



CASE REPORT

REVISED Case Report: Whole exome sequencing reveals a novel frameshift deletion mutation p.G2254fs in COL7A1 associated with autosomal recessive dystrophic epidermolysis bullosa [version 2; referees: 3 approved]

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Abstract

Dystrophic epidermolysis bullosa 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** channel.

Open Peer Review

Referee Status:

	Invited Referees		
	1	2	3
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	↑		
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- Regina Fölster-Holst**, Universität Kiel Germany
- Mohamed Badawy Abdel-Naser**, Venerologie und Allergologie Städtisches Klinikum Dessau Germany

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

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REVISED Amendments from Version 1

1. New references were added according to the reviewers comments
2. Removed the confusing generalised statements from the introduction

No other changes were made to the methods, findings or conclusion about the study

See referee reports

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*)¹. 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 deformities¹⁻³.

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 blistering 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

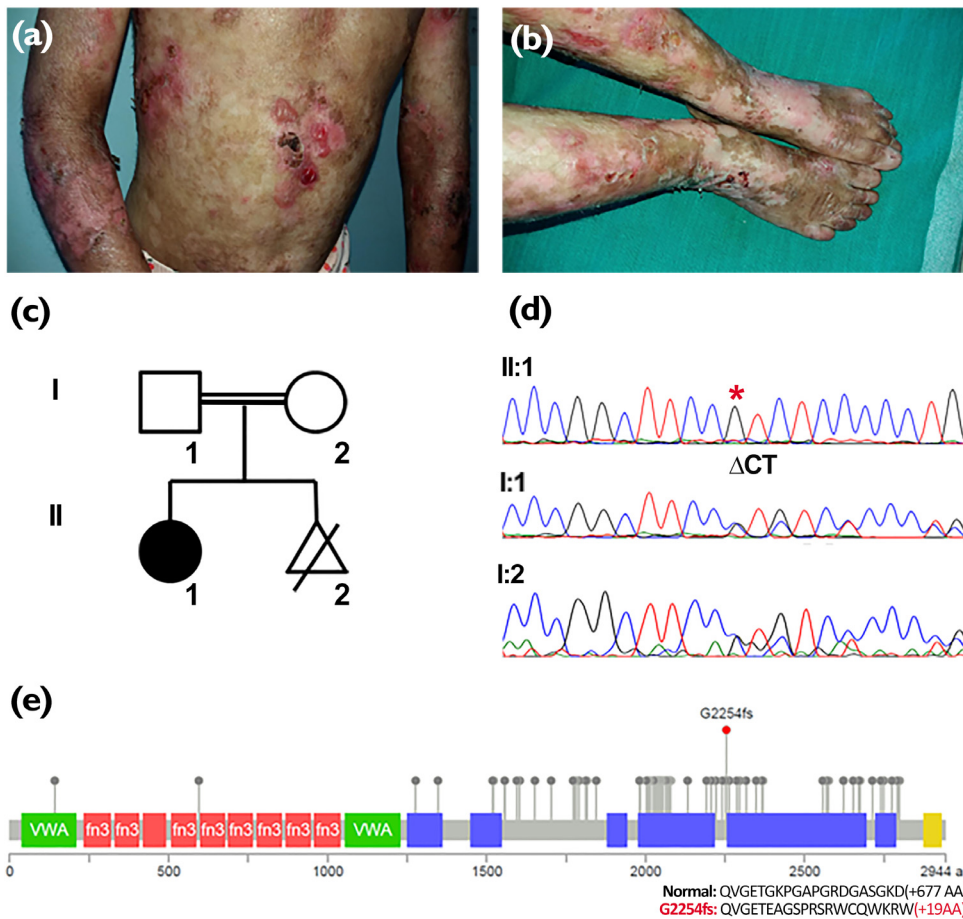


Figure 1. a) Hands and thoracic region showing generalized bullae, scarring and milia b) Lower legs showing scarring, bullae, milia and characteristic dystrophic nails c) Pedigree of the family d) The chromatogram depicting capillary sequencing results of c.6759_6760del in the trio. The mutation loci (Δ CT) is highlighted with asterisks e) Domain structure of COL7A1 protein showing Von Willebrand factor type A domain (VWA), Fibronectin type III domain (fn3), collagen triple helix domain (blue) and Kunitz domain (yellow). Each needle represents disease causing variation site and the red needle represent p.G2254fs (c.6759_6760del) variation. Panel at the bottom represents COL7A1 p.G2254fs induced PTC compared to the normal protein.

