

Two Novel Mutations in *LAMC2* Gene in Iranian Families Affected by Junctional Epidermolysis Bullosa

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Abstract

Background: Junctional epidermolysis bullosa (JEB) is an autosomal recessive skin disorder with defective adhesion of dermal- epidermal within the lamina lucida region of the basement membrane zone. The main characterization of JEB is blistering and fragile skin and mucous membrane. Laminins are noncollagenous part of basement membrane and classified as a family of extracellular matrix glycoprotein. Laminins contain three chains: Laminin α , Laminin β and Laminin γ . *LAMC2* (laminin subunit gamma 2) gene encodes γ subunit of laminin and its mutation contributes to JEB. Here, we report a disease-causing nonsense mutation and a large deletion mutation in *LAMC2* gene in two families affected by JEB.

Methods: Whole exome sequencing (WES) was carried out on the mother of patient in family I and the patient himself in family II to detect the underlying mutations. Then, sanger sequencing was performed to confirm the identified mutations.

Results: Next generation sequencing (NGS) data analysis of the first family showed a novel, nonsense mutation in *LAMC2* gene (*LAMC2*: NM_005562: exon14:c.C2143T: p.R715X). The heterozygous state of the mutation was confirmed by sanger sequencing in the parents and unaffected brother. In Family II, NGS data had no coverage in the large area of *LAMC2* gene. Thus, to confirm the possible deletion sanger sequencing was done and blasting of sequence showed the deleted region of 9.4 kb (exon10-17) in *LAMC2* gene.

Conclusions: In summary, current study reported a novel disease-causing premature termination codon (PTC) mutation in *LAMC2* gene and a large deletion mutation in patients affected by JEB.

Keywords: Junctional Epidermolysis Bullosa, *LAMC2* gene, Novel mutation, Skin disorder.

Introduction

Epidermolysis bullosa (EB) is a group of heritable, severe fragile skin disorders in human and animal. It is classified into different subclasses based on the underlying genes and symptoms. Regarding to the severity and type of EB, blisters and skin deformity can appear in distinct part of the whole body (1, 2).

Junctional epidermolysis bullosa (JEB) is an autosomal recessive skin disorder with

defective adhesion of dermal- epidermal within the lamina lucida of the basement membrane zone. This can be attributed to defective proteins such as laminin 332, collagen XVII and integrin $\alpha 6\beta 4$ or integrin $\alpha 3$. The main characterization of JEB is blistering and fragile skin and mucous membrane along with little or no trauma. Clinically, JEB is classified into two major

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groups: Herlitz and non-herlitz. In the former type, early onset of severe symptoms is observed and lead to early death, while in the latter one affected individuals demonstrate blisters during their normal lifespan (3).

Laminines are noncollagenous part of basement membrane and classified as a family of extracellular matrix glycoprotein. Laminines contain three chains: Laminin α , Laminin β , and Laminin γ . Laminin 332 can act as a bridge between the epidermis and the underneath dermis. It is an extracellular adhesion ligand which associated with the hemidesmosome-anchoring filament complex. Mutations of three genes (*LAMA3*, *LAMB3*, and *LAMC2*) encoding the three subunits of laminin 332, affect dermal-epidermal adhesion and mechanically induced skin blistering and fragility, which are the main characteristic of JEB (4, 5). The herlitz type mainly shows bi-allelic premature termination codon (PTC) mutation in one of the laminin 332 gene, while non-herlitz forms have missense mutation in one or both alleles. This is why the herlitz type is more life threatening than non-herlitz type (3).

The *LAMC2* (laminin subunit gamma 2) gene with 23 exons is located on q25-q31 location of chromosome 1 and highly expressed in skin and lung tissues (6, 7). In this study, we report a disease-causing nonsense mutation and a large deletion in *LAMC2* in two families affected by JEB.

Materials and Methods

The Ethics Committee of the Islamic Azad University of Kazerun approved the study protocol. The patients and all family members signed a written informed consent to participate in this study.

