

Targeted next-generation sequencing identifies a novel mutation of *LAMB3* in a Chinese neonatal patient presented with junctional epidermolysis bullosa

Hairong Wang, MS^a, Yun Yang, MD^{a,b}, Jieqiong Zhou, MD^c, Jiangxia Cao, MD^c, Xuelian He, MD^c, Long Li, MS^a, Shuyang Gao, MS^d, Bing Mao, MD^e, Ping Tian, MD^c, Aifen Zhou, MD^{c,*}

Abstract

Rationale: Epidermolysis bullosa (EB) refers to a group of rare inherited mechanobullous disorders that present with great clinical and genetic heterogeneity. Its severity ranges from mild blistering to life-threatening. However, the clinical symptoms of different types of EB overlap significantly, especially at an early stage. Thus it is important to clarify the diagnosis for prognostic implications, patient management, and genetic counseling.

Patient concerns: Here, we report a 10-day-old male neonate from a nonconsanguineous Chinese family. He showed a bulla on the left lower limb lasting for 3 days, erosions around fingertips and toe tips at birth (predominantly on fingers), with the progressive spread of generalized blisters over the body as well as the development of the illness.

Diagnosis: The patient was diagnosed with suspected epidermolysis bullosa according to the blisters and erosions of the body as well as the pyogenic fingernails and toenails.

Interventions: The patient was performed targeted next-generation sequencing (NGS) with 9 candidate known genes, subsequently, his parents were screened for the mutations identified in the patient by Sanger sequencing. Then, prenatal diagnosis with amniotic fluid was performed in the subsequent pregnancy by Sanger sequencing.

Outcomes: Targeted NGS revealed a previously unreported splice site variant c.822+1G>A (IVS 8) and a known recurrent nonsense variant c.124C>T (p.Arg42Ter, exon 3) in *LAMB3* gene. The patient's father possessed a heterozygous c.822+1G>A mutation, his mother possessed a heterozygous c.124C>T mutation. For the subsequent pregnancy, the analyses of amniotic fluid sample indicated that the fetus carried neither of the mutations.

Lessons: Our finding will further enlarge *LAMB3* genotype-phenotype correlations spectrum. Targeted capture sequencing is a valuable method to illustrate precise molecular pathology in patients with EB disorders, especially at an early stage of the clinical evaluation of complex disorders to avoid unnecessary and economically wasteful tests.

Abbreviations: DEB = dystrophic epidermolysis bullosa, DNA = deoxyribonucleic acid, EB = epidermolysis bullosa, EBS = epidermolysis bullosa simplex, JEB = junctional epidermolysis bullosa, LE = laminin epidermal, NGS = next-generation sequencing, PTC = premature termination codon.

Keywords: generalized severe junctional epidermolysis bullosa, genotype-phenotype, *LAMB3*, prenatal diagnosis, targeted next-generation sequencing

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HRW and YY contributed equally to this work.

The study was approved by the BGI-Shenzhen ethics committee (no: BGI-IRB 17232) and performed with written informed consent for participation from the proband's parents.

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^aBGI-Wuhan, BGI-Shenzhen, Wuhan, Hubei, ^bDepartment of Obstetrics and Gynecology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, ^cDepartment of Obstetrics and Gynecology, Wuhan Medical & Health Center for Women and Children, Wuhan, Hubei, ^dBGI Genomics, BGI-Shenzhen, Shenzhen, Guangdong, ^eDepartment of Neurology, Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.

* Correspondence: Aifen Zhou, Department of Obstetrics and Gynecology, Wuhan Medical and Health Center for Women and Children, Wuhan, Hubei 430015, China (e-mail: 2395676763@qq.com).

