Substantiating atypical phenotypes of epidermolysis bullosa

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Chapter 4

In-frame exon skipping in *KRT5* due to novel intronic deletion causes epidermolysis bullosa simplex, generalized severe

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TO THE EDITOR

Basal epidermolysis bullosa simplex (EBS) is the most common type of epidermolysis bullosa (EB) comprising 31.8% of all EB patients with an identified mutation in the Dutch National EB registry (unpublished). Basal EBS is characterized by intra-basal splitting of the epidermis. Mutations in *KRT5* and *KRT14* are responsible in 75% of the cases. [1] The clinical phenotype of the most severe variant, EBS, generalized severe (EBS-gen sev, previously reported as EBS, Dowling-Meara) consists of congenital generalized mechanobullous skin and mucous membrane fragility, typically presenting with sero-hemorrhagic vesicles in a circinate distribution, and late-onset palmoplantar keratoderma. [2] Characteristic of EBS is that most symptoms tend to diminish in severity after adolescence. [3] Although often seen, the exact molecular mechanism for improvement of disease by adulthood for EBS and other keratin disorders is not fully understood. More than 86% of reported mutations in *KRT5* and *KRT14* causing EBS are dominantly-acting missense mutations. The majority are located in the helical initiation and termination domains H1, 1A, and 2B. [4,5] Here, we report a four-generation Dutch kindred affected with EBS-gen sev caused by a novel heterozygous intronic deletion in *KRT5*. The mutation leads to in-frame skipping of exon 6 encoding 42 amino acids of the 2B helical domain.

The female index patient (II-3, EB-092-01) now in her late 50's, was born to Caucasian parents, and had a history of generalized blistering from birth. She suffered from cutis aplasia congenita of the legs and persistent oral blistering in infancy. Later in life, occasional blistering without scarring occurred all over the body, with predilection sites on hands, inguinal folds and feet. Blistering was most severe during puberty; around the same time she developed palmoplantar keratoderma. Severity and extent of affected integument all subsided during early adulthood. The family history was positive for blistering (Figure 1a). All affected family members suffered similar symptoms as the index patient and observed gradual improvement in both severity and extent with age (Figure 1 b-g). After obtaining informed consent, skin biopsies of fresh blisters from the index patient (II-3) and her eldest daughter (III-3) were obtained for immunofluorescence antigen mapping (IF) and transmission electron microscopy (TEM). IF using BL18 antibody (gift from Dr.P. Ogden, Dundee, UK) directed against keratin 5 of lesional skin showed intraepidermal cleavage with similar expression (3+) as in control (3+). Staining was positive in the blister roof and floor. Staining performed with antibodies directed against keratin 14, BP230 (dystonin), laminin-332, and type VII collagen were normal. TEM of lesional skin showed intraepidermal cleavage, lateral aggregation of keratin filaments and insufficient tonofilament insertion into the hemidesmosomes (Figure 1h). Tonofilament clumping and acantholysis (as earlier reported in [6]) were also observed. Genomic DNA was extracted from peripheral blood lymphocytes from several affected individuals (Figure 1). PCR amplification of *KRT5* (GenBank NM_000424.3) revealed a 30-bp heterozygous intronic deletion in intron 6, (c.1218+2_1218+31del). Subsequent mRNA analysis from frozen skin biopsies (from patients II-1, II-3, III-3, III-5, IV-1, and IV-2) showed besides the wild-type transcript, a shortened transcript lacking the 126-bp exon 6 (Figure 2). The intronic deletion occurring in intron 6 affects the highly conserved consensus donor splice site at position c.1218+2, leading to aberrant splicing. Consequently, the transcript resulted in an internally truncated keratin 5 polypeptide (p.Tyr365_Gln406del). The intronic deletion was confirmed by Sanger sequencing for all other affected individuals from whom DNA was available.

The novel intronic deletion in *KRT5* described here led to a classical EBS-gen sev phenotype showing marked improvement with age in this four-generation Dutch kindred. To our knowledge, there have been only three earlier reports of splice site mutations in *KRT5*. Schuilenga-Hut described a patient with EBS generalized intermediate (formerly called EBS-Koebner) caused by a heterozygous acceptor splice site mutation, c.556-1G>C. 7 The effect on RNA level was in-frame skipping of the first 6 amino acids encoded by exon 2 (18bp). The second splice site mutation also reported by our group was c.1474+4A>G in a patient with EBS localized (previously EBS, Weber-Cockayne). [1] The effect on RNA was not further investigated. Lastly, a heterozygous splice site mutation was identified in a large family exhibiting a similar clinical phenotype to the here reported family. [8] In this report, abnormal in-frame splicing of exon 1 occurred by G to A transition in the consensus donor splice site of exon 1 (c.555+1G>A). This led to the use of an upstream cryptic splice site and subsequent deletion of 22 amino acids of the H1 and 1A rod domains. The reported patients displayed, just as our family classic EBS gen sev. [8]