Family I, patient I

The patient I, was a product of full-term vaginal delivery. She had nail dystrophy, red scars on the face, abdomen, and legs. Loss of serum protein, electrolytes and dermal sepsis seemed to have been responsible for death one month after birth, with extensive blisters and erosions. Unfortunately, the skin biopsy of the

patient and blood sample were not accessible. The parents were second- degree cousins and had one healthy child. The mother also reported three previous spontaneous abortions. The patient was passed away at the time of genetic evaluations.

Family II, patient II

The male infant was born with numerous skin erosion and fragility of mucous membrane. There was extensive absence of skin on the face, trunk, and limbs. There was positive history of EB in his extended families, thus he was referred to genetic counseling to find the underlying mutation for his severe condition. The patient died 1 week after birth due to severe wounds and respiratory failure.

Whole-Exome Sequencing (WES)

Whole exome sequencing (WES) was carried out on the mother of patient in family I and the patient himself in family II to capture and enrich all exons and crucial flanking site of *LAMC2* gene. Next generation sequencing (NGS) was conducted using Illumina Hiseq 2000 machine to sequence about 100 million reads and standard Illumina protocol for pair-end 99 nucleotide sequencing. Data was aligned with a reference genome by BWA aligner, variants was identified by GATK and then annotated by ANNOVAR as a functional bioinformatics tool (8-10).

Sanger Sequencing

Sanger sequencing was performed on parents and healthy child of family I in order to confirm the WES results and also find the possible inheritance manner of the mutations. Patient was expired at the time of experiment thus we do not have access to the sample. Whole blood samples were collected from participants in Ethylenediamine tetraacetic acid (EDTA) tubes. DNA was extracted from blood using QIAamp DNA Minikit (Qiagen, Germany) according to the instructions. DNA concentrations were assessed using NanoDrop C (Thermofisher, USA) and then kept at -20 °C until use. Primer pairs are listed in Table 1.

Table 1. Sequences of primers.

| Gene, Exon number | Primer Sequence | Length (bp) | TM (°C) |
|--------------------------|--|-------------|---------|
| <i>LAMC2</i> , Exon 14 | F: TTTGACCATAAGCCAGTCAA R: TCCCAACACAGCAGTATTC | 345 | 54 |
| <i>LAMC2</i> , Exon 9-18 | F: CAGTGTATATGTCCTGTTGGG R: CGCTTGAACATCCATATG (Use Primer pairs of exon 14 as inner primers). | 10660 | 54 |

Results

Next generation sequencing (NGS) data analysis of the first family showed a novel, nonsense (stop gain) mutation in *LAMC2* gene (*LAMC2*: NM_005562: exon14:c.C2143T: p.R715X). The heterozygous state of the mutation was confirmed by sanger sequencing in the parents and unaffected brother. Therefore, this mutation might be inherited as autosomal recessive manner and mutation in this gene is associated with recessive JEB disease (Fig. 1a). Although we did not access to patient's sample, according to bioinformatics tools such as mutation taster (11) and mutation assessor this substitution variant is disease causing. Moreover, comparative amino acid alignment of *LAMC2*

protein was performed using T-coffee multiple sequence alignment online program across different animal kingdoms. The result showed that Arg715 residue of the protein was highly conserved through the evolution (Fig. 1b). In Family II, NGS data had no coverage in the large area of *LAMC2* gene. Thus, to confirm the possible deletion primer pairs were designed to identify exact deletion region by sanger sequencing (Fig. 2a). Blasting the sequence data showed the deleted region of 9.4 kb in *LAMC2* gene (Fig. 2b), which encompasses exon 10-17 and so lacking several functional exons would definitely cause severe symptoms in affected individual.