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1. Introduction

Epidermolysis bullosa (EB) manifests as a group of rare inherited bullous disorders that skin is extremely fragile with blistering formation due to tiny or no apparent mechanical trauma. It is classified into 4 main types based on the identification of the level of skin cleavage: epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JEB), dystrophic epidermolysis bullosa (DEB), and Kindler syndrome. JEB is usually considered as the most severe type of EB in which blisters occur within skin basement membrane at the level of the lamina lucida. JEB is subdivided into JEB generalized subtypes, JEB localized subtypes based on clinical phenotypic features and relative severity from mild blistering to life-threatening disease.^[1] Generalized severe JEB type (formerly Herlitz type, OMIM 226700) is a lethal skin-blistering of JEB generalized subtype with mucous membranes in autosomal recessive inheritance, it leads to the early demise of patients, usually within the 1st few months of life due to serious complications.^[2,3] The incidence of generalized severe JEB is about 0.41 per million live births in the United States.^[4]

It is caused by loss-of-function mutations in 3 genes: *LAMA3*, *LAMB3*, *LAMC2*, encoding the subunits of laminin-332 (designated the $\alpha3$, $\beta3$, $\gamma2$ chains),^[5] which belongs to a family of basement membrane proteins involved in adhesion of epidermal-dermal cells, forming scaffolds for migrating cells. Mutations in *LAMB3* account for 80% of generalized severe JEB according to the reported cases,^[6–9] especially with 2 recurrent mutations R635X and R42X in Caucasian patients' *LAMB3* alleles.^[8]

The EB disease groups are clinically and genetically heterogeneous; however, the clinical features of all types of EB overlap significantly, no matter how different they are in severity, elucidation of mutations in different EB forms that can help to improve diagnostics, prognostic implications, and patient management, so it is necessary to establish the molecular diagnosis of EB. Current approaches available for diagnosis of EB are 3 steps involving skin biopsy for electron microscopy, immunohistochemistry, and Sanger sequencing of the candidate gene. They are slow, labor-intensive, expensive, and incapable of identifying pathogenic mutations in ~15% of cases.^[10–12] Next-generation sequencing (NGS) is a fast, precise, and efficient complementary diagnostic which has been applied widely in the clinical examination in recent years, and has already shifted the diagnostic paradigm. A growing number of EB cases were successfully identified disease-causing mutations by NGS strategy which improve diagnostic sensitivity in EB.^[10,13–15]

Herein we described a Chinese male infant with suspected EB. We designed a customized panel including 9 known candidate genes such as *KRT5*, *KRT14*, *PLEC*, *ITGB4*, *LAMB3*, *COL17A1*, *LAMC2*, *LAMA3*, *ITGA6* associated with EB. Then we used the panel to identify the causative mutations in this patient by targeted capture sequencing. To confirm the mutations revealed by the targeted capture sequencing, co-segregation analysis of the proband and his parents was performed by the Sanger sequencing. Detection of gene mutations is important for genetic counseling of family recurrence risk and prenatal diagnosis.^[16,17] When the proband's mother was pregnant again, she was referred to hospital for genetic counseling at 18-week gestation. She expressed a great desire to know whether she might be carrying another affected fetus or not, the amniotic fluid was performed for prenatal diagnosis, then the fetal carrying status of the mutations was identified by Sanger sequencing.

2. Case report

2.1. Clinical findings

The proband was born at 38-week gestation, spontaneous delivery by a 25 years old mother. His birth weight was 2800g. His family had no history of genetic skin diseases. The patient attended the Wuhan Medical and Health Center for Women and Children for evaluation at the age of 10 days.

The patient presented a bulla on the left lower limb lasting for 3 days, erosions around fingertips and toe tips at birth (predominantly on fingers). Physical examination: temperature was 36.5°C, heart rate was 140/min, respiratory rate was 38/min. The infant showed premature appearance with clear consciousness. Anterior fontanelle was 2 cm × 1.5 cm, posterior fontanelle was 0.2cm. Skin blisters continued developing to most of the body surface, including the scalp, ear, napkin, hands, and feet region (Fig. 1A–D). Fingers and toes were pyogenic, the left second to 3rd fingernail and the right 3rd fingernail were lost (Fig. 1C). The napkin region was completely affected, purulent secretion was present in the umbilical region (Fig. 1D). Laboratory examination (Table 1): blood analyzer showed eosinophilia, serum myocardial zymogram results showed the concentrations of CK-MB were 36U/L (normal 0–24) which signaled the existence of infection. Liver function test results were TP*48.4g/L (normal 63–82), ALB*29.7g/L (normal 35–50), ALT*13U/L (normal 21–72), respectively. All results of coagulogram, blood and pus bacterial culture, electrolyte, renal function, stool routine, TORCH, and imaging examination were tested normally.

