

Identification of multi-exon deletion in the COL7A1 gene underlying dystrophic epidermolysis bullosa by whole-exome sequencing

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ABSTRACT

Dystrophic epidermolysis bullosa (DEB) is a rare form of genodermatosis characterized by skin blisters, milia, scarring over the entire body, and nail dystrophy. In this study, a pedigree with one affected member with skin blisters, and a clinical diagnosis of epidermolysis bullosa who was a result of a non-consanguineous marriage, was investigated by whole-exome sequencing (WES). This survey revealed that the proband is a compound heterozygote for a previously reported heterozygous missense variant (c.6205C>T) and a heterozygous deletion of exons 13–24 in the COL7A1 gene. This study indicates that the use of WES along with copy number variation (CNV) analysis gives a higher diagnostic yield for such patients. Moreover, considering the autosomal recessive and dominant forms of the disease, both caused by variants in one gene, proper interpretation and classification of novel variants in heterozygous as well as homozygous states is always a major challenge.

Key words: dystrophic epidermolysis bullosa; COL7A1; whole-exome sequencing; deletion; CNV analysis

INTRODUCTION

Epidermolysis bullosa (EB) is a group of heritable and acquired skin disorders of variable clinical severity. The heritable and severe forms of EB manifest themselves at birth or shortly thereafter as blistering and erosions of the skin and mucous membranes, with considerable morbidity during the early postnatal period, whereas the milder variants are characterized by skin fragility that does not influence the patient's overall lifespan [1]. On the basis of clinical observations and ultrastructural demonstration of the topographic level of blistering within the skin, the hereditary forms of EB have been divided into three categories: epidermolysis bullosa simplex (EBS) involving intra-epidermal blistering

with autosomal dominant inheritance; junctional epidermolysis bullosa involving tissue separation within the dermal–epidermal basement membrane with autosomal recessive inheritance; and the dystrophic forms of epidermolysis bullosa involving sublamina densa blister formation within the upper papillary dermis with autosomal dominant or recessive inheritance. There is extensive heterogeneity in the severity of the phenotype and the outcome of the disease in these subgroups of EB. The phenotypic heterogeneity of different forms of EB reflects the fact that as many as twenty different genes encoding the components of the dermal–epidermal attachment complexes harbor mutations in different subtypes of EB [2]. Investigation on the genetic aspect of

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diseases provides the opportunity to explore the relationships between complex phenotypes and genomic variations [3]. Herein, with the help of next-generation sequencing (NGS) technology, we report the case of a fifteen-year-old male who had been suffering from chronic skin disease with skin blisters resulting from a compound heterozygote for a previously reported heterozygous missense variant (c.6205C>T) and a novel heterozygous deletion of exons 13–24 in the COL7A1 gene.

CASE REPORT

A pedigree with one affected member was investigated. A fifteen-year-old male with skin blisters, and a clinical diagnosis of epidermolysis bullosa who was a result of a non-consanguineous marriage, was referred to Watson Genetic Laboratory in Tehran, Iran, for genetic counseling and/or analysis. Written informed consent was taken from the patient's parents and peripheral blood samples were collected from the patient and his family (Fig. 1). Genomic DNA was extracted from whole peripheral blood with the GeneAll® Exgene™ kit (GeneAll Biotechnology Co., LTD, Seoul, Korea) [4]. Human whole-exome enrichment was performed with the Twist Human Core Exome kit and the library was sequenced on the Illumina HiSeq 4000 platform with a raw coverage of 345X and a mean on-target coverage of 149X, performed by CeGaT GmbH, Germany. Detected variations included single-point mutations and small indels (within 20bp). Furthermore, copy number variation (CNV) detection of the aforementioned genes was performed for the patient with breakpoint analysis. Breakpoint analysis was conducted by long-range PCR followed by Sanger sequencing. Long-range PCR was performed with the TaKaRa PrimeStar GXL kit (TaKaRa Bio Inc., Shiga, Japan). The thermal profile for long-range PCR was as follows: denaturation for 8 min at 96°C, then 35 cycles of denaturation (30 s at 96°C), annealing (30 s at 61°C), and extension (4 min at 72°C), followed by the final extension step of 10 min at 72°C. Primers used for genomic amplifications by which the deletion was found were as follows: F, 5'-CCCAGTACCGCATCATTGTG-3' (exon 12); R,

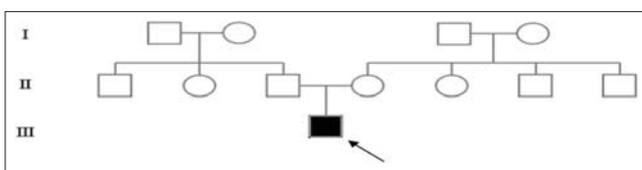


Figure 1: The investigated pedigree, with the proband indicated by filled squares and an arrow.

