Polymorphism

RESEARCH

A novel variant (c.1966C>T) in the COL7A1 gene identified by exome sequencing in a patient of dystrophic epidermolysis bullosa

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ABSTRACT

Epidermolysis bullosa (EB) is an inherited disorder. It involves a heterogeneous group of the rare genetic dermatoses are characterized by the mucocutaneous brittleness and the blister development, which are often inducible by the minimal trauma. A wide-ranging phenotypic diversity has been defined, with possibly severe extracutaneous appearances, morbidity and the mortality in some cases. In this study, we have documented a case of the EB with a novel variant (c.1966C>T; p. Gln656Ter) in the COL7A1 gene detected by whole exome sequencing. This novel mutation has been authenticated by Sanger sequencing. This case highlights the importance of whole exome sequencing for confirmatory molecular diagnosis and adds a novel variant (c.1966C>T; p. Gln656Ter) to the genotypic spectrum of COL7A1 gene mutations in epidermolysis bullosa.

KEYWORDS: Epidermolysis bullosa, COL7A1 gene, c.1966C>T, p. Gln656Ter

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INTRODUCTION

Epidermolysis bullosa (EB) is a collection of genetic skin illnesses that originate in the skin as blisters with skin erosion on minimal trauma. Aberrations of the macromolecules, which anchor the dermis to the epidermis lead to the reduced cohesion of the skin layers, blister formation, and fragility. The ruthlessness of the skin appearances can be highly variable and is dependent on the mode of inheritance and the underlying mutation. In the people with EB, blisters form in retort to minor injuries or friction, such as rubbing or scratching. There are four main types of the EB, which are categorized based on the complexity or the level of blister formation (Bruckner-Tuderman L 2019), EB simplex, Dystrophic EB (DEB), Junctional EB and Kindler Syndrome. Most of the patients presenting to a genetic/dermatological unit belong to the DEB subtypes owing to the augmented morbidity accompanying with this subtype (Nilay et al. 2021).

EB may be further categorized on the basis of its severity and with the help of specific symptoms, such as the distribution and whether parts of body other than skin are exaggerated in the affected individual. The specific sub-types may then be determined on the basis of categorizing the exact protein that is defective in a person with the EB. The identifications of EB may be done by the tests performed on a skin biopsy, and supplemented by the genetic testing (Bruckner-Tuderman L 2019). Even the direct genetic testing is feasible in paediatric populations owing to avoidance of the painful skin biopsies and the advantage of the molecular confirmation. A person with EB may be mildly or severely affected, and the disease can range from being a minor inconvenience requiring modifying activities to completely disabling and even fatal in the some cases (Nilay et al. 2021). EB may be caused by variations (the changes called mutations) in genes that play vital roles in the structure, integrity, and the repair of skin. Till date, mutations in 18 genes have been identified as the causes of the EB in the human beings (Fine et al. 2008, Fine. 2010). Inheritance pattern may be autosomal dominant or autosomal recessive depending upon the type and the subtype of the EB a person has (Bruckner-Tuderman, 2019). Managing EB involves a multidisciplinary team of the health care workers comprising a dermatologist, EB nurse who specializes in the care of wound, professional therapist, nutritionist, geneticist and a social worker (Epidermolysis bullosa. NORD. 2013). The management should be individualized for each person depending on their age, severity of the symptoms, and the associated complications.

Presently there is no specific curative therapy for most forms of the EB. Extensive clinical research regarding the potential treatments is ongoing. At this time, management is often supportive. Monitoring for the complications with the laboratory testing and the imaging studies is also important, although the frequency of these tests will vary depending upon the type of EB and severity in each person. New-borns with EB should be taken care of in a neonatal or paediatric unit that has the expertise, staffing, and resources necessary to manage severe skin erosions and potential complications.

