# Recessive Epidermolytic Hyperkeratosis Caused by a Previously Unreported Termination Codon Mutation in the Keratin 10 Gene

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## TO THE EDITOR

Epidermolytic hyperkeratosis (EH; MIM 113800), also termed bullous congenital ichthyosiform erythroderma, is a keratinization disorder characterized by erythema and widespread blister formation at birth that is replaced by progressive hyperkeratosis later in life (Arin and Müller, 2007). The causes of EH in most cases are the dominant mutations in the genes encoding keratins, K1 and K10, which lead to clumping of keratin intermediate filaments in suprabasal keratinocytes. The majority of mutations are located at the highly conserved helix boundary motifs of both keratins, and a genetic "hotspot" has been identified in K10 affecting an evolutionarily highly conserved arginine residue (p.Arg 156) (Rothnagel *et al.*, 1993; McLean *et al.*, 1994). Recently, a recessive form of EH has been identified that is due to a nonsense mutation in the *KRT10* gene leading to loss of K10 expression in the affected homozygous individuals (Müller *et al.*, 2006).

In this study, we carried out molecular analysis in a family of Sudanese descent with several lines of blood relations (Figure 1a). At birth, the affected girl presented with widespread erythema and superficial erosions located predominantly on the trunk and on the proximal extremities, and in the face (Figure 1b). Shedding of thick plates without erosions starting around the umbilicus was reminiscent of a collodion presentation. During the initial days of life, she became increasingly somnolent and developed hypernatremia requiring intensive neonatal care. The consanguineous parents as well as the 3-year-old brother did not show any skin disease. A similar severe picture was seen in a previously reported family with recessive EH (Müller *et al.*, 2006), and diffuse skin blistering and erosions at birth were also reported in another child with recessive EH (Tsubota *et al.*, 2008). Palmoplantar sites were not affected in all four patients described to date, similar to autosomal dominant EH caused by *KRT10* mutations. Skin biopsy specimens were taken by excision biopsy under local anesthesia from the affected proband, and were processed for routine histology, immunofluorescence, and electron microscopy, essentially as described in Müller *et al.*, 2006. Genomic DNA from all family members was extracted from peripheral

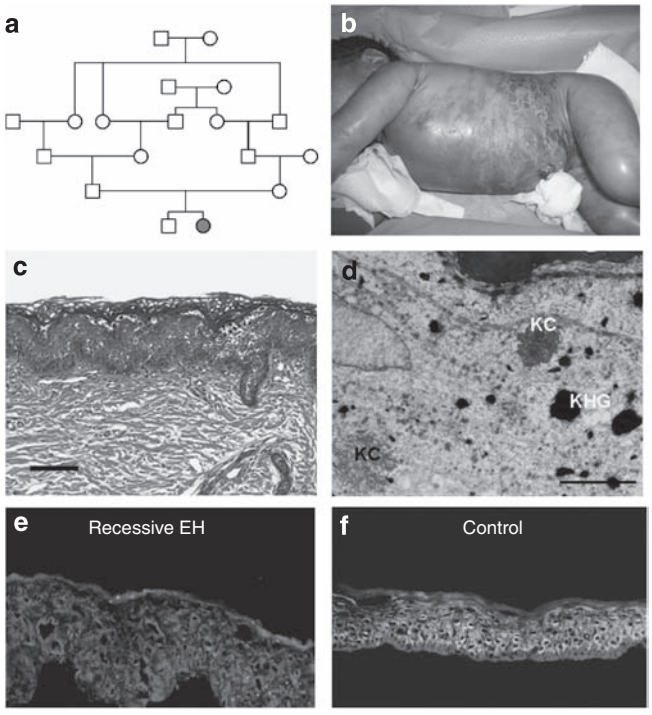


**Figure 1. Clinical presentation, ultrastructural and immunofluorescence analysis in recessive EH.** (**a**) The affected girl is the second child of consanguineous parents. The simplified pedigree shows several lines of blood relations within the parental families. The parents as well as the brother do not show any skin disease. (**b**) The clinical presentation at birth was reminiscent of a collodion baby. (**c**) Histopathology is typical of epidermolytic hyperkeratosis (EH) with suprabasal cytolysis. Magnification ×100. Bar = 100 μm. (**d**) Electron microscopy of the granular layer reveals two distinct aggregates within keratinocytes: keratin clumps (KC) and more electron dense keratohyalin granules (KHG). Bar = 2.5 μm. (**e**) Immunohistology shows absence of epidermal K10 protein in the affected girl. Normal control (**f**). Bar = 100 μm.

