A Novel Mutation in the L12 Domain of Keratin 5 in the Köbner Variant of Epidermolysis Bullosa Simplex

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Epidermolysis bullosa simplex (EBS) is a group of hereditary bullous diseases, characterized by intraepidermal blistering due to mechanical stress-induced degeneration of basal keratinocytes. Three major subtypes have been identified with autosomal dominant inheritance: Weber-Cockayne type, Köbner type (EBS-K), and Dowling-Meara type. These three EBS subtypes are all caused by mutations in either keratin 5 gene or keratin 14 gene, the major keratins expressed in the basal layer of the epidermis. We describe a female newborn with generalized blistering over the whole body since birth. Based on the clinical features of widespread blistering at birth, histopathological finding of intra-basal vesicle and ultrastructural findings of basal cell cytolysis without prominent clumping of tonofilaments, EBS-K was diagnosed. Mutational analysis revealed a novel keratin 5 mutation (967G>A) that produces an amino acid change (valine to methionine) at position 323 (V323M) of the seventh residue within the L12 linker domain. (Dermatol Sinica 23: 32-35, 2005)

Key words: Epidermolysis bullosa simplex, Köbner type, Mutation analysis, Keratin 5 gene

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INTRODUCTION

Epidermolysis bullosa (EB) is a group of cutaneous disorders characterized by blister formation caused by minor mechanical trauma. It has been traditionally divided into three broad categories, epidermolytic (simplex), junctional, and dermolytic (dystrophic) categories, based on the level of blister formation.\(^1\) Epidermolysis bullosa simplex (EBS) is characterized by blister formation due to cytolysis of the epidermal basal cells and the majority of cases are transmitted autosomal dominantly. Clinically, EBS is divided into three main subtypes based on the severity, distribution and the onset age of blistering. Weber-Cockayne EBS (EBS-WC) is the mildest and most common form of the disease; blisters are usually confined to the hands and/or feet and often do not occur until well into childhood. Köbner EBS (EBS-K) is more severe; blistering is more generalized and the onset is generally earlier than that of the EBS-WC. The most severe form of this disease is Dowling-Meara EBS (EBS-DM).\(^7\) EBS-DM is characterized by widespread herpetiform blisters which appear at or shortly after birth. It can be distinguished ultrastructurally from other forms of EBS by the presence of large clumps of tonofilaments in the cytoplasm of the basal keratinocytes.

Almost all EBS subtypes are caused by mutations in either keratin 5 (\(KRT5\)) gene or keratin 14 (\(KRT14\)) gene\(^3\) with the exception of EBS with muscular dystrophy, which is caused by mutations in the plectin gene (\(PLEC1\)).\(^4\) More than 74 different mutations in \(KRT5\) and \(KRT14\) have been reported.\(^5,6\) The mutations are clustered in four regions in \(KRT5\) and \(KRT14\), the H1 domain of the head region, the two segments of the rod domain (1A and 2B) and the linker region L12.\(^7\) Most (31 out of 34) of the reported mutations in \(KRT5\) are nucleotide substitutions and resulting in missense mutations.\(^6,7\)

EBS as a group is uncommon, and EBS-K is rare. In this report, we describe a sporadic case of EBS-K with characteristic clinical, histopathological and electron microscopic features, and the detection of a point mutation (V323M) in the L12 linker domain of \(KRT5\).

CASE REPORT

A 3-day-old, female newborn, dizygotic twin A, was born at gestational age 36 weeks via cesarean section due to breech presentation. She presented with flaccid bullae and erosions on the face, neck, trunk and extremities at birth (Fig. 1). Involvement of the oral mucosa and tongue (Fig. 1) and shedding of fingernails were noted. Her parents' and her twin sister's skin were normal. Skin biopsy of the baby revealed extensive subepidermal and focal suprabasal separation of the epidermis from the dermis with an eosinophilic-rich inflammatory infiltrate in the papillary dermis. The findings were consistent with EBS. Electron microscopic study showed a suprabasal vesicle and cytolysis of basal cells without prominent clumping of tonofilaments, and the basement membrane...
zone appears normal with normal anchoring fibrils (Fig. 2). These ultrastructural findings were also consistent with EBS.

For mutation analysis, genomic DNA was extracted from peripheral blood (QIAamp Midi kit, QIAGEN, USA) of the proband and members of the nuclear family. The KRT5 gene and KRT14 gene were amplified by polymerase chain reaction (PCR) followed by direct sequencing (ABI 377 automatic sequencer, Advanced Biotechnologies, Columbia, Md.) The KRT5 primer sets used were 5'-ATG AGA TTA ACT TCA TGA AGA TG-3' and 5'-CCA TTC TTA GTG TCG TCA TG -3'.

A nucleotide transition of G to A (967G>A) in one allele of exon 6 was detected in the patient (Fig. 3). The mutation is predicted to result in amino acid change from valine to methionine (V323M) in the L12 linker domain of KRT5. The mutation was not found in either parent or in 50 unrelated samples. The other regions of KRT5 and KRT14 were unremarkable in this family.

DISCUSSION

EB is uncommon or rare; about one in 20,000 people suffer from some form of EB. EBS was first distinguished from other subtypes of inherited EB in 1898. Blistering is due to fragility of the basal keratinocytes and on ultrastructural examination, the level of split is within the basal keratinocytes itself and generally within the subnuclear cytoplasm. There are three main subtypes of EBS. The incidence of all types of EBS is about 1:50,000. EBS-K (OMIM 131900) is clinically apparent at birth or in early infancy. Despite its rather generalized distribution, the extremities tend to be more severely involved than the face or trunk in most patients. Milia, atrophic scarring and nail dystrophy may be seen in some patients. In the present case, the clinical diagnosis of EBS-K was based on the clinicopathological features and the lack of prominent keratin filament aggregates in the keratinocytes ultrastructurally.

Mutation analysis in our case found a nucleotide transition of G to A (967G>A), resulting in V323M in the L12 linker domain of KRT5 (Fig. 3). Since both parents did not carry the mutation, the mutation in the proband was likely to be due to a germ line mutation in one of the parents.

The valine at position 7 of the L12 domain is absolutely conserved in all type II keratins, and in other intermediate filament subunits as well. The findings suggest that this particular residue is critical in the integrity of keratin filament. Ten different mutations in the L12 domain of KRT5 (V323A, V324D, L325P,
M327T, M327K, D328V, D328H, D328E, N329K, R331C) have been reported.\(^{13-20}\) It has been postulated that these structural changes may result in a more rigid molecule with less resistance to torsion stress.\(^{21}\) These mutations may also impair the normal packaging of dimers in the filament and result in diminished interactions between residues critical for maintaining tensile strength. In vitro modeling of K5 L12 mutations, V323A, M327T and N329K substitutions tend to cause filament unraveling and result in a loosely packed, shortened filaments with an apparent increase in diameter.\(^{15,18}\)

In summary, we report a case of EBS-K and detect a novel mutation within the L12 region of K5 that is associated with a clinical EBS-K phenotype. The identification of this substitution adds to the catalog of disease-associated mutations involving residues of the KRT5 and KRT14 L12 linker domain.

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