In this paper, we used the Whole Exome sequencing (WES), which is a widely used next-generation sequencing (NGS) method that involves sequencing of all protein-coding regions of the genome (Rani et al. 2019). Owing to its high throughput nature and the ability to instantaneously sequence multiple genes, NGS, specifically the whole exome sequencing (WES), has modernized the molecular approach and provides a fast and well-versed diagnostic strategy in a number of genodermatoses (Takeichi, T et al. 2015, Tenedini, E et al. 2015). WES is a proficient approach to selectively sequence the coding regions (exons) of the genome to discover rare or common variants associated with a disorder or phenotype (Biesecker. 2010, Illumina. 2013).

METHODS

Case summary

The subject was a three years old, female child (non-consanguineous born) with family pedigree presented in fig 2, who was brought to the clinician with complaints of failure to thrive, skin blisters and peeling of skin on the trunk and limbs with minimal friction since birth. She (proband) also had milia and pseudo syndactyly of fingers and toes, her blood sugar, liver functioning test (LFT), kidney functioning test (KFT), CBC, Vit D and TSH reports were negative. She (proband) was not able to eat solid food and had urine and stool retention. The parent's karyotype report was normal. Oral mucosal lesions and nail dystrophy were evident on examination. There was no similar complaint in the family. A provisional diagnosis of the DEB was made and the Whole exome sequencing was performed to ascertain the exact molecular diagnosis as the parents did not give consent for skin biopsy.

Test methodology

The DNA extraction from the blood was used to perform the targeted gene capture with the help of a custom capture kit. The libraries were sequenced to >100X coverage on Ilumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh37/hg19) and variant analysis was performed using a set of Bioinformatics pipeline.

Variant prioritization

Golden Helix VarSeq 2.2.0 is a clinical genomics interpretation and reporting platform from Golden Helix. The variant annotation engine includes algorithms to identify the impact of variant on gene using both public content (ClinVar, HPO, links to dbSNP, gnomAD and in-silico predictors - GERP++, PhyloP, PhyloP LRT, SIFT and PolyPhen2. VarSeq allows quick filtering and evaluation of variants. The clinically relevant variants were annotated using published variants in literature and a set of diseases databases – dbSNP, ClinVar, OMIM and HGMD. The cutt off of allele frequency in variant exploration was less than 5%. Common variants were filtered based on allele frequency in gnomAD. Only nonsynonymous and splice site variants found in the exome panel consisting of specific set of genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region were not reported.

RESULTS

We found a novel variant (c.1966C>T; p. Gln656Ter) in *COL7A1* with the help of widely used technique whole exome sequencing, which was successfully validated by the gold standard method of Sanger sequencing (Fig. 1). The individual carries two copies of a nonsense variant in the *COL7A1* gene, which is predicted to cause the premature truncation of the protein. This variant seems to be novel as it has not been previously reported in the scientific literature and in the public databases. We were not able to perform the genotyping of parents because they refused to give blood sample.

Since, this variant is prophesied to produce a truncated protein which might result in the loss-of-the function. Since other truncating variants in this gene are also known to cause similar phenotype, this variant has been labelled as pathogenic (disease causing) (Table 1) according to the ACMG guidelines. This variant is also present in the important Fibronectin III domain of the COL7A1 protein. This variant is evolutionary conserved in different species.

DISCUSSION

DEB is an extremely rare subtype of EB, which is caused by mutation of the *COL7A1* gene. EB is characterized by blistering with scarring predominantly which can occur in the childhood or the adulthood stage (Rizzo et al. 2008). Initially, the diagnosis of DEB was suspected in the patient because it overlapped with other subtypes with

respect to the symptoms. Biopsy is very important to confirm EB (Kon, A et al. 1997, Kon, A et al. 1997) but we could not obtain biopsy from the patient because the parents did not provide the required consent. A number of studies across the world have reported mutations in different exons of the *COL7A1* gene in EB (Christiano, Christiano et al. 1997, Mellerio et al. 1998; Varki et al. 2007, Yenamandra et al. 2018; Nilay et al. 2021) and other similar disease (Chuang et al. 2004, Lin et al. 2012). We found a novel mutation in exon 15 of the *COL7A1* gene.

