Known and novel mutations responsible for epidermolysis bullosa simplex cases in a Chinese population

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Abstract. Epidermolysis bullosa simplex, generalized severe (EBS-gen sev) is one of the major forms of EBS, caused by mutations of the keratin 5 (*KRT5)* or keratin 14 (*KRT14)*. However, it is rarely reported in the Chinese population. The current study was performed on three unrelated Chinese families with five patients clinically suspicious for distinct stages of EBS. Mutation screening was performed by direct sequencing of the entire coding regions of *KRT5* and *KRT14* genes. A diagnosis of EBS-gen sev for patients in these three families was confirmed by revealing missense mutations c.373C>T (p.Arg125Cys), c.374G>T (p.Arg125Leu), and a novel frameshift mutation c.1231delG (p.Glu411Argfs*31) in *KRT14*. Considering two previously reported cases and the results of the current report, amino acid residue 125 is likely the most frequent hotspot of EBS-gen sev in the Chinese population. The current study further indicated that the symptoms of EBS-gen sev patients decline with age.

Introduction

Epidermolysis bullosa simplex (EBS) belongs to a group of inherited disorders characterized by the high occurrence of bullous lesions after a certain degree of friction or trauma. It is most commonly caused by mutations in keratin 5 (*KRT5*) or keratin 14 (*KRT14*) (1,2). The protein products of these genes, keratin 5 and keratin 14, are paired intermediate filaments expressed in basal keratinocytes that contribute to mechanical stability of keratin filament networks (1,2). EBS is comprised of three major forms: i) Localized and mild subtype, called EBS, localized (EBS-loc; OMIM 131800) or EBS Weber-Cockayne; ii) generalized and severest subtype, EBS Dowling-Meara (EBS-DM; OMIM 131760); and iii) generalized but relatively milder subtype known as EBS, generalized intermediate (EBS-gen intermed; OMIM 131900) or EBS-Koebner (3). EBS, generalized severe, formerly known as EBS Dowling-Meara (EBS-gen sev; OMIM 131760), is the most severe form of EBS. Its cardinal features include large, generalized blisters, mucous membrane involvement, and dystrophic nails. Bullous lesions are usually most severe in the neonatal and infancy stages, diminishing in severity with age, especially during later childhood and adulthood (3). The ultrastructural pathogenesis of EBS-DM includes clumping or collapsing of keratin filaments in the basal epidermal cells, leading to basal cell cytolysis and sequent intraepidermal blister formation (4,5). In most cases, the clinical severity is related to the location of the mutations. Most of the mutations responsible for this subtype are located in the highly conserved α-helical end segments of helix 1A and 2B (including amino acid residue 125) of *KRT5* and *KRT14*, which are critical for proper intermediate filament structure (6). The current study reported the distinct clinical features of three Chinese probands suspected of having EBS and diagnosed with the EBS-gen sev subtype by molecular analysis.

Patients and methods

Patients. Three unrelated Chinese families with three probands clinically suspected of having EBS subtypes were enrolled in the current study at the outpatient department of Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine from October 2016 to October 2018, including two young males (age, 4 and 10 months), and a 19-year-old female.

PCR and Sanger sequencing. Primers flanking all coding exons and intron-exon boundaries of *KRT5* and *KRT14* were designed using Primer Premier 5.0 software (Premier Biosoft International). Sequences of primers used in the current study are presented in Table SI. Sanger sequencing was performed to identify the mutations in all patients and to verify the sequences of unaffected family members. Peripheral blood samples of all index patients were collected in EDTA anticoagulant tubes (Insepack™; Sekisui Medical Co., Ltd.) and

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frozen at -20˚C. TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd.) was used to extract genomic DNA from 600-μl blood samples according to the manufacturer's instructions. Genomic DNA samples were amplified by PCR using Takara Ex Taq DNA polymerase (Takara Bio, Inc.). The following thermocycling conditions were used: Initial denaturation at 94˚C for 5 min; 31 cycles of denaturation at 94˚C for 30 sec, annealing (temperature for each primer is listed in Table SI) for 30 sec, extension at 72˚C for 1 min, and a final extension at 72˚C for 1 min; and 4˚C for 5 min. PCR products were purified with AxyPrep DNA Gel Extraction kit (Corning, Inc.) according to the manufacturer's instructions. The Sequencing Reaction system was based on BigDye® Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Purified PCR products were directly sequenced using an ABI PRISM® 3730 automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.).

Results

The proband in family 1 was a 10-month-old male. His mother first came to the Department of Dermatology, Xinhua Hospital in April 2014 complaining of generalized skin blisters on the baby's hands and feet since birth (Fig. 1A and B), especially after friction or trauma. Seven months later, the skin lesions over his extremities were slightly improved (Fig. 1C). His 26-year-old father with a similar history of blistering was also present at this visit. Dermatological investigation showed no scarring, but only mild, post-inflammatory pigmentation on skin regions previously covered in blisters (Fig. 1D), although he presented with widespread blisters as an early infant.

The second proband, a 19-year-old female, presented with diffuse pigmentation, scar formation after rupture of the blisters, and scattered new blistering, resembling dermatitis herpetiformis (Fig. 1E and F). With age, the blistering diminished.