5'-T GTCACGGATCCTTTGCAAGA - 3' (exon 25). For PCR amplification, 1 ml (50 ng/ml) of genome DNA was used as a template in 20 ml of reaction mixture, 1 ml of each primer (5 pM), and 8 μL DH₂O. The PCR products, examined by 2% agarose gel electrophoresis, were 4,899 bp (for the normal allele) and 836bp (for the mutant allele) in size, respectively. The PCR products were sequenced by an ABI 3500 automated sequencer (Pishgam Biotech Company, Tehran, Iran). Sequences obtained were analyzed with publicly available NCBI Blast, UCSC BLAT, the sequence alignment tool on UCSC (<http://genome.ucsc.edu/cgi-bin/hgBlat?>). In order to validate the detected missense variant, polymerase chain reaction was followed by Sanger sequencing. The sequences of the designed primers were as follows: forward primer: GAGTGAGGGAAGAGGGGTTG; reverse primer: ACAGGACTAAGGCAGGGATG. PCR reaction was performed in 20 μL of the total volume containing 8 μL of the Taq DNA Polymerase 2× Master Mix (Ampliqon A/S, Odense, Denmark), 8 μL DH₂O, 1 μL of each 5 pM primers, and 1 μL of 50 ng/μL DNA. The conditions for PCR were as follows: initial denaturation at 96°C for 6 min; 32 cycles of denaturation at 96°C for 30 s, the annealing step at 60°C for 30 s, elongation at 72°C for 30 s, and the final extension at 72°C for 5 min. The PCR products were sequenced by an ABI 3500 automated sequencer (Pishgam Biotech Company, Tehran, Iran).

Whole-exome sequencing revealed a heterozygous missense variant in the proband. The variant was a C-to-T transition at the first base of codon 2069 in exon 74 of the COL7A1 gene that caused the substitution of Arg (CGT) with Cys (TGT) (Fig. 2). According to our survey, this variant (c.6205C>T) in the COL7A1 gene has been reported in the HGMD as well as several other publications as a pathogenic variant [6-11]. The bioinformatic investigation was performed with online tools, including Mutation Taster and CADD, to predict the possible effect of the variant on the function of the protein. The variant was predicted with high confidence to be “disease-causing” by Mutation Taster and with CADD to yield a Phred score of 25.6. The variant is absent in population databases (ExAC, 1000G). In addition, this variant has a frequency of zero in the largest available local database of genomic variations in the Iranian population (Pishgam Biotech Company, Tehran, Iran). Furthermore, a heterozygous deletion of exons 13–24 was detected in the COL7A1 gene. CNV analysis of multiple exon deletions in the

COL7A1 gene for the patient with breakpoint analysis is shown in Fig. 3. Long PCR analysis of genomic DNA indicated 4063 bp deletions of the COL7A1 gene in the patient (Fig. 4). This variant had been previously reported for its pathogenicity in homozygous status [5]. The nature of the deletion and multiple lines of *in silico* computational analysis support the deleterious effect of the variant on the gene or gene product(s). This variant is absent in population databases (ExAC, 1000G) and our local database.