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 is fast, less tedious, and cost-effective and also provides a holistic view of the mutation spectrum in the patient⁴⁻⁶.

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 method⁷. 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 using 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*)⁸. The mean mapped coverage on target region was 12.2x. Variants were called using Platypus pipelines (version 0.7.9.1)⁹. 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 SIFT¹⁰. Homozygous PTCs in *COL7A1* is previously reported to reduce overall stability of anchoring filaments and cause mild to very severe generalised RDEB¹. 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)¹¹.

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 form¹. Several cases of DEB have been reported from India. A recent paper reported a cohort of 17 DEB patients using immunofluorescence mapping¹², though the patients were not genetically characterized. Our earlier report characterized a novel mutation in *KRT5* associated with epidermolysis bullosa (EB) simplex in West India⁶. 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 the all the experiments and analysis and contributed to writing the manuscript.

Competing interests

The authors declare that they have no competing interests.

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

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Current Referee Status:



Version 2

Referee Report 13 July 2016

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Robert Sidbury

Dermatology Division, Seattle Children, Seattle, WA, USA

The authors have responded satisfactorily.

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.

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 27 June 2016

doi:[10.5256/f1000research.9013.r14177](https://doi.org/10.5256/f1000research.9013.r14177)



Mohamed Badawy Abdel-Naser

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- 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.
-

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.

Competing Interests: No competing interests were disclosed.

Referee Report 14 June 2016

doi:[10.5256/f1000research.9013.r14364](https://doi.org/10.5256/f1000research.9013.r14364)



Regina Fölster-Holst

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The authors describe a 5 year old boy with dystrophic epidermolysis bullosa (DEB), which is due to a novel mutation in the *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 *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?

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.

Competing Interests: No competing interests were disclosed.

Referee Report 06 June 2016

doi:[10.5256/f1000research.9013.r14175](https://doi.org/10.5256/f1000research.9013.r14175)



Robert Sidbury

Dermatology Division, Seattle Children, Seattle, WA, USA

Though the authors distinguish dominant (DDEB) and recessive (RDEB) subtypes of dystrophic epidermolysis bullosa (DEB) at the outset in their abstract, they lapse into speaking of DEB in more monolithic terms later and conflating findings that are specific in some cases only to one subtype. There is a reason for this as there is considerable overlap but there are differences particularly with certain features like the propensity for developing skin cancer. The authors state "the development of skin cancer....in almost all affected members in the third or fourth decade of life." This is true for RDEB phenotype but not DDEB in whom the development of squamous cell carcinoma as well as the distinctive psuedosyndactyly type of scarring much less common. This may not be the forum for parsing such details but this struck me.

Similarly, while I realize this is not an EB review article a brief internal reference to the Vander Oever article might allow interested readers easy access to a therapeutic update.

Finally, I would want someone other than I with expertise in the genetic methods used to weigh in on their suitability. The methods and results to my untrained --relative to a geneticist-- eye appear sound.

Otherwise I approve indexing this article.

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[PubMed Abstract](#) | [Publisher Full Text](#)
2. Vanden Oever MJ, Tolar J: Advances in understanding and treating dystrophic epidermolysis bullosa. *F1000Prime Rep.* 2014; **6**: 35 [PubMed Abstract](#) | [Publisher Full Text](#)

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.

Competing Interests: No competing interests were disclosed.

Author Response 27 Jun 2016

Vinod Scaria, Institute of Genomics and Integrative Biology, Delhi, India

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.