused by compound heterozygous mutations: one PTC mutation and one missense mutation. As a result, the full-length type VII collagen polypeptides can be synthesized by one allele with missense mutation, but have a different conformation and affect AF stabilization by disulfide bonding or other structural changes.

The position of the PTC within *COL7A1* correlates with the clinical severity. When both PTC are located upstream of the triple helical region, they result in severe clinical symptoms such as complete pseudosyndactyly. Downstream PTCs affect patients with partial pseudosyndactyly (Tamai et al., 1999; Dang and Murrell, 2008). The presence of some functional protein appears to be the most important factor in ameliorating the disease severity (Tamai et al., 1999).

In this study, the phenotype of the affected child resulted from compound heterozygosity: one frameshift (c.4232delC) and one splicing mutation (c.6573+1G>C) within the upstream triple-helical region. The splicing mutation has been reported previously, and to our knowledge, the single-base pair deletion leading to frameshift (c.4232delC; p.Pro1411Leufs*17) is a novel mutation. These two mutations induced PTC of both alleles and they were associated with the RDEB-HS phenotype (Christiano et al., 1994; Tamai et al., 1999). This report showed that molecular diagnosis can be beneficial to diagnose RDEB and predict prognosis. Moreover, this novel mutation of *COL7A1* in a Korean patient is an addition to the range of *COL7A1* mutations related to DEB.

ACKNOWLEDGMENTS

We are grateful to the patient and parents, and the Catholic Genetic Laboratory Center for assisting us in carrying out this study and compiling this report.

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