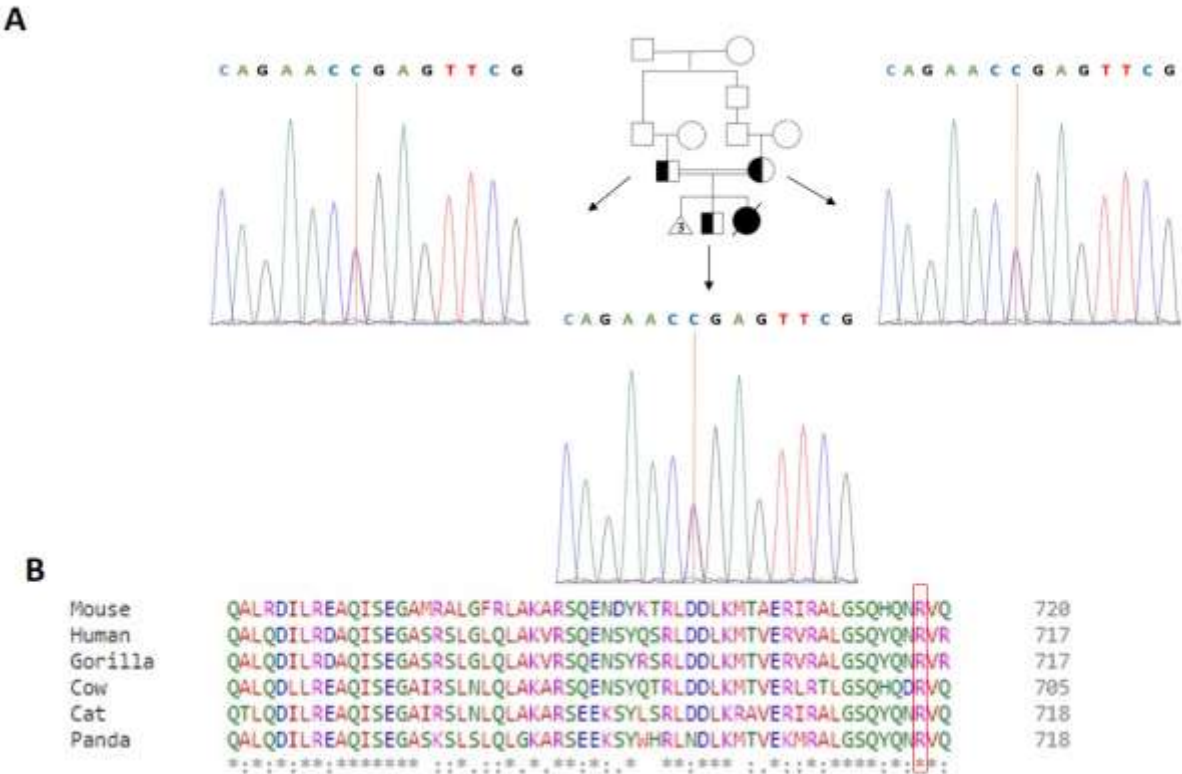


Fig. 1. A) Sanger Sequencing Chromatogram of Family I. B) Comparative multiple sequencing alignment of *LAMC2* protein across different animal kingdom.