Skipping of exon 6 leads to the exclusion of 42 amino acids of the highly conserved 2B domain of *KRT5*. This heterozygous deletion is expected to act in a dominant-negative fashion affecting the keratin intermediate filament assembly, similar to keratin polypeptides affected by heterozygous missense mutations. [9] Normally, a keratin 5 monomer will polymerize with a keratin 14 monomer by parallel assembly and winding of their rod domains into a coiled-coil structure. The stability of keratins is regulated by interactions between the two chains, which are held together by their hydrophobic residues. [10-12] These residues are organized as heptad repeats, labelled 'a' to 'g' with the strongest hydrophobic interactions occurring at heptad positions 'a' and 'd'. A molecular defect such as an amino acid substitution alters the keratin inter-chain interactions to different degrees, depending on the polarity and position of the expressed residue. [10,11] The residues occupying the 'a' position of the α-helix are apolar amino acids, which stabilize the coiled coil of keratins. Residues located in the 'g' position additionally contribute to the stability of the heterodimer by charged hydrophobic interactions. In the case of a heterozygous missense mutation, or in this case an internally truncated protein, the interchain assembly is distorted and the higher architecture of dimers and subsequent tetramers and filaments will be defective and prone to collapse. [12] To date, 28 of the 121 reported mutations in *KRT5* have been located in exons encoding the 2B domain of *KRT5*. 4 Of these, all but two published reports have been missense or nonsense mutations with the remaining being a deletion and an insertion deletion. [13,14]
In conclusion, we illustrate the first intronic deletion leading to exon skipping in *KRT5* causing EBS-gen sev. The described four-generation family exhibited classic EBS-gen sev phenotype and TEM and IF findings. With the presented casus, we would like to draw attention to introns of EBS genes, including *KRT5,* as they can harbour mutations that lead to altered splicing and thereby affecting intermediate filament assembly in the basal epidermis.

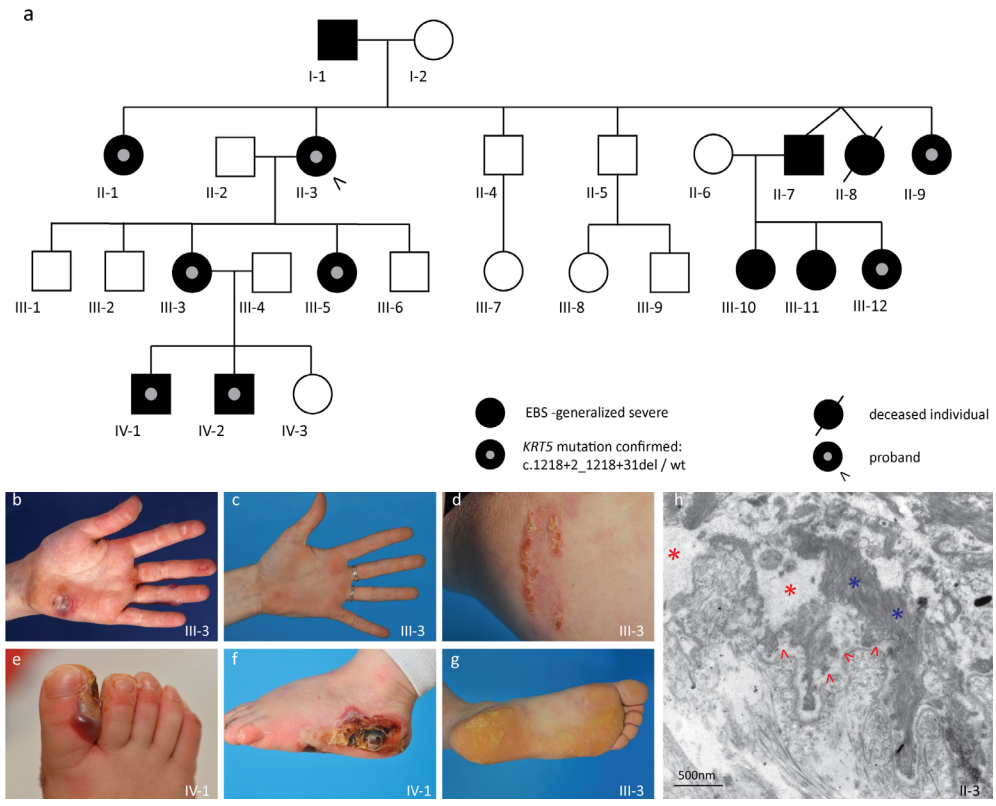

Figure 1: Family pedigree and clinical pictures of affected family members with ultrastructural view of affected skin in EBS-gen sev.