Since onset, his appetite, mental state, and sleep were good, stools and urination were normal. Flucloxacillin sodium and Ceftazidime was given to treat the bacterial infection, nursing care of skin was also performed. He was admitted with a clinical diagnosis suspected epidermolysis bullosa based on the clinical presentation. Targeted capture sequencing was further performed to help clinical refined classification. The patient died due to the complications of his disease at age <8 weeks.

The study was performed with written informed consent for participation from the proband's parents and approved by the BGI-Shenzhen ethics committee (no: BGI-IRB 17232).

2.2. Targeted NGS

Peripheral blood sample was obtained from the patient (2mL). Total DNA of peripheral blood was isolated using the QIAamp DNA extraction kit (Qiagen, Hilden, Germany). The genomic DNA sample was manipulated by washing, elution, and postcapture amplification according to NimbleGen protocols (NimbleGen; Roche Molecular Diagnostics, Pleasanton, CA). Library construction, sequencing, and data information analysis were performed as described.^[18]

The size of capture target region was 57,437bp with the mean sequencing depth 185.61-fold. The coverage of target region was 99.89% and the ratio of more than 30-fold sequencing depth of target region was 94.42%. We identified 2 compound heterozygous mutations in *LAMB3* (NM_000228) (c.124C>T, c.822+1G>A). The c.124C>T (p.Arg42Ter, chr1:209823368) mutation in exon 3 of the coding region was supported by 119 out of 200 reads, and the c.822+1G>A (chr1:209805927) mutation at IVS 8 was supported by 21 out of 33 reads covering the mutation site (Fig. 2). To the best of our knowledge, the c.124C>T (p.Arg42Ter) mutation changes an arginine codon to a premature stop codon which is one of the recurrent pathogenic mutations.



Figure 1. Clinical manifestations of the patient. (A, B) Blisters on the scalp and scab formed on the head. (C) Dystrophic shedding of nails. (D) Erosions on the groin region.

The c.822+1G>A mutation is a novel mutation which located in laminin epidermal growth factor-like (LE) domain. Prediction with SPANR (<http://tools.genes.toronto.edu>) showed that c.822+1G>A alters the normal donor splice site with the dPSI_per-

centile 0.07. Prediction with HSF (<http://umd.be/HSF3/>) showed that it probably affects donor splicing.

Table 1

Laboratory findings of the patient.

Characteristics	Result	Units	Reference range
HGB	127	10 ⁹ /L	140–170
HCT	37.3	%	41–51
RDW-SD	55.0	fL	32.2–42.4
TP	98.4	μmol/L	208–506
BUN	1.4	mmol/L	3.2–7.1
CREA	38.8	μmol/L	58–110
CK-MB	36	U/L	0–24
TP	48.4	g/L	63–82
ALB	29.7	g/L	35–50
ALT	13	U/L	21–72
IgE	19.6	IU/mL	0–15
HBsAb	198	IU/L	0–10
HbCAb	0.008	COI	>1.0
Purulent secretion culture	Negative		
Blood culture	Negative		
TORCH	Negative		

ALB=albumin, ALT=alanine transaminase, BUN=blood urea nitrogen, CK-MB=creatinase-MB, CREA=creatinine, HBsAb=hepatitis B surface antibody, HbCAb=hepatitis B core antibody, HCT=hematocrit, HGB=hemoglobin, IgE=immune globulin, RDW-SD=red-cell distribution with, TORCH=toxoplasmosis, other (viruses), rubella cytomegalovirus, herpes simplex viruses, TP=total protein.

