A Novel Keratin 5 Mutation in an African Family with Epidermolysis Bullosa Simplex Indicates the Importance of the Amino Acid Located at the Boundary Site Between the H1 and Coil 1A Domains

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Epidermolysis bullosa simplex (EBS) comprises a group of hereditary disorders characterized by mechanical stress-induced blistering of the skin, which results from tissue separation within the epidermal basal keratinocytes (1). The most severe subtype is the Dowling-Meara type (EBS-DM); the moderately severe variant is other generalized (EBS, gen-nonDM) with generalized blister formation; and the mildest variant is localized with blistering confined to the hands and feet (1, 2). EBS is mostly inherited in an autosomal dominant fashion and is caused by a single mutation in either *KRT5* or *KRT14,* which encode keratin 5 (K5) or keratin 14 (K14), respectively (3). K5/14 form heterodimers that assemble into intermediate filaments (IFs) of the basal keratinocytes (2). The amino acid sequences of K5/14 share a characteristic tripartite structure that includes a central α-helical rod domain flanked by non-α-helical N- and C-terminal end domains called the head and the tail, respectively (4). The α-helical rod domain consists of 4 segments (coils 1A, 1B, 2A and 2B) responsible for dimerization and higher-order polymerization, and it is interrupted by 3 non-helical linkers (L1, L1–2 and L2). The ends of the rod domain, known as the helix initiation motif (HIM) and the helix termination motif (HTM), are more highly conserved and have been known to be important areas for K5/14 to form heterodimers, stabilize the helix structure and assemble into filaments (5). Consequently, more severe EBS has been associated with mutations in HIM and HTM (3). In contrast, milder EBS is usually linked to mutations in less essential central rod domains, the L1-2 non-helical linker region, and the H1 region, which is immediately adjacent to the N-terminal end of coil 1A in K5 (3). In this study, we identified a p.Glu168Asp mutation at the boundary site between the H1 and coil 1A domains of K5 in an African family with EBS, gen-nonDM.

CASE REPORTS

Case 1. An 8-year-old African girl of Zulu ethnicity, presented with skin fragility since birth was referred. Examination revealed generalized blisters and erosions on her whole body (Fig. 1a). She had several dystrophic fingernails and toenails, with subungual hyperkeratosis and ridged thickening of the nail plates. There was diffuse palmoplantar hyperkeratosis on the palms and soles. She had occasional intraoral blisters.

Case 2. A 40-year-old African man of Zulu ethnicity, the father of patient 1, had had blisters since the neonatal period. He had been diagnosed with vesicular pemphigoid and diffuse non-epidermolytic palmoplantar hyperkeratosis based on clinical manifestations. The blisters and erosions had ameliorated with age and now developed only occasionally (Fig. 1b). The palmoplantar keratoderma has remained unchanged. He had dystrophy of the toenails, but normal fingernails. He did not have oral lesions. He and his daughter were diagnosed with EBS, gen-nonDM from their clinical findings and mutation analysis.

Direct sequencing of the *KRT5/KRT14* genes revealed that both patients were heterozygous for the mutation c.504G>C transversion (p.Glu168Asp) in *KRT5* (Fig. 1c). Sequence analysis of 200 alleles from normal unrelated individuals could not detect any nucleotide change at this codon (6).

DISCUSSION

The behaviour of the amino acid residues near the ends of the α-helical segment plays an important role in stabilizing the α-helix of the polypeptide (7). A small electric dipole exists in each peptide bond. These dipoles are connected through the hydrogen bonds of the helix, resulting in a net dipole extending along the helix that increases with helix length. For this reason, negatively charged amino acids are often found near the amino terminus of the helical segment, where they have a stabilizing interaction with the positive charge of the helix dipole (8). The coil 1A fragment of K5 contains 5.5 heptad repeats (5 *a* positions and 6 *d* positions) (7, 8). All coiled coil core positions in this fragment are hydrophobic, with the exception of amino acid residue Glu^{168} (*d* position) and residue Asn^{193} (*a* position), and the negative charge of Glu^{168} is considered to compensate the helix dipole (8). In this study, we identified p.Glu168Asp mutation, which can affect the stability of an α-helix.

The residue is highly conserved among other IFs, type II keratin families and diverse species. In fact, at the same amino acid position, the other substitution mutation (p.Glu168Lys) has been reported as a causative mutation for EBS-DM (9). In addition, the p.Glu178Lys mutation in *KRT2* and p.Glu163Lys in *KRT6a*, located in the amino terminus of coil 1A of each keratin, have been identified in a patient with superficial epidermolytic ichthyosis, previously termed ichthyosis bullosa of Siemens, and in a patient with pachyonychia congenita, respectively (10, 11). These findings suggest that the amino-terminal residue of coil 1A plays a critical role in cytoskeletal function.