(a) Family pedigree (b) Blistering of the palm at 6 years of age with surrounding subtle hyperkeratosis. Bullae of the fingers with visible desquamation. (c)The same patient at 11 years of age where amelioration of symptoms is seen. (d) Circinate blistering seen on the inner thigh. (e) Haemorrhagic blistering of the large toe, with focal hyperkeratosis and subtle onycholysis at 2 years of age. (f) Grouped haemorrhagic blisters at the lateral aspect of the foot of the same patient. (g) Generalized plantar keratoderma at 15 years of age. (h) Ultrastructure of affected skin shows a blister cavity (red asterisks) in the basal cell with the plasma membrane in the blister floor (red arrowheads), and lateral aggregation of tonofilaments (blue asterisks) .

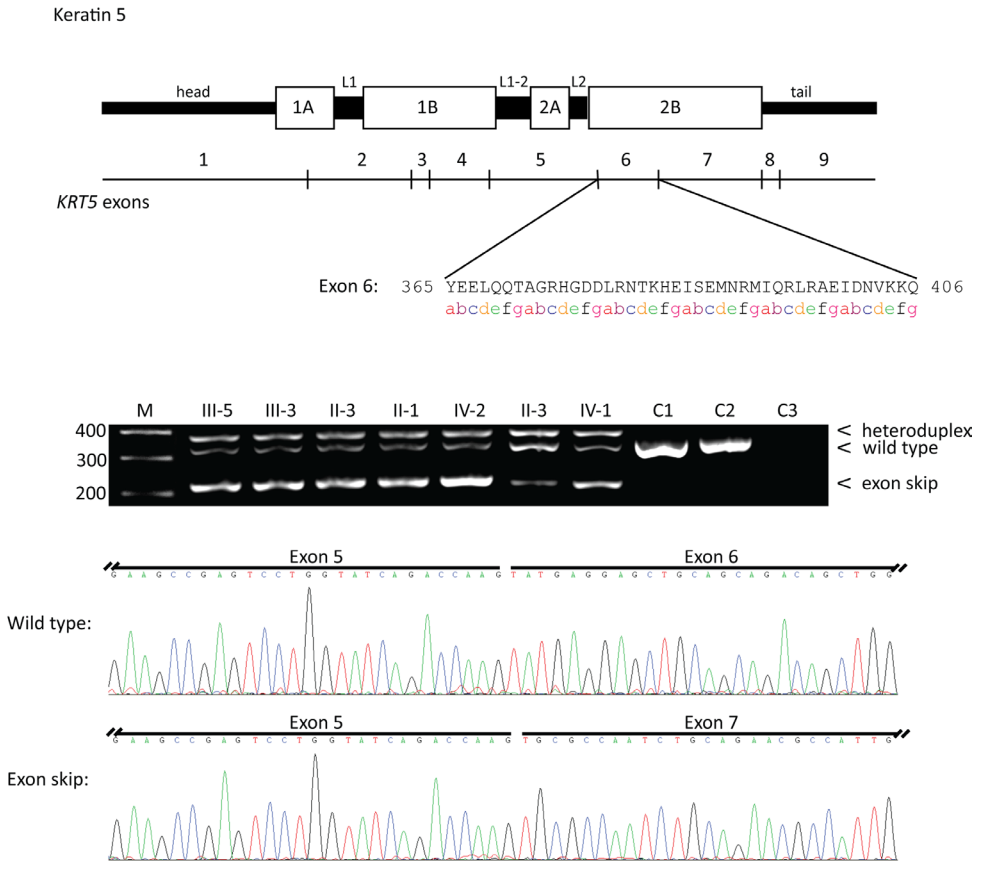

Figure 2: Schematic representation of keratin 5 and mRNA studies

(a) Schematic representation of the keratin 5 polypeptide and below the nine exons comprising the encoding gene *KRT5*. Exon 6 containing 42 amino acids (codons 365-406), comprises exactly 6 heptad repeats and is depicted in colour courier font.

Using frozen skin biopsies, nested RT-PCR analysis (b) and Sanger sequencing of patient and healthy control mRNA. Lanes are marked with pedigree numbers of patients. C1 and C2: healthy control, C3: negative control. *KRT5* primers used: forward 5'-CGCAACCTGGACCTGGATAG- 3' reverse: 5'-CCATGTCCTGCTTGGCCTTC- 3' (wild type: 349 bp length, exon skip 223 bp length).

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