2.3. Sanger sequencing

To confirm the mutations that were revealed by the targeted sequencing and examine the molecular basis of the family, cosegregation analysis was performed by the Sanger sequencing for the patient and his parent. Peripheral blood samples were obtained from the parent (5 mL). Total DNA of peripheral blood was isolated by the QIAamp DNA extraction kit (Qiagen). Primers (Table 2) for 2 mutations were designed according to the *LAMB3* gene sequence. Polymerase chain reaction products were sequenced by 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, MA). The heterozygous c.124C>T mutation was observed in the proband's mother, the heterozygous c.822+1G>A mutation was confirmed in the proband's father (Fig. 3). The result indicated genotype-phenotype segregation in the family.

2.4. Prenatal diagnosis

For the subsequent pregnancy, amniotic fluid was extracted (20 mL) from the pregnant mother at 18 weeks gestation under ultrasound guidance for prenatal diagnosis. The fetal DNA was isolated from the amniotic fluid using the QIAamp DNA Mini kit (Qiagen). Sanger sequencing was performed with the previously mentioned primer pairs for cosegregation analysis. The result

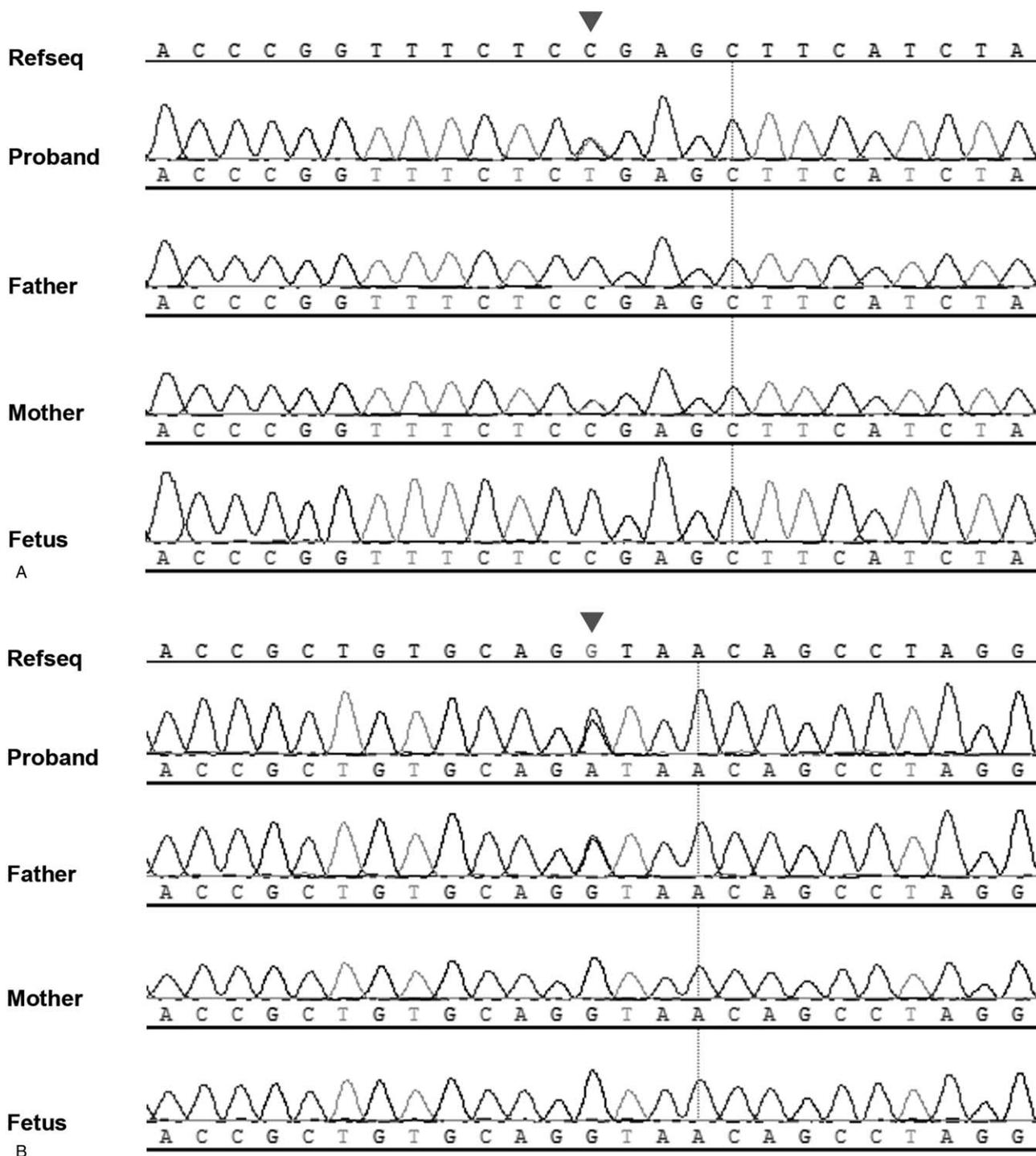


Figure 3. Cosegregation analysis of the mutations in the family by Sanger sequencing. The mutated bases are indicated by a triangle. (A) The heterozygous mutation c.124C>T. (B) The heterozygous mutation c.822+1G>A.

weeks. Based on clinical presentations and mutation analysis, we preliminarily concluded the clinical diagnosis as generalized severe JEB.

The mutation c.124C>T is a recurrent pathogenic mutation observed in the Caucasian group. The mutation c.822+1G>A is a novel disease-causing mutation which has not been reported previously. Generally, generalized severe JEB tends to predominantly harbor premature termination codon (PTC) mutations in

