MUTATION ANALYSIS IN THE FAMILY OF A TAIWANESE BOY WITH EPIDERMOLYSIS BULLOSA SIMPLEX DOWLING-MEARA

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Abstract: Epidermolysis bullosa simplex (EBS) is a group of hereditary bullous diseases characterized by intraepidermal blistering due to mechanical stress-induced degeneration of basal keratinocytes. The major subtypes of EBS, including EBS Dowling-Meara (EBS-DM), are caused by mutations of the basal keratin genes, keratin 5 (KRT5) or keratin 14 (KRT14). Here, we describe the first reported pedigree of EBS-DM in Taiwan. The proband was a 5-day-old newborn, who presented with numerous blisters of various sizes, some of which were hemorrhagic, as well as erosions on the extremities and hard palate since birth. Biopsy of a new vesicle showed subepidermal and basal cleavage with infiltration of eosinophils and neutrophils. Electron microscopy revealed cytolysis of basal cells and clumping of tonofilaments forming thick bundles and peculiar electron-dense round or oval basket-weave bodies. These features are characteristic of EBS-DM. The proband’s mother had also suffered from a similar blistering disorder since birth, with gradual appearance of mottled pigmentation on the trunk, diffuse irregular or linear palmoplantar hyperkeratosis, and nail dystrophy. Mutation analysis revealed a heterozygous point mutation (R125C) in helix 1A of keratin 14 in the proband and his mother. The detection of this pathogenic point mutation enables future prenatal diagnosis in this family.
this report, we describe a pedigree of EBS-DM with characteristic clinical, histopathologic, and electron microscopic features, and the detection of a point mutation (R125C) in helix 1A of KRT14.

Case Report

A 5-day-old newborn male was admitted to the hospital because of extensive blistering and erosion of the skin since birth. Physical examination revealed many small and large blisters and denuded areas on the face, hard palate, back, buttocks, and all extremities. The lesions were more pronounced on the acral parts of skin (Fig. 1). The clinical diagnosis of EB was made, and biopsy of a new vesicle from the right thigh was performed. Histopathologic examination showed a subepidermal and suprabasal vesicle with infiltration of eosinophils and some neutrophils and extravasation of erythrocytes (Fig. 2). At the periphery of the vesicle, there was also intraepidermal infiltration of eosinophils. Direct immunofluorescence test for immunoglobulin G and complement was negative. Electron microscopy showed subnuclear cytolysis of basal cells (Fig. 3A) and clumping of tonofilaments forming thick bundles and peculiar electron-dense, round or oval basket-weave bodies (Fig 3B), features characteristic of EBS-DM. Other structures of the dermal-epidermal junction, including hemidesmosomes and anchoring fibrils, appeared normal. With supportive care, the severity of blistering improved gradually. Some vesicles healed with milia formation and some of the nails were shed or deformed. Later, herpetiform vesicles were noted over the face and trunk at the age of 1 year.

In the patient’s family (Fig. 4), the mother had also suffered from a similar blistering disorder since birth but the condition improved with age. She was 26 year old, and had a few vesicles and a mottled hyperpigmentation on the trunk and forearms. In addition, diffuse irregular or linear palmar and plantar hyperkeratosis and nail dystrophy were noted.

For mutation analysis, genomic DNA was extracted from peripheral blood of the proband and members of the nuclear family (QIAamp Midi kit; Qiagen, Valencia, USA). The KRT14 gene was amplified using the polymerase chain reaction (PCR) followed by direct sequencing. The K14 primer sets used were 5’-TAC CCG AGC ACC TTC TCT TC-3’ (257–276 bp) and 5’-TGC TGG AGA ACA AGT AGC TGC -3’ (1223–1203 bp) to amplify exon I, and 5’-CAG TAT TCA GGC CTA AGG AAC A 3’ (3161–3182 bp) and 5’-GGA AGA GGT GGG AAG AGG AC-3’ (4622–4613 bp) to amplify exons III, IV, V, and VI.

Approximately 200 ng DNA was used per 50 µL reaction containing 10 µL Buffer Q (Qiagen), 5 µL 10X buffer (Qiagen), 8 µL dNTPs (1.25 mM/µL), 4 µL each primer (3.2 pmole/µL), and 0.5 U Taq polymerase (Qiagen). PCR conditions were 94°C for 1 minute followed by 10 cycles of 10 seconds at 94°C, 30 seconds at X°C, 2 minutes at 68°C; 25 cycles of 10 seconds at 94°C, 30 seconds at X°C, and 2 minutes at 68°C increased 10 seconds/cycle; and 8 minutes at 68°C. (X = 58 to amplify exon I; X = 55 to amplify exons III, IV, V, and VI) A point mutation causing a substitution (R125C) of the arginine (CGC) at codon 125 with a cysteine (TGC) in helix 1A of KRT14 was detected in the patient and his mother (Fig. 5). The same mutation was not detected in his elder sister and father.