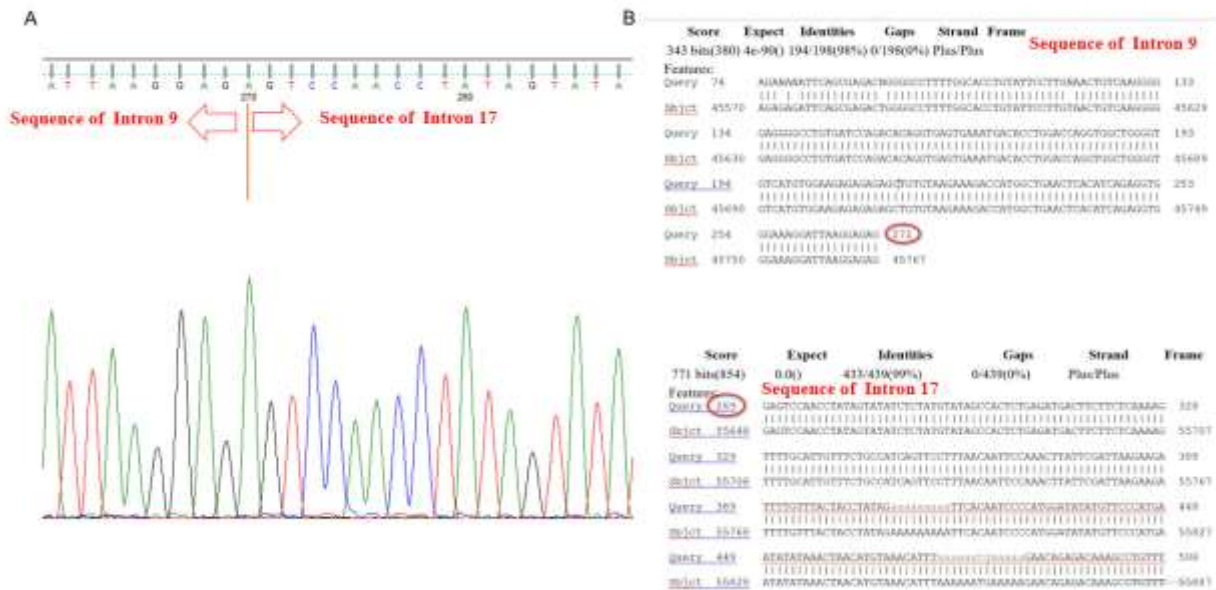


Fig. 2. A) Sanger Sequencing Chromatogram of patient of Family II. B) Blast result of sequence of patient II.

Discussion

Inherited Junctional epidermolysis bullosa is an autosomal recessive subtype of epidermolysis bullosa disorder, characterized by defective dermal-epidermal adhesion. Herlitz (MIM# 226700) and non-Herlitz (MIM# 226650) are two major subgroups of JEB. Herlitz JEB is a severe form which identified with serious blistering over wide regions of the body and mucous membranes of digestive and respiratory tracts, leading to feeding and breathing difficulties, from birth or early ages. The affected individuals usually survive just a few weeks or months (12). The main underlying mutations of JEB occur in the three genes, *LAMA3*, *LAMB3* and *LAMC2*, encoding the $\alpha3$, $\beta3$, $\gamma2$ chains of laminin 332. *LAMC2* is located on 1q25.3, and the total genomic size is approximately 59 kb, according to the UCSC Genome Browser (hg38). In 2002, Nakano et al had investigated JEB mutations in two main forms. In Herlitz form, 13 out of 15 individuals had PTC mutation (3) Jeon et al reported a compound heterozygote mutation (one missense mutation c.79G> A and an insertion one (382 insT) in *LAMC2* gene, which also resulted in PTC mutation (13). Other studies also showed the PTC mutation as a dominant mutation among Herlitz patients in human (14).

In this study we introduce another nonsense mutation which is responsible for causing Herlitz JEB (*LAMC2*: NM_005562: exon14: c. 2143C> T: p. R715X). This mutation was reported in dbSNP (rs1035597347) but not reported in ClinVar, Human Genome Variation Database and Human Genome Mutation Database. The global minor allele frequency of this SNP is 0.000004 for T allele. According to mutation taster, this mutation can lead to nonsense mediated decay (NMD) process. In the normal protein the stop codon amino acid is at 1194 while in mutant one is located at position 715. Therefore, protein truncation is obvious.

In the second family, the large deletion was confirmed in affected person and the mutation evaluation showed the deletion of exons 10-17. For further deletion evaluation, the blast result of patient's sanger sequences reveals that the first segment of this sequence (up to the nucleotide 270) is located in the intron between 9 and 10 exons, while the rest of the sequence is located in the intron between 17 and 18 exons. Therefore, we came to the conclusion that 10-17 exons were omitted in the homozygous status (Fig 2). This is the first large deletion mutation of *LAMC2* gene in human.

In conclusion, current study reported a novel disease causing PTC mutation in *LAMC2* gene and a large deletion mutation in patients affected by JEB-Herlitz type. This finding expands the mutation spectrum of *LAMC2* which will be useful for prenatal molecular studies. Further practical studies are needed to evaluate the

effects of this mutation on mRNA and protein expression.

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