A novel homozygous nonsense deletion/insertion mutation in the keratin 14 gene (Y248X; 744delC/insAG) causes recessive epidermolysis bullosa simplex type Köbner


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Summary We report the sixth case of a human keratin 14 'knockout' mutation resulting in recessive epidermolysis bullosa simplex (EBS). A novel, homozygous nonsense mutation resulting from a deletion/insertion mutation (744delC/insAG) leads to a premature termination codon in the KRT14 gene (Y248X). The patient suffers from generalized cutaneous blistering since birth, mild nail dystrophy, involvement of mucous membranes and multiple epidermolysis bullosa naevi. The clinical variability noted in K14-deficient EBS patients suggests phenotypic modulation by additional genetic and/or epigenetic factors.

Report The proband, an 8-year-old male, is the child of unaffected parents of Austrian origin without apparent consanguinity and with a negative family history of blistering skin diseases. He has been presenting with generalized cutaneous blistering since birth, most pronounced on the extremities and the face. Skin fragility has been lessening with age. Mild nail dystrophy and involvement of mucous membranes have been noted. Hair and teeth are not affected. The blisters heal without milia but with mild atrophic scarring. Interestingly, the patient has been developing multiple, rapidly enlarging, asymmetrical, irregularly pigmented melanocytic naevi with poorly defined borders, i.e. epidermolysis bullosa (EB) naevi, since the age of 3 years. One of these naevi is shown in Fig. 1.

Epidermolysis bullosa simplex (EBS) is an inherited mechanobullous skin disorder characterized by cytolysis of basal keratinocytes and intraepidermal blistering following minor trauma. EBS is usually inherited in an autosomal dominant fashion. Various mutations in the genes encoding keratin 5 (KRT5) and 14 (KRT14), most of which are missense mutations that act in a dominant negative way to perturb K14–K5 heterodimer formation and hence keratin filament assembly, have been identified to underlie EBS. To our knowledge, five kindreds

Figure 1 An approximately 4 cm × 3 cm large EB naevus on the right thigh showing asymmetry, irregular borders with stippled pigmentation, prominent protrusions and satellite lesions.
Verification of the deletion/insertion mutation 744delC/insAG in exon 3 (Y248X). (a) The pedigree of the nuclear family is consistent with the recessive mode of inheritance. (b) Verification of the mutation by restriction enzyme digestion with Ddel. The mutation creates a new restriction site for Ddel that results in digestion of the 327-bp PCR product to 232- and 95-bp fragments. The father and the mother are heterozygous for the mutation, resulting in the appearance of both bands as well as the undigested band of 327 bp. Lane MW, molecular weight marker; lane M, mother; lane C, child; lane F, father; lane Co, control DNA from unrelated healthy donor. (c) Sequence analysis of the PCR products from the father and the affected patient encompassing exon 3 of the keratin 14 gene shows a homozygous C deletion and an AG insertion at position 744 resulting in a change of tyrosine (TAC) to a stop codon (TAA). This deletion/insertion is detected on one allele of the father’s DNA resulting in a heterozygous sequence pattern.

deletion/insertion (744delC/insAG) in KRT14. This mutation leads to a premature termination codon of K14 in the 1B segment of the alpha-helical rod domain (Y248X) (Fig. 2). Presumably as a result of nonsense mediated mRNA decay, this mutation causes complete loss of K14 protein expression in basal keratinocytes of the patient (Fig. 3).

The mutation analysis in this family was performed by polymerase chain reaction (PCR) amplification of the coding sequence of KRT14 from genomic DNA, followed by heteroduplex scanning and automated sequencing of PCR products containing heteroduplexes. The mutation was verified by Ddel restriction enzyme digestion (Fig. 2). The following primers, complementary to the flanking intronic sequences, were used for amplification of exon 3 of the K14 gene: 5'-ACA AGG CAC CAG CTC TGG-3' (forward), and 5'-TAT GGG CAC CCA CCA CTG-3' (reverse). PCR conditions were: 94°C for 10 min, followed by 94°C for 45 s, 65°C for 45 s and 72°C for 45 s (AmpliTaq Gold™, Applied Biosystems, Foster City, CA; 40 cycles).

Immunohistochemistry on de-paraffinized skin sections of the patient revealed negative staining with a monoclonal anti-keratin 14 antibody (CKB1/072 H-4860, Sigma-Aldrich Corp., St. Louis, MO) using a biotin-streptavidin system and AEC (3-amin-9-ethylcarbazole) labelling with haematoxylin counterstain. In comparison, bright K14 staining of basal keratinocytes was visible in a normal skin specimen from an unrelated healthy control (Fig. 3).

Despite the finding that, in all recessively inherited K14 ‘knockouts’, K14 expression of the epidermis was completely ablated,3-7 not all patients demonstrated the same degree of severity of disease. For example, the patient reported here had severe and generalized EBS
the patient with mutation Y248X further underscores that the clinical phenotype of K14-deficient individuals may vary and that other genetic and/or epigenetic factors modulate the phenotype.

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References


