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Novel and recurrent mutations in Keratin 5 and 14 in Korean patients with *Epidermolysis bullosa* simplex

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ABSTRACT

Backgrounds: *Epidermolysis bullosa* simplex (EBS) is a group of hereditary bullous disorders caused by mutations in the keratin genes *KRT5* and *KRT14*. A significant genotype–phenotype correlation has been noted in previous studies of EBS.
Objective: In order to identify additional EBS mutations and elucidate the genotype–phenotype correlations in Korean EBS patients, we performed the first large scale mutational analysis of EBS patients of Korean origin.
Methods: We investigated fifteen Korean EBS patients by performing a sequence analysis of the entire coding sequences of *KRT5* and *KRT14*.
Results: We identified six novel mutations, four within *KRT5* (p.V143F, p.R265P, p.C479X and p.Asn177del), and two within *KRT14* (p.R125L and p.L401P). In all, 13 missense, 1 nonsense, and 1 small deletion mutation were found. Five mutations in Dowling–Meara type (K14-p.R125H, K14-p.R125L, K5-E477K, K5-p.C479X and K5-p.Asn177del) were located in the highly conserved ends of the alpha-helical rod domain, the helix initiation (HIP), or helix termination (HTP) peptides of *KRT5* and *KRT14*. Further, seven and three mutations were identified in EBS-generalized type and EBS-localized type, respectively. The positions of the mutations in both subtypes were more widely distributed within the rod domains and in the L12 linker domains of both keratin genes.
Conclusions: This study should provide useful data and enhance our understanding of the EBS genotype–phenotype relationship. The genotype–phenotype correlation in Korean EBS patients was similar to previous studies performed in other ethnic groups. Lastly, our results confirmed that the mutational location in *KRT5* or *KRT14* is the most important factor in determining the phenotype severity.

1. Introduction

Epidermolysis bullosa (EB) comprises a group of inherited blistering disorders classified into three main subtypes on the basis of the ultramicroscopic cleavage level of blistering, namely, simplex, junctional and dystrophic [1]. *Epidermolysis bullosa* simplex (EBS) is the most common subtype of EB, predominantly following an autosomal dominant inheritance pattern, due to mutations in the keratin 5 (K5) or keratin 14 (K14) genes [2]. These two keratin genes code for intermediate filament cytoskeleton proteins in basal keratinocytes, maintaining mechanical integrity of the epidermis against frictional forces. Recently, a number of additional functions of intermediate filaments have been discovered. These include regulation of key signaling pathways controlling cell survival, cell growth, and vectorial processes associated with protein targeting, vesicle transport, and cell adhesion [3,4].

The heteropolymeric keratin filament network is assembled in the cytoplasm from type I and type II keratin proteins, both of which have a common structure consisting of a discontinuous α-helical rod domain flanked by non-helical head and tail domains [5].

EBS is traditionally classified into three main subtypes [2,6]. EBS-localized type, formerly named EBS Weber-Cockayne, is the mildest form of EBS, in which blistering mainly affects the hands and feet; EBS-generalized type, formerly named EBS-Koebner, is the intermediate form and exhibits a more generalized pattern of blistering; and the third and most severe form of EBS is Dowling–Meara type (EBS-DM), in which extensive and severe blistering occurs in herpetiform clusters. These main subtypes of EBS largely follow an autosomal dominant inheritance pattern. However, a few cases of recessive EBS subtypes, representing about 5% of all EBS mutations [7], have been reported.

Detailed information on the pathogenic mutations in K5 and K14 is available at the following web site (see http://www.interfil.org, Szeverenyi et al., 2008). The pathogenic mutations of EBS are

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clustered in specific regions or hotspots in the protein molecule [5]. It has been noted previously that there is a close correlation between the position and nature of the mutation and the severity of the disease. Indeed, the helix boundary motifs at the beginning and end of the rod domain are critical for normal filament formation [8], and mutations in these areas cause the most severe forms of EBS (EBS-DM). Phenotype–genotype correlations for EBS-localized and EBS-generalized are less specific.

Although many mutations in EBS have been reported in Western countries and Japan [9–15], a large scale mutational analysis in Korean patients with EBS has not yet been conducted. To identify additional EBS mutations for genotype and phenotype correlation in Korean patients, we performed mutational analysis of 15 EBS patients of Korean origin by sequence analysis of the entire coding regions of the *KRT5* and *KRT14* genes.

2. Materials and methods

2.1. Patients

Fifteen unrelated Korean EBS patients were evaluated in this study. Patients were clinically diagnosed with EBS and classified into corresponding subtypes. These diagnoses were further confirmed by immunofluorescence antigen mapping and electron microscopy.