Table 1: A novel variant identified by WES (Whole Exome sequencing) in the *COL7A1* gene.

Gene	Chromosomal Coordinates	Exon	Variant*	Zygosity	Significance (ACMG Classification)	Inheritance	Coverage
COL7A1 (NM_000094.3)	chr3:48627730: G>A	15	c.1966C>T p. Gln656Ter	Homozygous	Pathogenic (PVS1,PM2,PP3)	Autosomal Recessive	111X

*The nucleotide sequence numbering for *COL7A1* is as per NM_000094.3

Gene *COL7A1* is present on chromosome 3p21.1 position and spans approximately 32 kb and encompasses 120 exons. *COL7A1* gene encrypts a type-VII collagen which is a major structural component of the AFs (Anchoring Fibrils). It is responsible for the cohesion of the epidermis and dermis. Kon et al. reported the first mutation in the *COL7A1* gene in EB (Kon, A et al. 1997, Kon, A et al. 1997). To date, more than 730 pathogenic mutations have been detected in the *COL7A1* gene in different variants of the DEB (http://www.col7.info). Nearly all of them are family-specific, although a small number of recurrent mutations have been reported. However, different cases may have the same subtype of DEB (Christiano AM. 1997, Varki et al. 2007).

The DEB is a severe skin disorder commonly present since birth, which is categorized by recurrent blistering at the level of the sub-lamina densa beneath the cutaneous basement tissue membrane. The recessive DEB has severe phenotype (Nilay et al. 2021) with generalised involvement; scarring and the patients may have contractures of the hands, feet and the joints. Patients may also develop strictures of the gastrointestinal tract from the mucosal involvement, which leads to dysphagia-poor nutrition-failure to thrive. The affected individuals also have an augmented risk of the developing aggressive squamous cell carcinoma [OMIM 226600]. The onset of these clinical features has been evident in the early childhood, but in some cases it is delayed till the second or third decade of the life.

Despite well-characterized genetic studies from different ethnic backgrounds, identifying several recurrent and region-specific mutations and molecular diagnosis of DEB is still challenging (Vamsi et al. 2018). Preceding to the initiation of the next-generation DNA sequencing (NGS) technologies, molecular diagnosis of DEB was based on the traditional Sanger sequencing of either the hot-spot regions or the entire *COL7A1* gene, requiring more than 70 primer sets; a process that is tedious, time consuming and expensive. Due to its high throughput nature and ability to simultaneously sequence multiple genes, NGS, especially whole exome sequencing (WES), has revolutionized the molecular approach and provided a fast and efficient diagnostic strategy in several genodermatoses (Takeichi et al. 2015;

Tenedini et al. 2015). In view of the scarcity of and need for molecular studies on DEB in India, the aim of this study was to explore the probable use of WES in the correct diagnosis of DEB, as part of a larger effort in understanding the mutational spectrum of patients with EB in Indian sub-continent. This study will serve as a basis for the future large-scale molecular studies on EB patients in the Indian population.

CONCLUSION

This case highlights the importance of whole exome sequencing for confirmatory molecular diagnosis and adds a novel variant (c.1966C>T; p. Gln656Ter) to the genotypic spectrum of COL7A1 gene mutations in epidermolysis bullosa. The meticulous molecular diagnosis would help in the management, prognosis, and prenatal diagnosis and also as a potential source for the therapeutic trials in the area of precision medicine and therapy.

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Author's contribution

PV has made substantial contributions to conception and design, who worked and compiled the data and drafted this manuscript. MN was involved in drafting the manuscript or revising it critically for important intellectual content. SD reviewed the study. AD contributed to finalizing and reviewing of the manuscript. DK gave her valuable inputs in data acquisition, analysis and interpretation. AJ reviewed the study thoroughly. VKM performed bioinformatics data analysis.

Conflict of interest

Authors have no conflict of interest.

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Declaration of originality

The authors have declared that the data/text presented in this manuscript is original and no text, figure or data has been copied from any other source without appropriate citation.

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