*Abbreviations: EH, epidermolytic hyperkeratosis; K, keratin protein;* KRT*, keratin gene; NMD, nonsense-mediated decay; PTC, premature termination codon*

blood samples using standard methods. The study was conducted after obtaining a written informed consent and following Institutional Review Board approval, and followed the Declaration of Helsinki Principles. Histopathology of a skin biopsy taken shortly after birth was typical for EH with vacuolar degeneration of suprabasal keratinocytes and coarse keratohyalin granules in the thickened granular layer (Figure 1c). Electron microscopy in low magnification showed cytolysis, and loose and irregularly shaped electrodense clumps within the keratinocytes of the suprabasal layers of the epidermis. The keratin clumps showed a nearly homogenous, amorphous structure (Figure 1d), which is not seen in autosomal dominant EH, wherein the clumps regularly maintain a filamentous, thready aspect. Immunofluorescence analysis using specific monoclonal mouse antibodies (LH2 and DE-K10), which are directed against the amino-terminal part of the K10 protein, showed the absence of K10 protein in the epidermis of the affected child (Figure 1e).

For *KRT1* and *KRT10* mutation analysis, the PCR fragments were amplified using exon-specific primer pairs as described earlier (Müller *et al.*, 2006). In the affected child, direct sequencing disclosed a homozygous insertion of C at nucleotide 1,325 in *KRT10* (c.1325insC). This frameshift mutation leads to a premature termination codon (PTC) (TGA) six codons downstream in the same exon. In terms of its effect on protein translation, the mutation is designated as p.Lys439fsX6. Both parents and the unaffected brother are heterozygous carriers of the mutation (Figure 2a). A common polymorphism could be excluded by the absence of the mutation in 50 unrelated control DNA samples, and no other mutation was found in the entire *KRT1* and *KRT10* genes.

To confirm that the reported mutation causes nonsense-mediated decay of the mRNA (NMD), we carried out quantitative real-time reverse transcriptase PCR using a 7300 real-time PCR System (Applied Biosystems, Foster City, CA). Total RNA was extracted from keratinocytes according to the manufacturer's protocol (Qiagen, Hilden, Germany). Primers specific for *KRT10* exon 2/3, exon 4/5, and exon 5/6 were used, and the samples were analyzed in triplicates. S26 mRNA was used as the endogenous reference gene, as it does not exhibit significant expression changes between wild type and recessive EH. The transcript levels of wild type vs. recessive EH were assessed by unpaired two-tailed *t*-test. A significant reduction of specific *KRT10* mRNA was found in the skin of the homozygous patient compared with a normal control (Figure 2b). This finding confirms that the here described nonsense mutation does not generate a truncated K10 gene product, but leads to instability and degradation of the mutant transcript by NMD in homozygous patients. The NMD detects mRNAs harboring PTCs and commits these transcripts to rapid decay. During mRNA splicing, protein complexes called exon junction complexes assemble close to each exon–exon junction (Lejeune and Maquat, 2005). During the initial round of translation, the translation machinery displaces the exon junction complexes associated with the transcript as it proceeds with elongation. If the mRNA contains a PTC located more than a certain range upstream the last exon–exon junction (for example, 50–55 nucleotides in the beta globin gene), at least one exon junction complex will remain on the mRNA thereby triggering NMD (Silva and Romao, 2009). For the *KRT*s, the first description of a PTC leading to transcript instability has been shown for *KRT14* in autosomal recessive epidermolysis simplex bullosa (Chan *et al.*, 1994). The present mutation (p.Lys439fsX6) together with both previously reported mutations in recessive EH, p.Gln434X (Müller *et al.*, 2006) and p.Cys427X (Tsubota *et al.*, 2008), are located 461, 476, and 497 nucleotides upstream to the 3′-most exon–exon junction, which supports the concept that in *KRT*s, PTCs are expected to be targeted by NMD when

