CASE REPORT

Case Report: Whole exome sequencing reveals a novel frameshift deletion mutation p.G2254fs in COL7A1 associated with autosomal recessive dystrophic epidermolysis bullosa [version 1; referees: 2 approved, 1 approved with reservations]

Shamsudheen Karuthedath Vellarikkal1,4, Rijith Jayarajan1, Ankit Verma1, Sreelata Nair2, Rowmika Ravi1, Vigneshwar Senthivel1, Sridhar Sivasubbu1,4, Vinod Scaria3,4

1Genomics and Molecular Medicine Unit, CSIR Institute of Genomics and Integrative Biology, Delhi, India
2Department of Fetal Medicine, Lifeline Super Specialty Hospital, Kerala, India
3GN Ramachandran Knowledge Center for Genome Informatics, CSIR Institute of Genomics and Integrative Biology, Delhi, India
4Academy of Scientific and Innovative Research, CSIR-IGIB South Campus, Delhi, India

Abstract

Dystrophic epidermolysis bulosa simplex (DEB) is a phenotypically diverse inherited skin fragility disorder. It is majorly manifested by appearance of epidermal bullae upon friction caused either by physical or environmental trauma. The phenotypic manifestations also include appearance of milia, scarring all over the body and nail dystrophy. DEB can be inherited in a recessive or dominant form and the recessive form of DEB (RDEB) is more severe. In the present study, we identify a novel p.G2254fs mutation in COL7A1 gene causing a sporadic case of RDEB by whole exome sequencing (WES). Apart from adding a novel frameshift Collagen VII mutation to the repertoire of known mutations reported in the disease, to the best of our knowledge, this is the first report of a genetically characterized case of DEB from India.

This article is included in the Rare diseases collection.
Corresponding authors: Sridhar Sivasubbu (s.sivasubbu@igib.res.in), Vinod Scaria (vinods@igib.in)

Competing interests: The authors declare that they have no competing interests.


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Grant information: Authors acknowledge funding from the Council of Scientific and Industrial Research (CSIR), India through Grant No. BSC0212 (Wellness Genomics Project) granted to SS and VS.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Introduction
Dystrophic epidermolysis bullosa (DEB) is an extremely rare subtype of epidermolysis bullosa with an estimated incidence of approximately 6.5 per million newborns. The disease is caused by mutations in collagen VII (COL7A1). Collagen VII is a major structural macromolecule of the skin and plays an important component of the anchoring fibrils which connect the epidermis and dermis of the skin. The disease affects the skin, the mucosa (including that of the oral cavity) and gastrointestinal tract. The blisters are further followed by scarring and development of deformities. The disease also predisposes individuals to development of skin cancer and it is estimated that almost all affected members develop cancers in the third or fourth decade of life.

Case Report
A 4.5-year-old South Indian female child presented to the outpatient clinic with a history of multiple vesicular and bullous lesions induced by trauma since perinatal period. The child was born out of a third degree consanguineous marriage with no known history of similar illness. The child had severe blistersing and scarring all over the body, nail dystrophy and milia. The oral mucosa was involved along with tongue blistering, dental calculus, and chipping of teeth with difficulty in opening the mouth. The child also had flexural deformities resulting in contractures and pseudo-syndactyly of the fingers. The clinical picture (Figure 1a,b) corroborated the diagnosis of dystrophic epidermolysis bullosa (DEB). There is no center in India offering genetic diagnosis for the disease using targeted gene sequencing. Given that targeted gene sequencing can be quite expensive, tedious and time-consuming to standardise, we attempted whole-exome sequencing (WES). Moreover no background genetic map of mutations in the disease from India was available. Previous reports, including from our laboratory suggest WES as an alternative to traditional approaches; WES being fast, less tedious, and cost-effective while also providing a holistic view of the mutation spectrum of the patient.

Approximately 5 ml of blood was collected from the affected individual and the parents after obtaining signed informed consent...
and approval from the institutional ethical committee (BSC0212 IHECC proposal No.08). Genomic DNA was isolated by using salting out method. 50ng of high quality DNA was used for whole exome sample preparation using a Nextera (Illumina Inc, USA) expanded exome kit according to manufacturer supplied instruction. The exome was sequenced by Illumina HiSeq2500 according to the manufacturer’s protocols (Illumina Inc, USA). Paired-end reads of 150 bases were generated, which was quality and adapter trimmed at a Phred quality score of 20. Alignment was performed on the human reference genome (hg19) using Burrows-Wheeler Alignment (version 0.5.10-evan.9). The mean mapped coverage on target region was 12.2x. Variants were called using Platypus pipelines (version 0.7.9.1). Analysis revealed a novel homozygous frameshift deletion (chr3:g.48610366CT->) c.6759_6760del (p.G2254fs) in COL7A1 gene. The c.6759_6760del was predicted to be deleterious (confidence score 0.858) and introduce a premature termination codon (PTC) at 2273th amino acid position according to SIFT. Homozygous PTCs in COL7A1 is previously reported to reduce overall stability of anchoring filaments and cause mild to very severe generalised RDEB. Secondary structure analysis shows that p.G2254fs resultant PTC leads to loss of function of several collagen triple helix repeats and kunitz domain (Figure 1e). We also found a homozygous nonsynonymous variation c.5716C>T (p.P1906S) in COL7A1 which was predicted to be ‘tolerated’ by SIFT (0.5).

