Epidermolysis bullosa simplex in Slovenia

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ABSTRACT

Background. Epidermolysis bullosa is a genetic disease where blisters occur spontaneously or after minor trauma. In epidermolysis bullosa simplex (EBS) the cleavage occurs in basal keratinocytes due to a mutation in genes encoding keratins 5 or 14.
Materials and methods. Clinical records of the Department of Dermatology, Medical Center Ljubljana as well as from other dermatology departments in Slovenia were reviewed. Molecular defects in 10 Slovenian EBS patients were investigated.
Results. Molecular defects were detected in K14 in five patients and in K 5 in two patients, clinically diagnosed with EBS - Weber Cockayne. In three patients the investigation of the hot-spots 1A, L12 and 2B as well as of H1, did not reveal any changes.
Discussion. A frequency of 14 cases per million was calculated for EBS in Slovenia, and in correlation with the data published in the literature, mutations were found within one of the main hot-spot regions linked to EBS, the L12 linkers of K5 and K14.

KEY WORDS

epidermolysis bullosa simplex, keratins 5 and 14, mutations, Slovenian population

Introduction

Epidermolysis bullosa (EB) comprises a group of genetically determined disorders characterized by blisters appearing on clinically uninvolved skin and on oral or esophageal mucosa. They are provoked by minor mechanical trauma or pressure. The blisters are indolent and are followed by erosions and crusts. Based on histopathology, electron microscopy and molecular biology three main types (groups), each one including a number of variants, can be differentiated (1).

Epidermolysis bullosa simplex (EBS). The blister is localized in the epidermal cells of the basal layer. The majority of cases are inherited in an autosomal dominant mode, but cases of recessive EBS also exist (2).

Epidermolysis bullosa junctionalis (JEB). The split is situated in the basement membrane zone. The inheritance is mostly autosomal recessive.

Epidermolysis bullosa dystrophica (EBD. The bullae are localized below the basement membrane and

arise due to deficient anchoring fibrils. The great majority of cases are inherited, as an autosomal recessive diseases but an autosomal dominant form also exist (2).

Over the past years our understanding of the molecular pathology underlying different clinical subtypes of EB has greatly increased. Mutations in several different genes encoding protein components of the anchoring fibrils have been implicated in this disease

Epidemiology of EBS

The estimation of prevalence of EBS depends to a large extent on the accuracy and precision of the diagnosis, which is based on modern biochemical, ultrastructural and molecular criteria and on the screening of a population. The best epidemiological studies were done in Scandinavia (2). Several different subtypes of EBS were recorded, an incidence of 1-14 per million was found. In Scotland the prevalence of EBS is 28.6 per million (3) and in the United States of America it is 10.8 (4). Most of the epidemiological studies performed to date are probably statistically not entirely exact, as in the past the diagnosis was based primarily on clinical symptoms. Also, the prevalence appears to differ in various regions.

Clinical characteristics

There are several subtypes of EBS which are expressed as different phenotypic variations. The most common EBS subtypes are:

Weber-Cockayne subtype (EBS-WC) is the mildest form and is characterized by onset in childhood or later in life. It is the most common variant. Thick walled blisters on the palms and soles usually appear after exercise. Secondary infections of blisters is a common complication.

Köbner subtype (EBS-K) starts at birth or in early infancy. The predilection zones are hands, feet and extremities. Nail, teeth and oral mucosa are usually spared or there is a minimal involvement.

Dowling-Meara or herpetiform subtype (EBS-DM) is characterized by blisters distributed all over the body and oral mucosa is often involved as well. The onset is at birth. Grouped or herpetiform blisters mostly appear spontaneously on the trunk and on proximal parts of extremities, mostly in the folds, and they heal without scarring. Heat does not contribute to exacerbation as it does in the EBS-K variant.

Mottled pigmentation subtype (EBS-MP) is a rare variant of EBS, with only a few cases reported so far. The disease shares the following clinical features: cutaneous fragility, diffuse macular hyperpigmentation, palmoplantar hyperkeratosis and nail dystrophy.

The autosomal recessive type without muscular dystrophy (EBS-AR) is also rare.