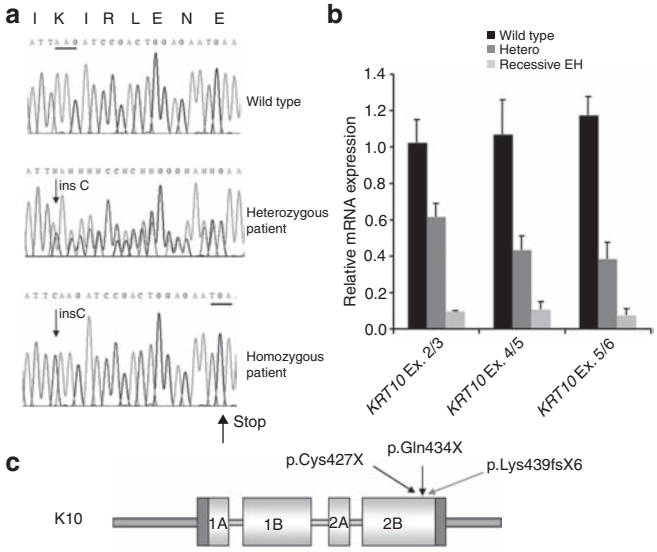


**Figure 2. Molecular basis of recessive EH in the present family.** (**a**) Direct sequencing revealed a homozygous nonsense mutation in exon 6 of *KRT10* in the affected individual, whereas the unaffected family members are heterozygous carriers of the mutation. The insertion mutation results in a frameshift mutation and a premature termination codon (TGA) six amino acids downstream (p.Lys439fsX6). (**b**) Quantitative real-time PCR analysis shows a significant decrease of specific *KRT10* mRNA in the skin of the homozygous patient compared with a normal control. Levels of the heterozygous father were about 50% of wild type. The data are presented as the fold change in *KRT10* mRNA level normalized to the S26 mRNA. The difference in expression of *KRT10* mRNA between wild type and recessive Epidermolytic hyperkeratosis (EH) was statistically significant (*$P<0.05$). Depicted are means and SEM of three experiments. (**c**) All mutations reported to date in recessive EH (present mutation, p.Lys439fsX6, together with both previously reported mutations, p.Gln434X (Müller *et al.*, 2006) and p.Cys427X (Tsubota *et al.*, 2008)) are located in close proximity at the end of the 2B domain of K10, just upstream of the highly conserved helix termination peptide (red area). The mutation ''hotspots'' of autosomal dominant EH are depicted in red. The color version of this figure is available on the html full text version of the manuscript.

located more than 92 nucleotides upstream to the 3′-most exon–exon junction (Müller *et al.*, 2006). K10 transcript levels in the normal skin of the heterozygous father were ~50% compared with the wild type (Figure 2b). Heterozygous family members are clinically unaffected, which indicates that one K10 allele is sufficient to maintain a normal phenotype.

In the previously reported homozygous EH patients (Müller *et al.*, 2006) and in the present proband, K6, K16, and K17 are upregulated in the absence of human K10 protein and show a maximal expression at the sites of cytolysis (data not shown). In addition, suprabasal persistence of the basal keratin, K14, was found in our patient and in the recessive EH patient reported by Tsubota *et al.* (2008). In the K10 knockout mouse, decreased proteolysis and suprabasal persistence of the basal keratins, K5, K14, and K15, were shown, and keratin aggregates were found to consist of residual K1 that formed atypical heterodimers with K14 (Reichelt *et al.*, 2001). Formation of instable atypical heterodimers between residual K1 and another type I keratin, for example, K16 or K17, which form relatively poor intermediate filaments compared with those built up from keratins constitutively expressed in the epidermis (Takahashi *et al.*, 1994; Paladini *et al.*, 1996), could additionally contribute to skin fragility and cytolysis in recessive EH.

In conclusion, the characteristic ultrastructural picture consisting of sparse keratin filaments and keratin clumps that show a nearly homogenous, amorphous structure should prompt a detailed analysis of the pedigree to search for parental consanguinity and a recessive inheritance. All reported mutations in recessive EH to date are located in close proximity in the 2B domain of K10 (Figure 2c), suggesting a genetic hotspot in recessive EH. Expanding the catalog of known mutations in this disorder is important with respect to molecular diagnosis and genetic counseling.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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*See related commentary on pg 2549*

# Desloratadine Inhibits Human Skin Mast Cell Activation and Histamine Release

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## TO THE EDITOR

Most symptoms of allergic disease are caused by mast cell (MC) activation and subsequent release of mediators, particularly histamine (Metz and Maurer, 2007; Metz *et al.*, 2008). Research shows that preformed histamine is stored in large amounts in cutaneous MC granules (Metz and Maurer, 2007). Currently, the most common way to treat MC-driven diseases, such as allergic

*Abbreviations: Ca, calcium; MC, mast cell*