Mutation in the KRT1 gene causing epidermolysis bullosa simplex

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Epidermolysis bullosa (EB) is a heterogeneous group of genetically determined bullous disorders characterized by spontaneous or trauma-induced formation of blisters. To date, four types of EB have been distinguished depending on the localization of blister formation in the epidermal-dermal junction and classified into more than 30 different entities on the basis of their inheritance and clinical symptoms [1].

Epidermolysis bullosa simplex (EBS) is characterized by superficial, intraepidermal blister healing without scars. In common dominant EBS variants dependent on mutations within the gene encoding keratin 5 or keratin 14 blisters arise through the basal keratinocyte layers whereas in rare recessive EBS variants blisters develop suprabasally.

Pathogenic mutations in more than 20 different genes have been identified as a molecular cause of EB [1–3]. However, the list is certainly not closed and novel reports indicating the contribution of other genes to EB, especially to suprabasal EBS etiopathology, are emerging [4].

Herein, we present the first case of a family with three members affected by EBS due to KRT1 mutation.

A twenty-four-year-old woman and her one-year-old sister initially presented to our department in 2005 with generalized flaccid blisters. In both sisters the blisters and erosions were localized in the extremities and trunk with predominantly involved lower abdomen due to mechanical friction with clothes (Figure 1 A). Erosions healed without scars and milia but some of them left post-inflammatory hyperpigmentation. Nikolsky’s sign was positive on the trunk and lower abdomen (Figure 1 B). Palmoplantar hyperkeratosis was observed in both patients without blisters on hands and feet (Figure 1 C).

Family history revealed that during a twelve-year follow-up both sisters presented severe relapses of the disease several times a year with short periods of remission. Bullous lesions developed in both sisters at birth and the course of the disease was particularly severe in the neonatal period. Moreover, the patients’ mother and grandfather manifested blisters at birth and during childhood, whereas palmoplantar hyperkeratosis persisted in adulthood.

Histology showed orthokeratosis and disruption of cytoplasm in keratinocytes of the stratum spinosum leading to vesicle formation and intraepidermal separation (Figure 2 A).

Double immunofluorescence mapping was performed on biopsy samples of apparently normal skin close to the fresh blister and perilesional skin using antibodies directed to the lamina densa (monoclonal antibody against collagen IV Sigma, clone 7.2) and the upper part of the lamina lucida (bullous pemphigoid serum against BP180 NC16A – positive, and BP230 – negative). Intraepidermal separation through the stratum spinosum of the epidermis was observed leaving normal basal keratinocytes attached to the dermal-epidermal junction labeled with both basal membrane zone (BMZ) markers (Figure 2 B).

Electron microscopic study of perilesional skin showed clumping of keratin intermediate filaments and cytolysis of keratinocytes of the stratum spinosum (Figure 3).

Based on the clinical picture and microscopic analysis the patients were diagnosed with EBS but the molecular analysis of KRT5 and KRT14 did not show any mutations (Sanger Sequencing, according to Hamada et al.) [5]. Recently, next-generation sequencing (NGS) was performed (using TruSight One Panel, Illumina), showing the heterozygous c.591+1G>A mutation within the KRT1 gene encoding keratin 1, leading to a change from guanine to

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adenine in a highly conserved donor splice site position of intron 1.

The presence of c.591+1G>A mutation in both sisters and their mother was further confirmed by Sanger sequencing (Figure 4, primers available on request). The DNA of the affected grandfather was unavailable.

The goal of our study was to determine the family mutation which triggered bullous genodermatosis in our 36-year-old patient and her family. Clinical observations and molecular studies were conducted for the first time in 2005. Immunofluorescence mapping analysis revealed intraepidermal blister formation characteristic of EBS ruling out dystrophic and junctional EB. The mode of inheritance of the disease, and the presence of palmoplantar hyperkeratosis along with flaccid blisters on the trunk and extremities strongly suggest keratin pathology. Nonetheless, molecular analysis of the KRT5 and KRT14 genes did not show any mutations.

Almost 10 years after the first molecular efforts it was possible to identify the KRT1 heterozygous c.591+1G>A mutation using a novel and powerful molecular strategy – next generation sequencing – allowing for a rapid analysis of several genes. This modern diagnostic method seems to be extremely useful in the molecular analysis of those genodermatoses in which several candidate genes are linked to similar pathology, e.g. different variants of ichthyosis or EB [5]. More importantly, in contrast to previously performed diagnostic procedures, more genes can be easily co-analyzed, enabling the identification of mutations in genes other than those selected initially by phenotype evaluation. Consequently, novel, yet unexpected, phenotype-genotype observations can be made.