The last proband was a 4-month-old infant, with clinical manifestations similar to those of proband 1 (Fig. 1G and H). His 25-year-old mother had a similar clinical history since birth, but currently showed only scattered blisters and mild, post-inflammatory pigmentation (Fig. 1I).

Pedigree charts of these three families are shown in Fig. 2. Mutation screening of *KRT5* was negative, whereas heterozygous missense mutations c.374G>T (Arg125Leu), c.373C>T (Arg125Cys) and c.1231delG (p.Glu411Argfs*31) in *KRT14* were identified in proband 1, proband 2 and proband 3, respectively, and were absent in unaffected family members (Fig. 3). Mutation delineation was based on comparisons with the reported cDNA reference sequence (GenBank accession number, NM_ 000526.4 for *KRT14*). Sequencing results were analyzed using Geneious, version 5.6.7 (Biomatters, Ltd.; http://www.geneious.com/).

Discussion

The underlying pathological mechanism of EBS-gen sev is intraepidermal blister formation via basal cell cytolysis (or, rarely, acantholysis). Other subtypes can be distinguished by clumping or significant collapse of keratin filaments in the basal epidermal cells, which can be observed with immunofluorescence mapping (IFM) or electron microscopy (EM) (7). However, these primary methods may lead to discordance with the actual diagnosis (7). In addition, skin biopsy in infants is usually regarded as a somewhat unacceptable trauma for parents, and complementary genetic testing is required.

EBS-gen sev is inherited in an autosomal dominant pattern. Except for those rare cases caused by truncated mutations, including nonsense/in-frame deletion/frameshift/splicing mutations, most EBS-gen sev cases are attributed to missense mutations that exert a dominant negative effect on functional protein structure by altering inter-chain interactions (8). There is a close correlation between the mutational locus and the severity of EBS. Compared with other two major forms, EBS-loc and EBS-gen, most EBS-gen sev cases were associated with mutations in the highly conserved end segments of *KRT5* or *KRT14* rod domain (6), which highlights the importance of molecular diagnosis.

The site Arg125 (CGC) of KRT14 contains a CpG dinucleotide, making it a hotspot for EBS-causing mutations due to the disposition of a spontaneous mutant. Mutations in Arg125 (including Arg125His, Arg125Cys, Arg125Ser, Arg125Gly and Arg125Leu) have been shown to be responsible for at least 40% of EBS-gen sev cases. In particular, Arg125His and Arg125Cys accounted for the majority of the mutations in Arg125 (1,6,9-14). Arg125 is located in a highly conserved region and strongly perturbs keratin network formation and keratin filament assembly (15). Despite the fact that other identical sites of mutations, even in one pedigree, may lead to distinct subtypes (1,16), Arg125 mutations are confined mainly to the most severe subtype EBS-gen sev with similar clinical courses. To the best our knowledge, only three pathogenic mutations of EBS-gen sev in the Chinese population (Arg165Ser in *KRT5*, and Arg125His and Arg125Cys in *KRT14*) have been reported so far. Only Arg125Leu has been reported in Korean and Polish populations. Phenotypes of patients with Arg125Leu and Arg125Cys in the current study were in accordance with the previously reported phenotypes (1,6,9-14).

Novel mutation c.1231delG (p.Glu411Argfs*31) located near the highly conserved α-helical end segments of helix 2B could cause a stop codon in this highly conserved region, which may result in a more severe phenotype, such as EBS-gen sev. EBS harbors risk of death at an early age (17). Currently, there is no well-established cure, other than general care to prevent trauma, infection control, and good nutrition (18).

Gene therapy research of EBS is rare, although gene therapy and bone marrow transplantation for the relatively severe dystrophic or junctional epidermolysis bullosa, having the possibility of cure, have been well investigated (19,20). These studies highlight that a corrective gene therapy could be an ideal therapy for EBS, however more studies are required before it can be developed and used in daily clinical practice. Prenatal or preimplantation genetic diagnosis is another sensible option for families at high risk of EBS.

The present study provided valuable information and assistance in genetic counseling such that the symptoms of EBS-gen sev patients declined with age. In clinical dermatology, a diagnosis of EBS-gen sev should be considered for patients with widespread, herpetiform, clustered blistering, especially in the neonatal period, diminishing in late childhood. It is

