A novel KRT5 mutation associated with generalized severe epidermolysis bullosa simplex in a 2-year-old Chinese boy

JIA ZHANG*, MING YAN*, JIANYING LIANG, MING LI and ZHIRONG YAO

Department of Dermatology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai 200092, P.R. China

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Abstract. Mutations in keratin 5 (KRT5) or KRT14 genes are responsible for the most severe form of epidermolysis bullosa simplex (EBS), which is EBS generalized severe (EBS-gen sev). To date, only four pathogenic mutations (p.Arg165Ser and p.Lys199Asn in KRT5; p.Arg125Cys and p.Arg125His in KRT14) have been reported to be responsible for EBS-gen sev in the Chinese population. In the present study, a 2-year-old Chinese boy was clinically suspected to suffer from EBS, and thus Sanger sequencing was performed in the extracted genomic DNA samples from the patient, his parents and 100 healthy controls. A novel de novo heterozygous missense mutation c.503A>G (p.Glu168Gly) located at the N-terminal end segment of the 1A domain in KRT5 was identified by molecular analysis. In silico analysis tools were used to predict the pathogenicity of the novel missense mutation. A diagnosis of EBS-gen sev was thus confirmed according to the clinical presentations and molecular results.

Introduction

Epidermolysis bullosa simplex (EBS) refers to a number of inherited disorders characterized by mechanical stress-induced blistering of the skin (1). EBS comprises three primary types: Localized [EBS-loc; Online Mendelian Inheritance in Man (OMIM) no. 131800], generalized severe (EBS-gen sev; OMIM no. 131760) and generalized intermediate (EBS-gen intermed; OMIM no. 131900) (1). The ultrastructural pathogenesis of EBS-gen sev is the collapse of keratin filaments in basal epidermal cells, resulting in basal cell cytolysis and sequent intraepidermal blister formation, which can differ from other subtypes (2-4). EBS-gen sev typically presents with characteristic features of large, generalized blisters in early infants, and small, clustered (herpetiform) blisters in childhood. In neonates and infants, EBS-gen sev is life-threatening as cutaneous lesions are typically severe, resulting in difficulties in feeding and care (5-7). Subsequent to infancy, particularly during late childhood and adulthood, the prognosis is favorable (5-7). Mutations located at the highly conserved α-helical end segments of the 1A domain of keratin 5 (KRT5) and 2B domain of KRT14, also known as helix initiation peptide (HIP) and helix termination peptide (HTP), respectively, typically result in EBS-gen sev (8,9). To date, four pathogenic mutations have been reported to be responsible for EBS-gen sev in the Chinese population, including p.Arg165Ser and p.Lys199Asn in KRT5, and p.Arg125Cys and p.Arg125His in KRT14 (10-13).

In the present study, molecular genetic tests were performed in a 2-year-old boy with suspected EBS, and a novel missense mutation c.503A>G (p.Glu168Gly) located at the N-terminal end segment of the 1A domain of KRT5 (HIP) confirmed a diagnosis of EBS-gen sev.

Materials and methods

Case. The proband was a 2-year-old boy that was referred to the Department of Dermatology of Xinhua Hospital (Shanghai, China) in October 2013. The patient had been reported to present generalized blisters throughout his body since birth (Fig. 1A-D), which were worsened by friction or trauma and were not treated. No other symptoms were observed other than blisters. At the 9-month follow-up visit a dermatological examination showed that there was no evident improvement (Fig. 1E and F). No other relevant medical history and consanguinity was reported in the patient's family. The patient was suspected to have EBS based on the clinical manifestations, and Sanger sequencing was performed to clarify the diagnosis.

Subjects. The proband, his parents and 100 population-matched healthy controls (the mean age of the healthy controls was 24 years old with a range between 18 to 30 years old and a gender ratio of females/males equal to 1.0) were enrolled in the present study. Subsequent to obtaining written informed consent from the participant's mother, peripheral blood
samples were collected for DNA extraction. The present 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.