The best of our knowledge this is the first report of a family with EBS caused by KRT1 mutation. Of note, the level of blister formation in the stratum spinosum reflects keratin 1 pathology. It is well known that KRT1 mutations usually cause a bullous variant of ichthyosis – a genodermatosis in which blisters appear at birth and in the neonatal period, but in childhood the skin lesions transform into ichthyotic scales and palmoplantar hyperkeratosis [6]. In contrast to the typical picture of bullous ichthyosis, none of the family members manifested scaly
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Figure 2. A – Histology shows orthokeratosis and disruption of cytoplasm in keratinocytes of stratum spinosum leading to vesicle formation and intraepidermal separation. B – Double immunofluorescence mapping shows intraepidermal separation through stratum spinosum of the epidermis leaving normal basal keratinocytes attached to the dermal-epidermal junction labeled with both basal membrane zone markers.

Figure 3. Electron microscopy shows clumping of keratin intermediate filaments (arrows in A, B) and cytolysis in keratinocytes of stratum spinosum (asterisks in C, D). Bars: 2 µm

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Skin. However, Nikolsky’s sign is present and flaccid blisters develop continuously after a mild trauma and persist in adulthood.

Moreover, histopathology showing blister formation though the stratum spinosum suggestive of suprabasal EBS and no signs of hyperkeratosis ruled out bullous ichthyosis.

It is well known that depending on the type of mutation and its localization within the gene, the clinical picture of certain genodermatoses may be strikingly different or even represent a distinct entity. For instance, a mutation in the tail of the KRT1 gene causes ichthyosis with confetti clinically characterized by scaling erythroderma and white spots due to revertant mosaicism;
Figure 4. A – NGS reveals the heterozygous c.591+1G>A mutation within the KRT1 gene encoding keratin 1 leading to a change from guanine to adenine in a highly conserved donor splice site position of intron 1. B – Sanger sequencing confirms c.591+1G>A mutation in KRT1 gene. C – Family pedigree.

Figure 4. A – NGS reveals the heterozygous c.591+1G>A mutation within the KRT1 gene encoding keratin 1 leading to a change from guanine to adenine in a highly conserved donor splice site position of intron 1. B – Sanger sequencing confirms c.591+1G>A mutation in KRT1 gene. C – Family pedigree.

However, blisters never occur in this disorder, contrary to bullous ichthyosis [7].

On the other hand, KRT5 and KRT14 mutations are strongly associated with EBS, whereas the loss of function mutations within KRT5 are mostly related to reticulate pigmentation without blister formation (Dowling-Degos disease) [8]. Finally, gene mutations in different types of epidermal keratins cause epidermolysis bullosa simplex, epidermolytic ichthyosis, superficial epidermolytic ichthyosis, epidermolytic palmoplantar keratoderma and pachyonychia congenita/focal palmoplantar keratoderma, which present with thickening of the palms and soles with underlying blister formation. The particular mechanism of blister formation in keratinizing diseases has not been well established, but we suggest that the compensation of an epidermal barrier defect may be the most reasonable explanation [9].

Our patients present a novel hereditary blistering disorder due to keratin 1 pathology confirmed by the disruption of cytoplasm in the stratum spinosum in histology and clumping of keratin filaments at the ultrastructural level.

Although this is the first study which associates KRT1 with EBS pathology, it is necessary to stress that the heterozygous c.591+1G>A mutation in the KRT1 gene was previously reported in 1 patient diagnosed with a bullous variant of ichthyosis [10]. The clinical details of a Swedish patient were not presented by the authors, who focused their study on a molecular analysis of 15 ichthyotic cases. It remains unclear why the same mutation causes two different entities – bullous ichthyosis in the Swedish patient and EBS in our Polish family. One explanation may be that the Swedish patient clinically presented palmo-plantar hyperkeratosis without blisters in adult life just as the mother of our two EBS sisters. It is also possible that some genetic or environmental factors may influence the course of genodermatoses.

In contrast to the Swedish authors, we present a 13-year follow-up of two adult sisters constantly developing blisters induced by mechanical trauma, without ultrastructural and histological signs of ichthyosis. Therefore, we propose including the heterozygous c.591+1G>A mutation in the KRT1 gene in the list of molecular defects underlying EBS pathology.

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Conflict of interest

The authors declare no conflict of interest.

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