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IDENTIFICATION OF A *DE NOVO* MUTATION IN *KRT5* GENE UNDERLYING EPIDERMOLYSIS BULLOSA SIMPLEX BY WHOLE EXOME SEQUENCING IN A VIETNAMESE PATIENT

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SUMMARY

Epidermolysis bullosa simplex (EBS) is a group of epidermolysis bullosa (EB) and accounts for 75-85% EB cases. Most EBS patients are caused by mutations in *KRT5* or *KRT14*, encoding for keratin 5 and keratin 14, respectively, which impair the structural entirety of paired intermediate filaments expressed in the fracture of basal keratinocytes and subsequent blistering of the epithelium. This study aimed to identify the causative mutation in a Vietnamese EB case. Whole exome sequencing (WES) was performed in the affected individual and revealed a *de novo* heterozygous pathogenic mutation in exon 7 of *KRT5* gene, resulting in an amino acid change at position 477, with glutamic acid to lysine substitution (p.E477K). The *KRT5* p.E477K was strong associated with the very severe or lethal of generalized severe EBS (GS-EBS), characterized by the severe symptoms at birth, improving with age and evolution to palmoplantar keratoderma and nail dysplasia. Our finding will aid the molecular diagnosis, prognosis prediction of the patient with GS-EBS due to p.E477K and significant genetic counselling the family concerning the recurrence risk for future pregnancies.

Keywords: *Epidermolysis bullosa simplex, KRT5, whole exome sequencing*

INTRODUCTION

Epidermolysis bullosa (EB) is a rare inherited disorder characterized by mechanical fragility of epithelial lined or surfaced tissues. Four major EB types have been described based on the certain layer in which the blisters form. Epidermolysis bullosa simplex (EBS) represents the most common EB type (75-85% of all cases). EBS typically affects hands and feet with blister formation first occurs within the basal keratinocytes. EBS is mainly associated with mutation in keratin encoding genes *KRT5* and *KRT14* resulting in dominantly inherited pattern. Other genetic mutations were found in genes encoding hemidesmosomal proteins such as plectin (*PLEC1*) and dystonin (*DST*), leading to recessive inherited pattern. The *EXPH5* gene, which encodes for exophilin 5 is also implicated in EBS. Furthermore, mutations in *KLHL24* and *CD151* genes, which expressed in increased keratin 14 degradation and the basolateral surface of basal keratinocytes within the hemidesmosomes, respectively, are also contribute to EBS (Bardhan *et al.,* 2020). Junctional epidermolysis bullosa (JEB) is a subtype with autosomal recessive inheritance and is characterized by the blisters within the lamina lucida of the basement membrane zone. In JEB, laminin coding genes are mutated (*LAMA3*, *LAMB3*, *LAMC2*). Additionally, it had been reported that mutations in genes encoding

for β4 integrin and α6 integrin chains (*ITGB4* and *ITGA6* genes) could cause JEB with pyloric atresia (Georges-Labouesse *et al.,* 1996; Vidal *et al.,* 1995). Dystrophic epidermolysis bullosa (DEB) is a subtype inherited in autosomal recessive or dominant manner, which caused by mutations in collagen type VII coding gene *COL7A1*, and the separation of tissue happens in the dermis layer. Finally, Kindler syndrome is the fourth major EB subtype, inherited in autosomal-recessive manner, is a subtype of EB with loss of function mutations in *FERMT1* encoding kindlin-1. Kindlin-1 is a component of adhesion contacts in basal keratinocytes, periodontal tissue and colon (Bardhan *et al.,* 2020; Fine *et al.,* 2014). Here, we report a Vietnamese patient with EBS causing by a *de novo* mutation in the *KRT5* gene.

CASE DESCRIPTIONS

The patient was a 3-year-old boy with a clinical diagnosis of EB in healthy nonconsanguineous Vietnam parents, recruited from Vietnam Military Medical University. Written informed consent was obtained from all family members before sample collection. This study was approved by the Institute of Genome Research Institutional Review Board, Vietnam Academy of Science and Technology. The patient's birth weight was 2.9 kg in the full term of gestational age and presented with severe lesions including peeling skin on limbs (Figure 1A and 1B) and widespread blisters, erosions in the body. In 14 days after birth, the level of lesions increased in two legs and signed of necrosis with smelly. However, his lesions gradually improved by taking well care with a bandage, clean properly and skin moisturizing. The syndactyly of fingers was seen and had to bandage for separation. The extent of these lesions improved over time, with primarily involving areas of friction (Figure 1C). The development of nail dystrophy in two index fingers and toes, and moderate palmoplantar keratoderma were observed around 6 years old.

Figure 1. Clinical features of Vietnamese EBS patient. Infant presented with generalized bullae, erosions in arms (A) and the peeling skin in legs (B). The improvement with age was observed and patient presented with fewer and milder lesions (C).