DISCUSSION

In this study, we attempted to target a group of genes responsible for epidermolysis bullosa to detect causative

mutations in an Iranian patient with skin blisters, and a clinical diagnosis of epidermolysis bullosa. As a result, a heterozygous missense variant (c.6205C>T) and heterozygous deletion of exons 13–24 in the COL7A1 gene were detected in the patient. Dominant and recessive mutations in gene COL7A1 encoding for collagen VII have been reported to cause DEB. Collagen VII is the major constituent of anchoring fibril presented below the basal lamina within the dermal–epidermal junction. Anchoring fibril appears in normal quantity and morphology on biopsy in Dominant DEB (DDEB). In Recessive DEB the amount of collagen VII was usually remarkably reduced on electron microscopy and immunofluorescence staining [12]. Mutations in the COL7A1 gene encoding for collagen

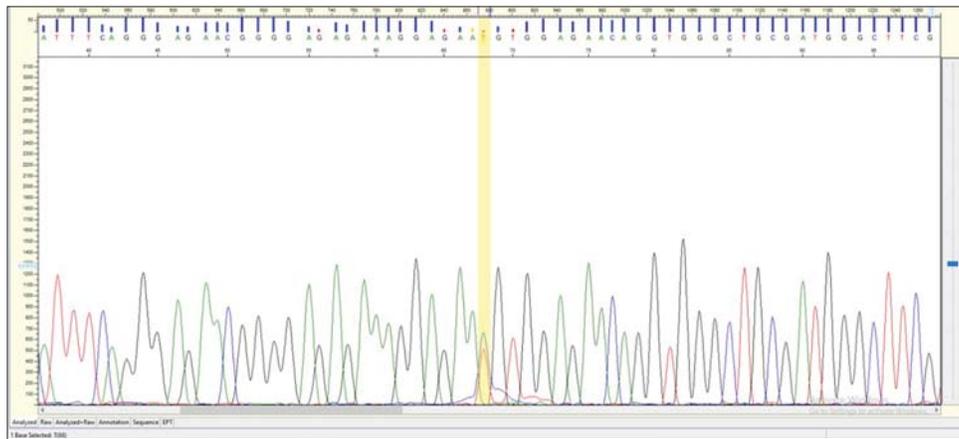


Figure 2: The result of DNA sequencing showing heterozygosity in the patient for variant c.6205C>T in the COL7A1 gene.

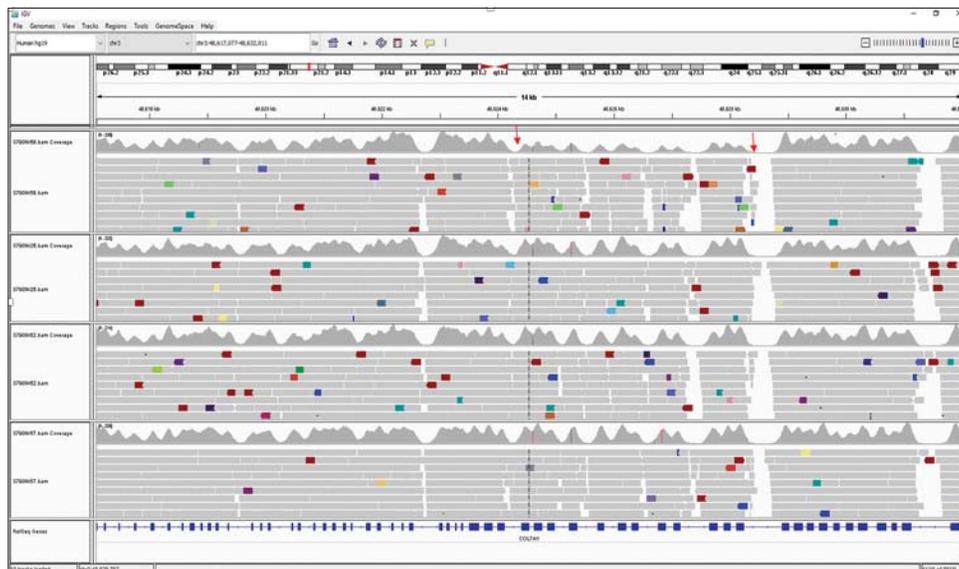


Figure 3: CNV detection of multiple exon deletions in the COL7A1 gene for the patient with breakpoint analysis. Coverage plots (IGV) of three control (ctrl) samples versus the patient (px) sample illustrate the statistical readout with a drop in coverage in the COL7A1 gene on exons 13–24 for the patient sample (double red arrows), compared to controls indicating a deletion event at this position. The 5' decrease in coverage is located at a clear-cut position at the beginning of exon 13 (red arrow). The COL7A1 gene is displayed from the right to left.