2.2. Mutation analysis

After obtaining informed consent from patients, genomic DNA was extracted from peripheral blood lymphocytes using a DNA extraction kit (QIAamp DNA Blood Midi kit, Qiagen, Hilden, Germany). Total DNA was used as a template for amplification of the genomic sequences of *KRT5* and *KRT14*. *KRT5* segments including nine exons and all exon-intron borders and *KRT14* segments including eight exons and all exon–intron borders were amplified as previously described [16,17]. Sequence analyses were performed using Big Dye terminator technology (ABI 3100 Perkin-Elmer, Warrington, UK). Genomic DNA samples from 50 normal healthy Koreans were used as controls.

3. Results

3.1. Clinical findings

Fifteen unrelated Korean EBS patients served as patients in this study. First, each patient was classified into the proper EBS subtype based on previously established diagnostic criteria [2,6]. The clinical features of the 15 Korean EBS patients are summarized in Table 1. Seven patients had a familial history of autosomal dominant trait. All of the EBS-DM patients were further confirmed by electron microscopy, which showed cytolysis in basal keratinocytes and keratin clumping within the cytoplasm of basal cells. The clinical features of the two EBS-DM patients, patient 13 (Fig. 1a), who was a 5-month-old female, and patient 15 (Fig. 1b), who was a 1-month-old female, were remarkable in that they exhibited severe generalized blisters, widespread erosions, loss of nails, milia formation, and general health problems such as feeding difficulty and malnutrition.

3.2. Mutation analysis

We investigated 15 EBS patients and families of Korean origin by performing sequence analysis of the entire coding sequences of *KRT5* and *KRT14*. Pathogenic mutations were identified in all patients (Table 2). The mutations identified through our analysis comprised 13 missense, 1 nonsense, and 1 small deletion mutation, and of these mutations, six were novel, consisting of four mutations in *KRT5* (p.V143F, p.R265P, p.C479X and p.Asn177del), and two in *KRT14* (p.R125L and p.L401P). None of these mutations were identified in the 50 unrelated healthy controls. Five of the 15 EBS patients were consistent with EBS-DM, and all mutations (K14-p.R125L, K-14p.R125H, K5-p.C479X, K5-p.E477K, K5-p.Asn177del) were located within the highly conserved ends of the alpha-helical rod domain, the helix initiation (HIP) or helix termination (HTP) peptides, respectively. Seven and three mutations were associated with EBS-generalized and EBS-localized, respectively. More specifically, the positions of the mutations in both of these subtypes were widely distributed within the rod domains and in the L12 linker domains of both keratin genes. Fig. 2 depicts the positions of the mutations identified in this study on both keratin genes.

4. Discussion

Mutation analysis of *KRT5* and *KRT14* in EBS is necessary to ensure precise diagnosis, prognostication, genetic counseling, prenatal diagnosis, and identification of future gene therapy trials that might benefit patients. To date, mutational analyses of *KRT5* and *KRT14* have been published only for certain ethnic groups in Western countries [9–14] and Japan, in which two Korean cases were also included [15]. Here, we present fifteen cases of novel and recurrent mutations in Korean EBS patients.

Pathogenic mutations were identified in all 15 EBS cases. These mutations included 13 missense mutations, 1 nonsense mutation,

Table 1
Clinical features of 15 Korean EBS patients.

Patient no.	Age/sex	Inheritance	Age of onset	Clinical features	EBS subtype
1	16Y/M	De novo	Infancy	Blisters on hands and feet	Loc
2	25Y/M	Familial	10 years	Blisters on hands and feet	Loc
3	18Y/M	Familial	4 years	Blisters on hands and feet	Loc
4	19Y/M	Familial	Infancy	Generalized blisters, plantar hyperkeratosis	Gen
5	21Y/M	Familial	Infancy	Generalized blisters	Gen
6	2mo/M	De novo	Infancy	Generalized blisters on the trunk, extremities	Gen
7	27Y/F	De novo	Infancy	Generalized blisters on the trunk, extremities	Gen
8	21Y/F	Familial	Infancy	Generalized blisters on the trunk, extremities	Gen
9	2Y/F	Familial	Infancy	Generalized blisters on the trunk, extremities	Gen
10	23Y/F	De novo	Infancy	Generalized blisters on the trunk, extremities, hyperpigmentation	Gen
11	16Y/M	De novo	Neonate	Severe generalized blister, palmoplantar keratoderma	D-M
12	47Y/M	De novo	Neonate	Severe generalized blister, palmoplantar keratoderma	D-M
13	5mo/F	De novo	Neonate	Severe generalized blister, oral mucosal erosion, nail loss	D-M
14	19Y/M	Familial	Neonate	Severe generalized blister, palmoplantar keratoderma	D-M
15	1mo/F	De novo	Neonate	Severe generalized blister, oral mucosal erosion, nail loss	D-M

Y; year, mo; month, M; male, F; female, Loc; localized, Gen; generalized, D-M; Dowling–Meara.