All the different subtypes have in common the formation of serous or serosanguinous vesicles or bullae. The vesicles can be provoked by sweating, friction or minor trauma, and generally healing occurs without scarring.

Pathophysiology

A three-component filament system forms the cytoskeleton of all mammalian cells: microfilaments (actin), intermediate filaments (IF) and microtubules. IF proteins are a large multigene family of proteins with a tissue specific expression pattern. Thus, intermediate filaments in all epithelial cells are composed of keratins. Keratins are subdivided into two types. The smaller, acidic, type I keratins (K9-K19) with a molecular weight 40-56 kD and type II keratins (K1-K8) which are larger, with a molecular weight 56-67 kD. Like all IF proteins, keratins contain a central alpha helical domain (particularly conserved amongst all IF proteins) interrupted by several non-helical linkers, and a carbo- and an amino-terminal end (5,6). Epithelial keratins are co- expressed in specific pairs, consisting of each one type I and type II molecules. In the epidermis K5 and K14 are expressed in the basal cell layer, while in the squamous layer these genes are shut down and K1 and K10 are expressed instead. In plantar skin K2 and K9 are also present. The regions located to the ends of the central alpha helical domain (the helix initiation and helix termination motifs) in keratins appear to correlate with the most severe EBS forms (7).

In patients with EBS, light microscopy will often reveal splitting of the epidermis whithin the basal cell layer. This is the result of a keratin filament structural deficiency in these cells, caused by mutations in genes encoding keratins K5 or K14. Most of these mutations are implicated in interfering with the various stages of keratin filament assembly. The milder types of the disease, such as the Weber-Cockayne, are associated with mutations coding for regions of keratins 5 and 14 that are less conserved (8-10). Mutations that code for a specific region of the amino terminus of keratin 5 are associated with mottled pigmentation (11) in some patients with EBS. Homozygotic mutation of K 14 have been demonstrated in a few cases of autosomal recessive EB simplex (12). Subtype of EBS associated with muscular dystrophy results from mutation in the plectin gene, a component of the hemidesmosome (12).

Slovenian EB cases

To our knowledge 28 cases of EBS have been recorded in Slovenia, which gives a prevalence of 14 cases

Table 1. Keratin mutations in Slovenian patients with epidermolysis bullosa simplex

Patients	Inheritance	Keratin	Mutations
Family I: H.M., H.B	Dominant	K14	D273G D273G
Family II: F.K., D.R	Dominant	K5 and K14investigated	No mutations detected H1, 1A, L12 and 2B
Family III: J.A., J.S.	Dominant	K14	D273G D273G
Family IV: T.A., T.I	Dominant	K14	D273G Healthy relative, no mutation
Family V: S.H., S.F	Dominant	K5	D328E D328E

per million. According to clinical manifestations they were EBS-K or EBS- WC subtypes. Additionally, 22 patients with recessive EBD and 10 dominant EBD were recorded. There were also 5 cases of JEB. The Slovenian families with the recessive subtypes EBD originated from remote mountain regions of Slovenia (14), where consanguinity was a possibility.

Materials and methods

The molecular investigations were performed in five Slovenian families with EBS. DNA was extracted according to the standard procedure (9) from whole blood samples of five affected families and their healthy relatives. Genomic DNA of patients and their healthy relatives was analyzed for K5 and K14 gene mutations. Initially the 1A, L12 and 2B hot-spot encoding domains were sequenced, but subsequently the sequencing was extended to the entire coding portion of the two genes (15)

Results

The patients' histories did not reveal consanguinity. In members of three families a mutation was found in the linker L12 of K14, K14 D273G, and in one family a mutation of the L12 linker of K5, K5 D328E, was detected. In one family clinically diagnosed with EBS, no mutations in K5 or K14 were detected. The mutations found are listed in Table 1.

In family I: the aspartic acid to glycin mutation in the L12 domain of keratin K14 was detected. Both patients are heterozygotic for mutation.

Family II: investigation of the hot spot domains H1, 1A, L12 did not reveal mutation of keratins K5 and K14.