The variant was verified independently using capillary sequencing in the child and parents. The variant was not found in ExAC or our internal cohort of 122 exomes, confirming its rarity and novelty. Parents were provided detailed genetic counselling by the consulting clinical geneticist.

Discussion
Dystrophic EB could be inherited in both recessive and dominant form. Several cases of DEB have been reported from India. A recent paper reported a cohort of 17 DEB patients using immunofluorescence mapping, though the patients were not genetically characterized. Our earlier report characterized a novel mutation in KRT5 associated with epidermolysis bullosa (EB) simplex in West India. Taken together, we suggest a large and potentially uncharacterized repertoire of genetic variations causing EB in India, which might benefit from genetic screening approaches.

In this study, we show the application of next-generation sequencing to identify the mutation in a sporadic case of autosomal recessive EB in clinical settings. Apart from adding a novel frameshift collagen VII deletion mutation to the repertoire of known mutations in the disease, to the best of our knowledge, this is the first report of a genetically characterized patient of DEB from India. We suggest that next-generation sequencing approach would significantly benefit the understanding and genetic characterization of this rare disease in India.

Consent
Written informed consent was obtained from the parent of the patients for publication of this case report and any accompanying images and/or other details that could potentially reveal the patient’s identity.

Data availability
The raw exome sequencing data are available at the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra), accession number SRX1584466.

Author contributions
SN performed the clinical evaluation and sent the blood for DNA analyses and provided genetic counselling. SKV, RJ, RR, AV and VSV isolated the DNA, conducted the quality checks, prepared the exome capture and sequencing library, performed the exome sequencing, performed data quality checks on the reads, reference alignments, variant call and computational prioritization of the variants, designed and performed the validation experiments and contributed to writing the manuscript. SS and VS conceptualized and oversaw all the experiments and analysis and contributed to writing the manuscript.

Competing interests
The authors declare that they have no competing interests.

Grant information
Authors acknowledge funding from the Council of Scientific and Industrial Research (CSIR), India through Grant No. BSC0212 (Wellness Genomics Project) granted to SS and VS.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
Authors acknowledge help from Dr. Vamsi Krishna for preparation of the manuscript.

References


Open Peer Review

Current Referee Status:  ?  ✓  ✓

Version 1

Referee Report 27 June 2016

doi:10.5256/f1000research.9013.r14177

Mohamed Badawy Hassan Tawfik Abdel-Naser
Klinik für Dermatologie, Venerologie und Allergologie Städtisches Klinikum Dessau, Dessau, Germany

- Apart from few typo errors, the manuscript is well written and informative.
- There are few too strong statements, e.g., "The disease also predisposes individuals to development of skin cancer and it is estimated that almost all affected members develop cancers in the third or fourth decade of life". Perhaps authors can insert a reference (s) that supports this statement.
- Fig. 1. needs some corrections. Please insert (a) to refer to the upper limb, thorax and abdomen (not hands and thoracic region). b. should refer to legs and feet.
- Is the mentioned "our internal cohort of 122 exomes" published or available online? Furthermore, they may mention some of the weaknesses of the Nextera platform, such as coverage bias.
- In the discussion sections, authors may mention the clinical relevance of their finding.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 14 June 2016

doi:10.5256/f1000research.9013.r14364

Regina Fölster-Holst
Dermatologische Klinik, Universität Kiel, Kiel, Germany

The authors describe a 5 year old boy with dystrophic epidermolysis bullosa (DEB), which is due to a novel mutation in the \textit{COL7A1} gene. The case is the first report of a genetically characterized case of DEB from India.

It is a well written manuscript which is worthy to be indexed with \textit{F1000Research}. To understand the new
mutation as the cause of severe DEB in the boy, it would be interesting to know what are the differences to other mutations of the COL7A1 gene. In other words, what does this mean on the protein level?

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**References**


**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
Dear Robert Sidbury,
Thank you for reviewing our article and your valuable comments. We have incorporated your suggestions in the recent version.

Thanks and Regards
Authors

**Competing Interests:** No competing interests were disclosed.