In-frame Exon Skipping in KRT5 due to Novel Intrinsic Deletion Causes Epidermolysis Bullosa Simplex, Generalized Severe

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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bullosa (EB), comprising 31.8% of all EB patients with an identified mutation in the Dutch National EB registry (unpublished).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 and mutations in KRT5 and KRT14 are responsible in 75% of 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 mechoanobullous 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). The exact molecular mechanism for the improvement 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). We report here a 4-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.

CASE REPORT
The female index patient (II-3, EB-092-01), now in her late 50s, was born to Caucasian parents, and had a history of generalized blistering from birth. The patient had cutis aplasia congenita of the hands, inguinal folds and feet. Blistering was most severe during puberty; around the same time she developed palmoplantar keratoderma. The severity and extent of affected integument all subsided during early adulthood. The majority of affected family members had similar symptoms to those of the index patient and observed gradual improvement in both severity and extent with age (Fig. 1b–g). After obtaining informed consent, skin biopsies (from patients II-1, II-3, III-3, III-5, IV-1 and IV-2) showed that, besides the wild-type transcript, a shortened transcript lacking the 126-bp exon 6 (Fig. 1h). 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.

DISCUSSION
The novel intronic deletion in KRT5 described here led to a classical EBS-gen sev phenotype showing marked improvement with age in this 4-generation Dutch kindred. To our knowledge, there have been only 3 earlier reports of splice-site mutations in KRT5. Schuilinga-Hut et al. (7) described a patient with EBS generalized intermediate (formerly called EBS-Koebner) caused by a heterozygous acceptor splice site mutation, c.556-1G>C. 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 family reported here (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, as in 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 fila-

¹https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2451
ment 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 2 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 2 published reports have been missense or nonsense mutations with the remaining being a deletion and an insertion deletion (13, 14).

With the presented case, we would like to draw attention to introns of EBS genes, including KRT5, as they can harbour mutations that lead to altered splicing, thereby affecting intermediate filament assembly in the basal epidermis.

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