both alleles of *LAMA3*, *LAMB3*, or *LAMC2* gene encoding the subunits of laminin-332. However, the patients with missense or splice junction mutations and PTC combination, have also been reported as JEB.^[20,21] In our case, the proband possessed one nonsense mutation, 1 splice site mutation. The consequences of the putative splice site mutation consist of in-frame exon skipping, out-of-frame deletion, PTC, or combinations of these.^[22] The consequence of the c.822+1G>A mutation was

not determined in mRNA level since the patient's sample was unavailable at the time. Therefore, we could not fully elucidate the cause of the severe manifestations in the patient.

Prenatal diagnosis was performed on the fetus as the parents wanted to have a genetic test when they were pregnant again. The fetus carried neither of the mutations. Based on this result, we gave the parents genetic counseling that the fetus was predicted as phenotypically unaffected.

In summary, we report a nonsense mutation c.124C>T and a novel splice site mutation c.822+1G>A in *LAMB3* gene. Our findings will broaden the genotype–phenotype correlations of *LAMB3* database. Targeted capture sequencing is a valuable method to illustrate precise molecular pathology in patients with EB disorders, especially in cases with an atypical clinical manifestation at an early stage of the clinical evaluation for complex disorders to avoid unnecessary and economically wasteful tests.

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Author contributions

Conceptualization: Xuelian He.

Data curation: Hairong Wang, Bing Mao.

Formal analysis: Jieqiong Zhou, Jiangxia Cao.

Investigation: Jieqiong Zhou, Xuelian He, Long Li, Bing Mao, Ping Tian.

Methodology: Yun Yang, Long Li.

Project administration: Aifen Zhou.

Resources: Yun Yang, Jieqiong Zhou, Jiangxia Cao, Bing Mao, Ping Tian, Aifen Zhou.

Software: Jiangxia Cao.

Validation: Long Li, Ping Tian.

Visualization: Xuelian He, Aifen Zhou.

Writing – original draft: Hairong Wang, Yun Yang.

Writing – review & editing: Hairong Wang, Yun Yang, Shuyang Gao, Aifen Zhou.

Hairong Wang orcid: 0000-0002-1616-5884.

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