Discussion

This is the first case of EBS-DM reported in Taiwan. The patient showed typical clinical and ultrastructural
findings of the disease. Clinically, the characteristic pattern or morphology of blistering of EBS-DM usually evolves from large hemorrhagic blisters on acral parts at birth to herpetiform vesicles and palmoplantar keratoderma during infancy and afterwards [5]. The typical mucocutaneous manifestations and the evolution of the lesions were well illustrated in our patient and his mother.

Pathologically, it is often difficult to be certain whether the blisters of EBS-DM are intra- or subepidermal from light microscopy [5]. In the present case, the vesicle appeared to be subepidermal in the center; however, close inspection revealed intrabasal separation at the edge of the vesicle, suggesting that the separation occurred initially within the basal cells. The diagnosis of EBS-DM was made after finding the characteristic ultrastructural features of EBS-DM, specifically, subnuclear cytolysis of basal cells (Fig. 3A) and clumping of tonofilaments forming thick bundles and peculiar electron-dense, round or oval basket-weave bodies (Fig. 3B).

EBS-DM is caused by structural abnormalities in keratin filaments; no inflammatory reaction is observed when a biopsy is performed on clinically unaffected skin where a blister or separation in the skin is induced immediately before the biopsy [6]. Interestingly, the blister in our patient was associated with a mixed inflammatory cell infiltrate containing eosinophils and neutrophils. A similar finding has been noted previously [5]. In our previous study, inflammatory cell infiltrates were also frequently seen in lesions of pretibial or prurigo-like EB dystrophica [7]. It is possible that cytolysis of basal cells in EBS-DM may induce some inflammatory cytokines, including chemotactic factors for eosinophils such as interleukin-5. Further work is needed to elucidate the mechanism involved.

In the present pedigree, the finding of mottled hyperpigmentation in the patient’s mother initially led us to consider another subtype of EBS, EBS with mottled pigmentation (EBS-MP). However, EBS-MP is characterized by generalized, irregular dyspigmentation and slight skin atrophy [8]. The pigmentary change appears in infancy and seems unrelated to blistering and sun exposure [9]. Furthermore, in EBS-MP there is no herpetiform blistering or the unique ultrastructural changes of electron-dense, oval basket-weave bodies of
Fig. 5. Automated sequencing of the KRT14 gene with a sense primer reveals A) a heterozygous change from C to T in the first position of codon 125 (KRT14 R125C) in the patient and B) no mutation in an unaffected family member. The altered nucleotide and amino acid are indicated above the respective normal sequence. The standard one-letter abbreviation for the amino acid is given.

aggregated tonofilaments. Moreover, mutation analyses in several patients with EBS-MP have all revealed a recurrent point mutation (P24L) in the keratin 5 gene [10].

Ultrastructural studies by Anton-Lamprecht of skin from patients with EBS-DM provided the first insight that EBS might be a keratin disorder [11]. Other studies showed that keratin mutants behave in a dominant negative fashion to perturb or disrupt keratin filament assembly and keratin architecture [12–14]. In the present study, the detection of a heterozygous R125C missense mutation in the proband and his mother is consistent with autosomal dominant transmission. As in previous reports, this particular point mutation in helix 1A of KRT14 has always been associated with EBS-DM phenotype and early onset of generalized blistering [15]. The point mutation has been shown to cause disruption of the keratin network formation in transfected epidermal cells, as well as perturbation of filament assembly in vitro [16]. This codon is a hotspot for mutagenesis, accounting for nearly 40% of all EBS-DM cases that have been analyzed so far. It is encoded by CGC, which in the epidermis is likely to be methylated at the coding and noncoding strand CpG dinucleotides. If spontaneously deaminated, methylated C nucleotides become thymidine residues, leading to permanent C to T transitions [17].

The treatment of EBS-DM is basically limited to trauma prevention and wound care, including antiseptics, antibiotics, protective moist dressings, and avoidance of exacerbating factors such as tight clothing, humid environments, sweating, and adhesive tapes [5]. Systemic cyproheptadine has been suggested to be beneficial [18]. Recently, Retief et al reported successfully treating two patients with oral tetracycline [19].

In summary, we have described a Taiwanese family with EBS-DM with characteristic clinicopathologic features, ultrastructural abnormalities, and a KRT14 gene mutation (R125C). The identification of this pathogenic mutation enables future prenatal diagnosis in this family.

References