A novel autosomal partially dominant mutation designated G476D in the keratin 5 gene causing epidermolysis bullosa simplex Weber-Cockayne type: A family study with a genetic twist

CEZARY KOWALEWSKI1, TAKAHIRO HAMADA2, KATARZYNA WOZNIAK1, YUKO KAWANO2, WERONIKA SZCZECINSKA1,3, SHINICHIRO YASUMOTO2, ROBERT A. SCHWARTZ4 and TAKASHI HASHIMOTO2

1Department of Dermatology, Medical University of Warsaw, Koszykowa 82a, 02-008 Warsaw, Poland; 2Department of Dermatology, Kurume University School of Medicine, 67 Asahi-machi, Kurume-shi, Fukuoka-ken 830-0011, Japan; 3Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland; 4Department of Dermatology, New Jersey Medical School, MSB H-576, 185 South Orange Avenue, Newark, New Jersey 07631, USA

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Abstract. Epidermolysis bullosa simplex Weber-Cockayne type (EBS-WC) is a genetically inherited skin disease characterized by blistering restricted to the palms and soles. Its inheritance in nearly all kindreds is caused by a dominant-negative mutation in either KRT5 or KRT14, the genes encoding keratin 5 and keratin 14 proteins, respectively. Rarely, recessive mutations have also been found. We described a family with EBS-WC caused by a novel autosomal dominant mutation (G476D) in the keratin 5 gene. One family member was first seen with mucosal erosions and generalized blisters localized on the anogenital area, trunk, face and sites of mechanical trauma. Molecular analysis in this patient showed the presence of an additional mutation, an autosomal recessive (G183E) one, in the same gene. This observation suggests an additional effect of a recessively inherited mutation modulating the phenotypic expression of EBS caused by a partially dominant mutation and is important for accurate genetic counseling.

Introduction

Epidermolysis bulosa simplex (EBS) is a heterogenous group of genetically inherited skin diseases characterized by blistering of the skin following mechanical trauma. Clinically, EBS is divided into severe generalized forms and mild localized ones, most notably one variant with blistering restricted to the palms and soles (Weber-Cockayne type; EBS-WC) (1). The inheritance in nearly all kindreds is caused by a dominant-negative mutation in either gene, KRT5 encoding keratin 5 and KRT14 encoding keratin 14 proteins (2,3). Rarely, recessive autosomal inheritance has also been described (4-6).

The signs and symptoms of the disease in patients of the same family are similar. Moreover, the same mutation causes similar phenotypic expression in different families. Interestingly, in a few reported EBS patients the blistering disease was more severe than in other affected family members. This difference was found to be associated with the presence of additional mutations. For example, a child of consanguineous parents with homozygous mutation M119I in the KTR14 gene had generalized EBS, whereas heterozygotes demonstrated localized EBS-WC. Partial dominance of this mutation was suggested (7).

The mutation E170K in the gene KTR5, which causes a mild form of EBS-WC, seems to be also partially dominant. One of the reported patients with the dominant heterozygous mutation E170K was also a carrier of a recessive heterozygous mutation in the stutter region of KRT5 (8). This patient with a compound dominant-recessive heterozygous mutation had a generalized form of EBS, while his father with the heterozygous mutation E170K had blisters restricted to the soles after intensive walking. Experimental studies on MDCK cells with cDNA-transfected keratin 5 genes revealed significant keratin aggregation associated with this compound dominant-recessive heterozygous mutation.

In contrast to the partially dominant mutations responsible for the EBS-WC phenotype, a family with generalized EBS caused by the fully dominant missense...
mutation designated as K173N in region 1A of the keratin 5 gene was described (9). This homozygosity for the mutant allele had clinical symptoms no more severe than that in heterozygotes. In addition, electron microscopic study did not reveal a significant difference in keratin filament formation between homozygotes and heterozygotes.

Materials and methods

Description of the family. The proband was a 14-year-old girl born at term, from non-consanguineous parents (Fig. 1). She had widespread skin fragility and generalized blistering from birth. At the time of consultation at the age of 14 years, she had mucosal erosions and generalized blisters localized on the anogenital area, trunk, face and the sites of mechanical trauma, especially on the palms and soles. Blisters on the feet were more severe during the summer than in the winter. On the face she had been constantly developing vesicles and bullae followed by erosions (Fig. 2). Lesions healed without scarring or milia formation. There was neither palmoplantar hyperkeratosis nor nail dystrophy. Skin fragility and blistering had improved with age. Her father and many of his relatives since adolescence had developed a few blisters restricted to the soles after extensive walking. The proband's mother and her relatives were unaffected.