Methods. Genomic DNA was extracted using a TIANamp
Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China).
Primers flanking all coding exons and intron-exon boundaries
of KRT5 and KRT14 were designed using Primer Premier
version 5.0 (Premier Biosoft, Palo Alto, CA, USA; Table I).
Genomic DNA samples were amplified using polymerase chain
reaction (PCR). PCR was performed as follows: A denaturation
step at 94˚C for 5 min; 31 cycles of denaturation at 94˚C for
30 sec, an annealing step for 30 sec (temperature was according
to the primers of each fragment), an extension at 72˚C for 1 min
and an extension at 72˚C for 1 min. Next, a final extension
step was performed at 4˚C for 5 min, and the experiment was
repeated 10-20 times. The PCR products were evaluated by a
2% agarose gel electrophoresis and were further purified using
an AxyPrep DNA Gel Extraction kit (Corning Life Sciences,
Corning, NY, USA), according to the manufacturer’s instruc
tions. Sanger sequencing was subsequently performed using an
ABI PRISM 3730 automated sequencer (Applied Biosystems;
Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sequencing
results were analyzed by Geneious version 5.6.7
software (Biomatters, Ltd., Auckland, New Zealand). An iden-
tified mutation was verified in the corresponding region of the
unaffected parents of the proband and 100 population-matched
healthy controls. The mutation was described by comparison
with the NCBI cDNA reference sequences NM_000424.3 for
KRT5 and NM_000526.4 for KRT14.

The potential impact of an amino acid substitution
on the structure and function of KRT5 and KRT14
proteins was predicted using the following in silico
analysis tools: PolyPhen2 (http://genetics.bwh.harvard.
edu/pph2), SIFT (http://sift.bii.a-star.edu.sg) and Mutation
Taster (http://www.mutationtaster.org/), which automatically
generated the results following input.

Results

Sequencing results. The results of the present study indi-
cated that mutation sequencing of KRT14 was negative,
whereas a novel heterozygous missense mutation, c.503C>T
(p.Glu168Gly), in KRT5 was presented in the patient. This
mutation was absent in the patient's unaffected parents and the
100 population-matched healthy controls (Fig. 2).

Functional consequence predictions. Analysis using PolyPhen2
indicated that the mutation c.503A>G (p.Glu168Gly) was
‘probably damaging’. Furthermore, SIFT and Mutation Taster
software predicted the mutation to be ‘deleterious’ and ‘disease
causing’, respectively.

Discussion

The locus of the mutation in KRT5 serves an important
role in the phenotype of EBS-gen sev. The majority of
causal variants of EBS-gen sev in KRT5 are missense
mutations that exist in the highly conserved regions of HIP and HTP (which are critical for the intermediate filament structure and integrity of the keratin cytoskeleton), and exert a dominant negative effect on the functional protein structure (2,8,9,14). Mutations in other regions are typically associated with the milder subtypes of EBS, namely EBS-loc and EBS-gen intermed (2,8,14). The Glu168 site (codon, GAG) is located at the boundary between the head and 1A domain (HIP) (Fig. 3). To date, three relevant substitutions have been reported. More specifically, mutations c.504G>T (p.Glu168Asp) and c.504G>C (p.Glu168Asp) result in EBS-gen intermed, while c.502G>A (p.Glu168Lys) is responsible for EBS-gen sev (15-17). Phenotype severity may be explained by the fact that Glu and Asp are acidic amino acids that share a similar structure and polarity, while Lys and Gly are very different with regards to these features. Contrary to Arg125Cys and Arg125His in the Arg125 site (a CpG-rich hotspot codon) accounting for the majority of mutations in KRT14 (8,16,18), Glu477Lys located at the C-terminal end segments of the helix 2B domain (HTP) was the most common mutation identified in KRT5 (19). This was more common than the corresponding region, Glu168 (Fig. 3; 8,14,16-18).