Family III: the aspartic acid to glycin mutation in the L 12 domain of the K14 gene was detected (L12 D273G)

Family IV: the same mutation in the L12 linker domain of the gene for K14 was found.

Family V: the novel mutation of aspartic acid to glutamic acid of keratin K5 in the L12 domain was detected (D328E).

Discussion

In EBS the mutations seem to change the configuration of keratin poplypeptides, thus causing formation of abnormal heterodimer units (K5/K14) and the keratin clumping (16). The correlations between the location of mutations and the corresponding phenotypes are still investigated.

The majority of mutations linked to EBS have been identified within the two main hot-spot domains, the 1 A and 2B helical domains in K5 and K14. Such mutations usually cause more severe phenotypes. The mutations detected in the L12 regions of K5 and K14 are linked to the milder EBS phenotype. Out of seven known mutations in the L12 domain of K5, two are affecting the positions 12D12V and D12T (10). Two mutations in K5, D328V and D328H, were reported as causing a mild EBS-WC phenotype. Both mutations alter the same amino acid residue in the L12 domain of K5 at position 12 (8-10,16). Recently a novel missense mutation D328E in the position L12 of K5 (17) was found.

Since our investigation of Slovenian EBS patients, there were additional reports on K5/K14 gene mutations: e.g. a missense mutation K14 R125C in a EBS-DM patient (18), a K14 A413T (alanin to threonin) in a EBS-K patient (19), a nonsense K14 3 G123 1T mutation creating a premature stop codon 411 (20). Two distinct K5 missense mutations E170K and E418K were detected in an EBS patient, while the mutation E170K was present in all the affected family members (21). A splice site mutation of K14 1842-2A—C causing a truncated K14 in a patient with recessive EBS and mosaicism (22) was also described. Specially interesting are the following two EBS cases in whom mutation were not in keratins, but in other proteins. In a EBS-Ogna patient the disease was attributed to a deficient plectin 1a (23), while in a case of EBS with further symptoms (dystrophy of the nails with onychogryphosis, enamel hypoplasia) it was attributed to a mutation of the integrin beta 4 gene (24).

As the database of keratin gene mutations is expanding, new attempts are made to correlate a specific genotype to a resultant phenotype. The determination of the molecular defect has a clinical relevance for genetic counseling, prenatal diagnosis and contributes to the future development of gene therapy strategies.

Diagnostic procedures and genetic counseling

The modern diagnostic approach to patients with EB integrates multiple steps: patient's history, course of the disease, histopathology electron microscopy, tratment and genetic counselling. A biopsy should be taken from clinically unaffected skin. A mild disruption of the skin should be induced by rubbing the area to be excised with a finger or a rubber for about one minute. After a delay of 5-10 minutes the biopsy should be taken (25). In most of the cases not all the diagnostic steps are necessary to get to a final diagnosis, however the information may be helpful for development of targeted gene transfer and ultimately gene therapy.

Genetic information provided by mutation analyses on EB candidate genes provides an immediate benefit to families of EB patients as prenatal diagnostic procedures are made easier. Fetal skin biopsy and fetoscopy with their increased risk to pregnancy can be avoided by analysis of chorionic vilus sampling as early as 8 to 10 weeks gestation.

The professional counselling in EB patients is also important. Avoidance of exposures to UV light, heat and pressure to palms and soles are to be considered.

Treatment

In patients affected with EBS, the treatment is typically supportive and preventive and consist of wound management, adequate nutrition and infection control. Fresh blisters should be drained after puncturing them with a sterile needle. The blister roof should be left in place. Bathing of blistered hand and feet in warm water containing potassium permanganate at a dilution of about 1:8000 is useful (23). Compresses with physiological saline, topical steroids, and topical antibiotics are commonly used in order to prevent secondary infections. Patients should maintain cool environment, avoid minor trauma, and wear comfortable shoes.

It can be expected that in the future genetic treatment may include the transfection of a recombinant protein. Recent advances in vector design, administration, immune modulation and regulation of gene expression have brought the field nearer to clinical utility (24). In the case of gene therapy delivery of genes target to restore normal protein expression is the goal.

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