Laboratory investigations

Immunofluorescence mapping study. To test the level of blister formation a biopsy specimen from the proband's perilesional skin was taken and immunohistochemical studies were performed using monoclonal antibodies against collagen IV (clone COL-94, Sigma) and ß4 integrin (Chemicon, Temecula, CA, USA) according to the method previously described (1).

Mutational analysis. To perform the mutational analysis, genomic DNA was extracted from blood samples obtained from the patient, her healthy mother and affected father using the protocol as previously described (10).

Electron microscopic study. Punch biopsy specimens from the involved and uninvolved skin of the proband were taken under local anesthesia, fixed in 2% glutaraldehyde and 1% osmium tetroxide, dehydrated with graded alcohol, substituted and embedded in epon. After polymerization, ultra-thin sections were cut, stained with uranyl acetate and lead citrate according to the method previously described (11) and observed under transmission electron microscopy (JEOL, model 1200EX).

Results

Immunofluorescence mapping study. The immunofluorescence mapping study confirmed the diagnosis of EBS on a biopsy specimen from the proband's perilesional skin showing the presence of collagen IV (a marker of the lamina densa) and ß4 integrin (a marker of the upper part of the lamina lucida) within the blister floor. Moreover, traces of
disrupted basal keratinocytes were also found on the floor of the bulla characteristic for intraepidermal separation.

**Mutational analysis.** Mutational analysis revealed that the proband with generalized EBS and her father with EBS-WC had a heterozygous missense mutation in exon 7 of the 2B domain in the KRT5 gene changing a G>A transition at the nucleotide at base 1427 that converts a glycine residue (GGC) to an asparginic acid residue (GAC); this mutation is designated G476D. The father was sequence identical with wild-type for comparison. Direct sequencing of exon 7 of the KRT5 gene in the proband with generalized EBS and her father revealed a G>A transition at nucleotide 1427 that converts a glycine residue (GGC) to an asparginic acid residue (GAC); this mutation is designated G476D. The healthy mother's sequence was identical with wild-type for comparison.
compound dominant-recessive heterozygous mutation. These sequence variants G476D and G183E were not found in any of the 100 control chromosomes sequenced.

Electron microscopic study. Electron microscopic study on a biopsy specimen performed from the lesional skin of the patient with generalized EBS showed the presence of an intra-epidermal blister and clumping of keratin filaments (Fig. 4a).

The ultrastructural study on a biopsy specimen from the proband's uninvolved skin disclosed gross alteration of keratin filament assembly including the reduction of the number of keratin filaments in some keratinocytes and aggregation of keratin filaments in others (Fig. 4b, c and d).

Discussion

The clinical findings and disease course in the affected parent and his many relatives were consistent with the diagnosis of EBS-WC, whereas the 14-year-old proband represented a generalized form of EBS (Koebner type). The diagnosis of EBS was confirmed by immunofluorescence mapping study.

The missense mutation in the KRT5 gene designated as G476D found in the proband and her affected father is a novel mutation. Since this mutation was not found in her unaffected mother, we consider it to be a pathogenic mutation responsible for the EBS-WC phenotype. In general, the mutations associated with the mild phenotype (EBS-WC) are found in the non-helical head or linker domains (12,13), whereas in generalized EBS the mutations reside in the distal end 2B (or 1A) domain of the keratin 5 gene, the so-called helix initiation or termination motif (14). However, in a separate study we detected an additional two novel missense mutations in the keratin 5 and 14 genes residing in the EBS-WC phenotype (10). Thus, the severity of EBS is associated not only with a functional site of the mutation, but also with the nature of the amino acid change and its size and structure (15).

Intriguingly, an additional missense mutation in region 1A of keratin 5 designated as G183E detected in the proband and also in her healthy mother seemed to be clinically silent when combined with the wild-type allele. However, the same mutation exacerbates the clinical severity of EBS combined with the G476D mutation on the other allele. To confirm this hypothesis, the ultrastructural organization of the keratin intermediate filament filaments of the proband was studied. Electron microscopic analysis of perilesional skin of the 14-year-old proband revealed gross alteration in the organization of the cytoskeleton of the basal keratinocytes including keratin aggregation and clumping and reduction in the number of intermediate filaments (Fig. 4).

In summary our study showed that an unusual course of EBS in our patient, more severe than in other family members, may have been the result of the presence of compound dominant-recessive heterozygous mutations. This observation suggests the additional effect of recessively inherited mutations modulating the phenotypic expression of EBS caused by partially dominant mutations. This finding may be important for accurate diagnosis and genetic counseling.

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