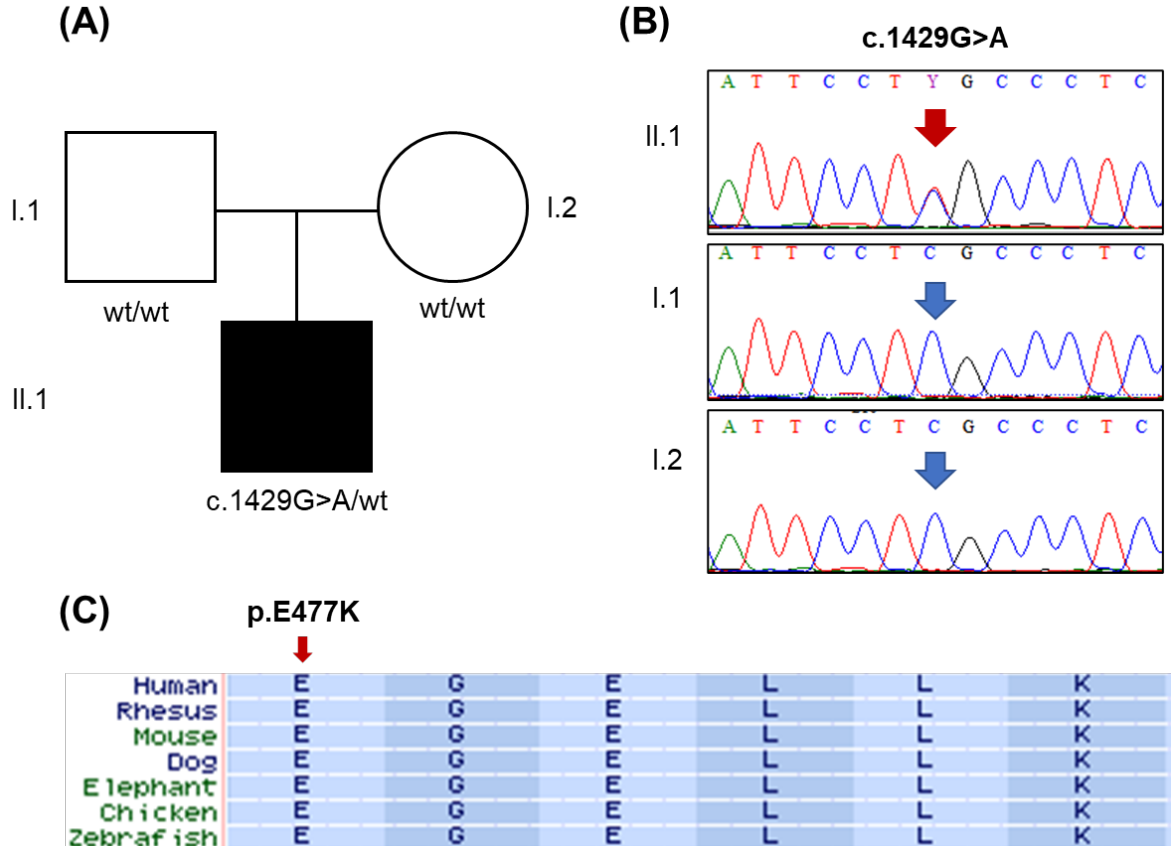

Figure 2. (A) Pedigree chart of the family with a GS-EBS case. Black/white represent patient/not affected individuals. Square and circle show male and female members. (B) Electropherogram of the patient (II.1) with a *KRT5* heterozygote, which was not inherited from the parent (I.1 and I.2). Mutated/wild-type (wt) nucleotides were marked with red/blue arrows. (C) The amino acid substitutions at the second glutamic acid residue (p.E477K) which was located in the most evolutionarily conserved KLLEGE motif among all types of intermediate filament proteins were marked with a red arrow.

In this study, whole exome sequencing (WES) in the affected individual was used for mutation identification (Figure 2A). Library construction was performed using Sure Select V6-Post (Agilent Technologies, Santa Clara, California, USA) and sequencing followed by Illumina platform (Illumina, San Diego, CA, USA) with paired reads of 150 bp. The raw sequencing data was over 8.7G, the base Q30 ratio greater than 90%, and the 20× sequencing depth was more than 90% of bases. WES analysis revealed a heterozygous variant of c.1429G>A in exon 7 of the *KRT5* (NM_000424) gene, leading to the substitution of glutamic acid to lysine (p.E477K). This variant corresponded to rs59190510 in the dbSNPs build 155, but the allele frequency was zero. Additionally, the c.1429G>A was described as pathogenic in ClinVar (VCV000021174.4) with at least 12 reported cases. Verification by Sanger sequencing also showed the same result in the proband, and the parents have not carried the mutation (c.1429G>A) (Figure 2B).