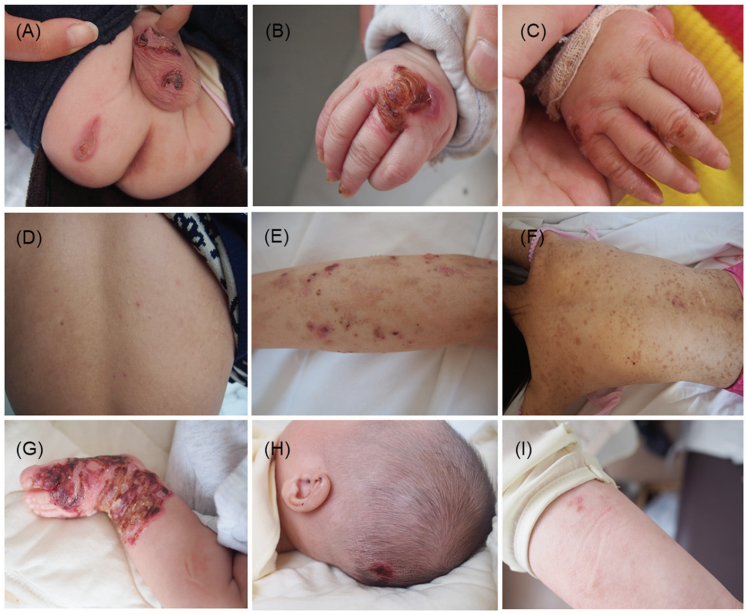

Figure 1. Clinical appearance of EBS patients. (A) The 3-month-old proband in family 1 at the first visit. (B) Proband 1 at their first visit. (C) Seven months later, bullous lesions over the extremities of proband 1 were slightly improved. (D) Twenty-six-year-old father of proband 1 showed no scarring, but only mild, post-inflammatory pigmentation on the skin regions previously covered with blisters. (E) Proband 2: A 19-year-old female presented. (F) A 19-year-old female presented with diffuse pigmentation. Scar formation occurred after rupture of blisters, accompanied with scattered new blistering. (G) Proband 3: A 4-month-old infant. (H) A 4-month-old infant with clinical manifestations similar to proband 1. (I) Twenty-five-year-old mother of proband 3 showed scattered blisters and mild post-inflammatory pigmentation where previously blistered.

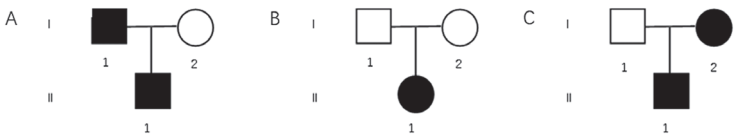

Figure 2. Pedigree charts of the three families. (A) EBS patients in family 1: A 10-month-old male and his 26-year-old father. (B) EBS patients in family 2: A 19-year-old female. (C) EBS patients in family 3: A 4-month-old infant and his 25-year-old mother. Sqaure, male; circle, female; shaded, affected; white, normal.

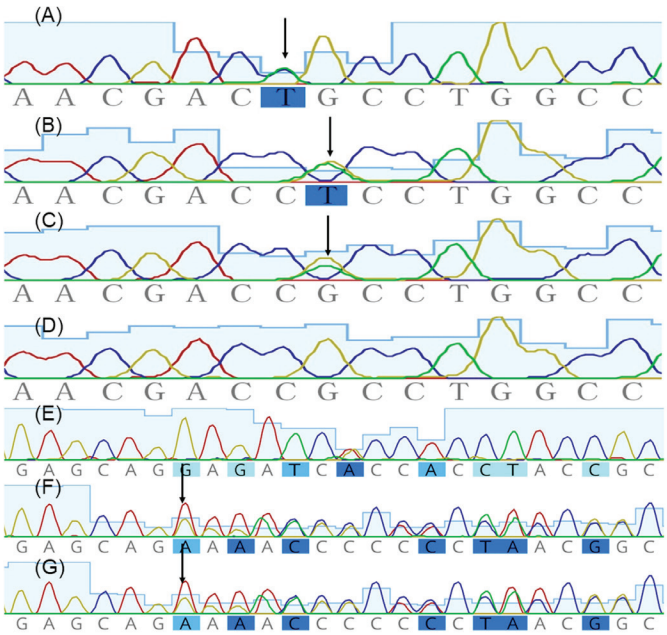

Figure 3. Sequencing results of *KRT14* by Geneious, version 5.6.7 (Biomatters, Ltd.; http://www.geneious.com/). (A) A Heterozygous missense mutation, c.373C>T (Arg125Cys), was revealed in the 19-year-old female in family 2. The c.374G>T (Arg125Leu) mutation was revealed in (B) a 10-month-old boy and (C) his father in family 1. (D) Unaffected mother in family 1. (E) Mutation c.1231delG (p.Glu411Argfs*31) in *KRT14* was (E) absent in his unaffected father but appeared in (F) the proband and (G) his mother in family 3. Mutation delineation was based on comparing with the reported cDNA reference sequence (GenBank accession number, NM_ 000526.4 for *KRT14*). Arrows indicate the site of mutation. *KRT14*, keratin 14.

generally challenging to distinguish EBS subtypes clinically in newborns. Related effective methods such as IFM and EM on freshly induced blisters combined with molecular genetic testing can be conducted to make a clear diagnosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YZ and ZY conceived and designed the present study. ML collected clinical data. JZ and YD assessed the results and wrote the paper.

Ethics approval and consent to participate

The current study was approved by the Institutional Review Board of Xinhua Hospital, Shanghai JiaoTong University School of Medicine and was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. All participants or their legal guardians gave their written informed consent for participation.

Patient consent for publication

Patients or patients' guardians provided consent for the publication of images in the present study.

Competing interests

The authors declare that they have no competing interests.

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