Dowling-Degos disease (DDD; OMIM no. 179850) or Galli-Galli disease (an acantholytic variant of DDD) are associated with haploinsufficiency of KRT5 caused by heterozygous frameshift/nonsense mutations (such as p.Met1?, p.Gln4* and p.Ile140Asnfs*39) in the head domain of KRT5 (20-22). Furthermore, p.Pro25Leu (in the head domain) and p.Gly550Alafs*77 (in the tail domain) result in a rare

Table I. List of the primers of the KRT5 and KRT14 genes.

| Primer name | Primer Sequence | Annealing temperature (˚C) |
|-------------|-----------------|---------------------------|
| keratin 5-E01_F | TGGGTAACAGAGCCACCTTC | 55 |
| keratin 5-E01_R | TTGCACAAAGCCAAAACATC | 55 |
| keratin 5-E02_F | TAGAGGGAGCGGAAAGAGGTG | 59 |
| keratin 5-E02_R | GGAGGTTGCCATGAGAGGATG | 59 |
| keratin 5-E03+4+5_F | CCCCTCCCACCTGAAAGTA | 57 |
| keratin 5-E03+4+5_R | GAGCCCCATTTCTAGGGT GCC | 57 |
| keratin 5-E06+7_F | AACCAGCCCCACACTTTTGG | 57 |
| keratin 5-E06+7_R | AGCAGCTTGCCTTTATGCA | 57 |
| keratin 5-E08_F | CGAATCATGAGGAGGATG | 55 |
| keratin 5-E08_R | GGAGGTTGCCATGAGAGGATG | 55 |
| keratin 5-E09_F | AGGAGGGGAGGAAAGAGGTG | 57 |
| keratin 5-E09_R | TTCTGCAAATTTGCTTGCTTC | 57 |
| keratin 14-E01_F | GACAGACATGAGGAGG | 57 |
| keratin 14-E01_R | CTGCTCCTCTGTCCGGAAAGG | 57 |
| keratin 14-E02+3_F | CCTTCCAGACACAGGGAGCAG | 57 |
| keratin 14-E02+3_R | CAGCGGATTGTTGTTCTTTAG | 57 |
| keratin 14-E04-8_F | TGTTGGAAACTCCTGACGTGG | 57 |
| keratin 14-E04-8_R | CCATGAAACCCATAGCATTT | 57 |

F, forward; R, reverse.

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subtype of EBS, known as EBS with mottled pigmentation (OMIM no. 148040), suggesting that specific regions in
KRT5 may be associated with melanin transportation and distribution, and malfunction of which can result in pigmentary
phenotypes (20,23).

Although variable phenotypes can arise from distinct
KRT5 mutations, even from an identical mutation in one
pedigree (13), substitutions in the Glu168 site primarily result
in the most severe subtype of EBS, which is EBS-gen sev.
However, there exist exceptions (for instance, Glu168Asp results
in EBS-gen intermed), suggesting that other factors, such as
epigene tic alterations, interchain interactions of protein struc-
ture, modifier genes, environmental interference and ethnic
background, may exert effects that result in distinct phenotypes.

In the present study, it can be suggested that Glu168Gly
is the pathogenic mutation present in the proband based
on the following: i) Glu168 is highly conserved among
different species (analyzed by the Mutation Taster software;
(www.mutationtaster.org/); ii) functional consequence predic-
tions are deleterious; iii) other pathogenic mutations in Glu168
have been reported (Fig. 3); and iv) the variant was not present
in the patient's unaffected parents and 100 healthy controls.
In combination with the generalized herpetiform blistering
occurring since birth and improving with age, the 2-year-old
male in the present study was diagnosed with EBS-gen sev.

The patient did not evidently improve after 9 months
from the first time that they appeared at the Department of
Dermatology of Xinhua Hospital (Shanghai, China), and this
may be attributed to the relatively long-term clinical course
of EBS-gen sev, or due to insufficient general management of
in EBS in the infant. Furthermore, no treatment was given within
these 5 months. Subsequent general therapy of EBS-gen sev
in this patient should concern the prevention of skin trauma,
infection control and the maintenance of good nutrition. Since
a favorable lifelong prognosis of EBS-gen sev is anticipated
following the high mortality rate period (within a year from
birth), prenatal diagnosis and potential gene therapy will be
available to the next generation in the family.

In conclusion, the current study successfully confirmed a
diagnosis of EBS-gen sev by revealing a novel de novo hetero-
yzogous missense mutation c.503A>G in the HIP of KRT5,
expanding the existing mutation spectrum.

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