DISCUSSION

EBS is a heritable disorder most commonly caused by autosomal dominant mutations in genes of basal cell keratin (*KRT5* and *KRT14*), with either of mutation in these genes result in

altered proteins, causing instability of the cytoskeletal structure and blistering at pressure sites (Coulombe and Lee 2012; Homberg and Magin 2014). Various subtypes of EBS are identified, from localized to generalized severe (GS) of skin fragility with increase of severity (Fine *et al.,* 2014).

In this case, the patient was diagnosed with EB since birth with severe lesions, however, he tends to be improved over time with milder lesions in the present (Figure 1C). The genetic analysis revealed a *de novo* heterozygote *KRT5* gene (c.1429G>A) in the proband, leading to the substitution of the conserved glutamic acid by glycine acid (p.E477K). Until now, four missense mutations at codon 477 in *KRT5* transcription associating to differ severity in phenotype have been found, with p.E477G results in localized EBS, while p.E477D, p.E477K and p.E477* cause GS-EBS (Hamada *et al.,* 2005; Kim *et al.,* 2017; Lalor *et al.,* 2019; Müller *et al.,* 1999; Sathishkumar *et al.,* 2016; Schumann *et al.,* 2012; Wertheim-Tysarowska *et al.,* 2016). The p.E477K mutation was high associated with mortality in GS-EBS (Sathishkumar *et al.,* 2016), which was reported to at least seven cases died within the first six months of life (Kim *et al.,* 2017; Komori *et al.,* 2018; Lalor *et al.,* 2019; Sathishkumar *et al.,* 2016). The severe phenotype causing by p.E477K would be explained by the substitution of acidic glutamate with a basic lysine residue that alters the second glutamate residue of the most evolutionarily conserved KLLEGE motif at the end of the 2B domain in the central alpha-helical rod, leading to severely disrupts basal keratin intermediate filaments (Lalor *et al.,* 2019; Stephens *et al.,* 1997). Despite the p.E477K predispose to a severe and potentially fatal phenotype of GS-EBS, survivals were also reported. The living cases with severe blisters in childhood were ameliorated when they increase in age and often evolution to palmoplantar keratoderma and nail dysplasia (Kim *et al.,* 2017; Komori *et al.,* 2018; Sathishkumar *et al.,* 2016). The clinical of patients in this study were followed the observation of survived EBS due to *KRT5* p.E477K.

In conclusion, the p.E477K *de novo* mutation was firstly identified in Vietnamese GS-EBS. A precise genetic diagnosis, in this case, could provide an additional scientific basis, prognostication for the affected child, and genetic counseling the family for future pregnancies.

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PHÁT HIỆN ĐỘT BIẾN *DE NOVO* TRÊN GEN *KRT5* GÂY RA LY THƯỢNG BÌ BÓNG NƯỚC ĐƠN HÌNH BẰNG GIẢI TRÌNH TỰ TOÀN BỘ HỆ GEN MÃ HÓA Ở MỘT BỆNH NHÂN VIỆT NAM

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TÓM TẮT

Ly thượng bì bóng nước đơn hình (EBS) là một phân nhóm của ly thượng bì bóng nước (EB), chiếm 75-85% các trường hợp EB. Nguyên nhân gây ra EBS ở hầu hết các trường hợp là do đột biến trên gen *KRT5* hoặc *KRT14*, mã hóa lần lượt cho protein keratin 5 và keratin 14, làm suy giảm tính toàn vẹn cấu trúc của các sợi trung gian ghép nối này và biểu hiện trong sự đứt gãy của các tế bào biểu mô sừng cơ bản và biểu mô bị phồng rộp sau đó. Nghiên cứu này tiến hành xác định đột biến gây bệnh ở một bệnh nhân EB Việt Nam, sử dụng phương pháp giải trình tự toàn bộ hệ gen mã hóa (WES). Chúng tôi đã phát hiện được một đột biến phát sinh mới (*de novo*) gây bệnh ở dạng dị hợp tử trên exon 7 của gen *KRT5*. Đột biến này dẫn đến sự thay thế axit glutamic thành lysine ở vị trí 477 (p.E477K) trên phân tử protein. p.E477K có liên quan chặt chẽ đến mức độ biểu hiện bệnh từ rất nặng đến tử vong ở các trường hợp EBS nghiêm trọng suy rộng (GS-EBS), đặc trưng bởi các triệu chứng nghiêm trọng khi mới sinh, được cải thiện khi lớn lên và tiến triển với biểu hiện dày sừng lòng bàn tay và loạn sản móng. Phát hiện này góp phần hỗ trợ trong chẩn đoán phân tử, dự đoán tiên lượng của bệnh nhân mắc GS-EBS gây ra bởi p.E477K, đồng thời có ý nghĩa trong tư vấn di truyền cho gia đình về nguy cơ tái phát cho những lần mang thai sau này.

Từ khoá: *Ly thượng bì bóng nước đơn hình, KRT5, giải trình toàn bộ hệ gen mã hóa*