Two substitution mutations at Glu[168] in *KRT5* (p.Glu168Asp (c.504G > T) and p.Glu168Lys (c.502G>A), respectively, leading to EBS, gen-nonDM and EBS-DM) have been reported (9, 12). Both Glu and Asp are highly polar, negatively charged acidic amino acids. The substitution of Glu with Asp, is termed a synonymous or conservative substitution and is unlikely to produce significant effects on the protein structure. In contrast, Lys has an amino group in a side chain and is categorized as a basic amino acid, which is widely different from Glu in isoelectric point. The similarity/difference between these amino acids may relate to the clinical severity. To investigate how the mutants p.Glu168Asp and p.Glu168Lys affect the conformation of K5, the secondary structures of the wild-type K5 and the p.Glu168Asp and p.Glu168Lys mutants were predicted by *in silico* analysis. Coil 1A and the structure of the area around the domain of K5 were predicted using the New Joint Method (13) (Fig. 1d). Interestingly, p.Glu168Asp leading to EBS, gen-nonDM supposedly had some effects on the coil 1A domain, meanwhile p.Glu168Lys leading to EBS-DM remarkably affect the α-helix stretch on the domain. The fact that p.Glu168Asp leads to EBS, gen-nonDM despite a cognate substitution mutation indicates that the Glu[168] is strictly regulated.

We report here the first mutational analysis of EBS in an African family. A recent report revealed EBS types in Israel that have a unique mutation spectrum and different patterns of inheritance, including a higher incidence of recessive cases than in families in Europe or the USA (14). In addition, the proportion of Japanese patients with EBS with *KRT5* mutations is 3 times higher than those with *KRT14*, even though mutations in these 2 genes have been reported as equally prevalent (3, 15). Further research into mutations in Africans is required in order to determine whether there are ethnic and geographical features of EBS.

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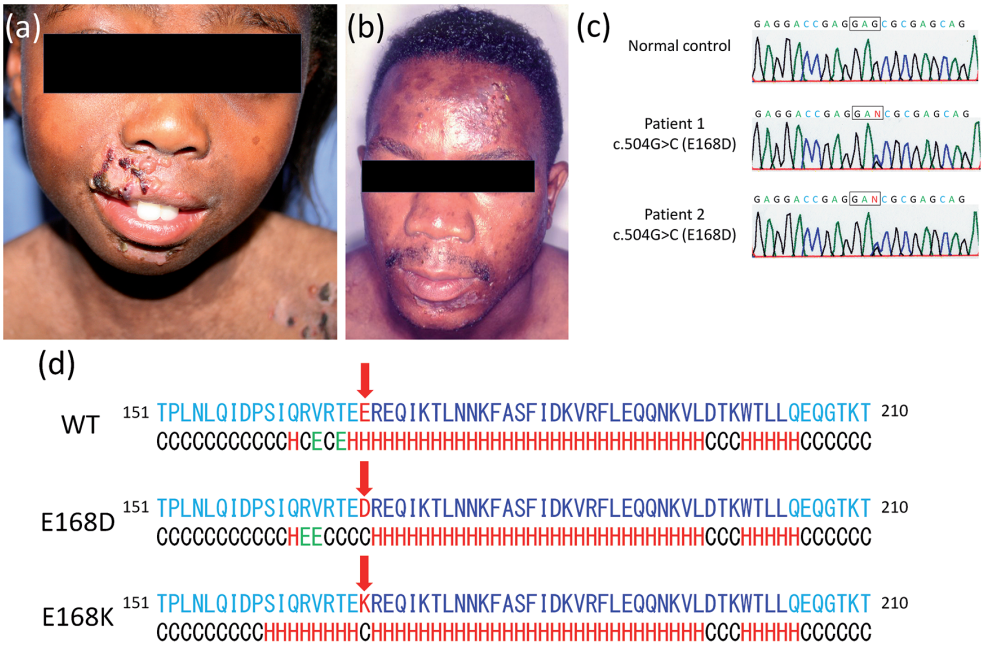

Fig. 1. (a) Clinical phenotype of patient 1. Generalized blisters and erosions are observed. (b) Clinical phenotype of patient 2 (father of patient 1). Blisters are randomly developed. Skin lesions ameliorated markedly with time. (c) DNA sequence of a normal control (*upper panel*), patient 1 (*middle panel*) and patient 2 (*lower panel*). c.504G>C missense mutation (p.Glu168Asp) in *KRT5* was detected in both patients. (d) Prediction of secondary structures of wild-type and mutant p.Glu168Asp and p.Glu168Lys keratin 5. p.Glu168Asp leading to EBS, gen-nonDM supposedly had some effects on the coil 1A domain, and p.Glu168Lys leading to EBS-DM remarkably affected the α-helix stretch of that domain. H, E and C represent the α helix, the β sheet and the coil, respectively.

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