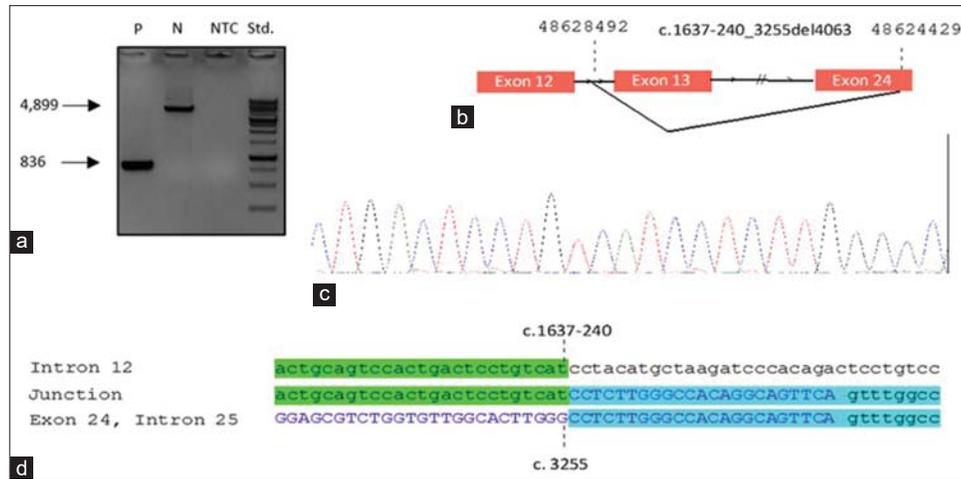


Figure 4: (a) Long PCR analysis of genomic DNA indicating 4063 bp deletions of the COL7A1 gene in the patient. Lane 1 (Std) is a 1 kb ladder. Lane 2 (NTC) is No Template Control. Lane 3 (Sample N) is amplified from the normal control. Lane 4 (Sample P) points to the abnormal fragments generated by the genomic deletion. (b) Schematic representation of the deletion regions in the COL7A1 gene. (c) The result of DNA sequencing showing heterozygosity in the patient for variant c.1637-240_3255del4063 in the COL7A1 gene. (d) Sequence analysis of junction fragments amplified by PCR revealing deleted sequences within nucleotide (c) at position c.1637-240 in intron 12 and nucleotide (g) at position c.3255 in exon 24.

VII cause dominant and recessive forms of DEB. Genetic investigations of the COL7A1 gene in several patients demonstrated that recessive DEB (RDEB) leads to highly severe phenotypes due to deletions or small insertions and nonsense mutations creating a premature termination codon [13]. On the other hand, the substitution of amino acids causes a dominantly inherited form of DEB with a milder phenotype [14]. Therefore, the EB type was identified by accurate molecular genetic investigation of the COL7A1 gene. In the present study, WES sequencing of the patient indicated previously detected heterozygous deletion of exons 13–24, coexisting with heterozygous Single-Nucleotide Variants (SNVs) elsewhere in the COL7A1 gene that predicted to be deleterious. We could not confirm compound heterozygosity, as parental DNA was not available, but some studies have suggested an autosomal recessive pattern of inheritance for the c.6205C>T variant [6-8]. In our patient, long-range PCR confirmed the loss of exons 13 to 24 on one allele and a recombination occurring between a short sequence of three nucleotides (CCT) in intron 12 (c.1637-240) and the same sequence at exon 24 (c.3255), resulting in a deletion spanning on 4063 bp (c.1637-240_3255del4063). Descriptions by Vahidnezhad et al. and ours demonstrates that the COL7A1 gene, containing numerous repeated sequences, and the affected regions might be prone to recombination. In addition, the geographic location of the family in our study was in the northwest of Iran that was located in the same location indicated in this study [5].

CONCLUSION

Our study suggests that CNV analysis would be required to reach molecular detection of epidermolysis bullosa. Especially for centers that already employ WES or targeted gene panels, we suggest extending their analysis to include CNV detection.

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Consent

The examination of the patient was conducted according to the principles of the Declaration of Helsinki.

The authors certify that they have obtained all appropriate patient consent forms, in which the patients gave their consent for images and other clinical information to be included in the journal. The patients understand that their names and initials will not be published and due effort will be made to conceal their identity, but that anonymity cannot be guaranteed.

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