A nonsense variant in the *KRT14* gene in a domestic shorthair cat with epidermolysis bullosa simplex

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Running title: *KRT14* variant in feline EBS

Summary

Epidermolysis bullosa simplex is a hereditary blistering disease affecting the skin and mucous membranes. It has been reported in humans, cattle, buffaloes and dogs, but so far not in cats. In humans, EBS is most frequently caused by variants in the *KRT5* or *KRT14* genes. Here, we report a case of feline epidermolysis bullosa simplex and describe the causative genetic variant. An 11-month-old male domestic shorthair cat presented with a history of sloughed paw pads and ulcerations in the oral cavity and inner aspect of the pinnae, starting few weeks after birth. Clinical and histopathological findings suggested a congenital blistering disease with a split formation within the basal cell layer of the epidermis and oral mucous epithelium. The genetic investigation revealed a homozygous nonsense variant in the *KRT14* gene (c.979C>T, p.Gln327*). Immunohistochemistry showed complete absence of keratin 14 staining in all epithelia present in the biopsy. To the best of our knowledge, this is the first report of feline epidermolysis bullosa simplex, and the first report of a spontaneous pathogenic *KRT14* variant in a non-human species. The homozygous genotype in the affected cat suggests an autosomal recessive mode of inheritance.

Key words: *Felis catus*, dermatology, skin, precision medicine, genodermatosis, keratin, whole genome sequencing

Main text

Epidermolysis bullosa (EB) is a group of hereditary diseases in humans and animals characterized by skin blistering due to defects in structural proteins of the epidermis (Medeiros & Riet-Correa 2015; Fine *et al.* 2014). EB is subdivided into four major

types based on the level of split formation: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler syndrome, of which only the first three types have been described in domestic animals (Mauldin *et al.* 2017). In humans, EBS, JEB and DEB are further classified into generalized and localized forms, a classification which has not been established for animals (Fine *et al.* 2014; Medeiros & Riet-Correa 2015). The major clinical finding of all forms in both animals and humans is blistering and sloughing of the epidermis upon minor trauma (Medeiros & Riet-Correa 2015; Fine *et al.* 2014). Significant oral lesions and ungual abnormalities have been described in all EB forms in animals, while in humans, these are primarily associated with generalized JEB and DEB (Medeiros & Riet-Correa 2015; Fine *et al.* 2014).

EBS is characterized by intraepithelial split formation and is associated with variants in the genes *DSP* (desmoplakin; human), *DST* (dystonin; humans), *EXPH5* (exophilin 5*;* humans), *JUP* (junction plakoglobin; humans), *KRT5* (keratin 5; humans, cattle), *KRT14* (keratin 14; humans), *PKP1* (plakophilin 1; humans, dogs), *PLEC* (plectin; humans, dogs), and *TGM5* (transglutaminase 5; humans) (Medeiros & Riet-Correa 2015; Fine *et al.* 2014; Mauldin *et al.* 2017). While pathogenic variants in the *DST, EXPH5, KRT5, KRT14*, and *PLEC* genes evoke intrabasal split formation, variants in the remaining genes result in suprabasal separation (Fine *et al.* 2014).

In cats, EB is a rare disease with isolated case descriptions of DEB in a Persian and a domestic shorthair cat and JEB in two unrelated domestic shorthair kittens (Alhaidari *et al.* 2005; Olivry *et al.* 1999; White *et al.* 1993). For none of the reported feline cases, the possible genetic cause was investigated. Here, we describe the first case of feline EBS with clinical, histopathological and genetic findings.

An 11-month-old male castrated domestic shorthair cat was referred for painful feet caused by lymphocytic pododermatitis (previous biopsy result). Topical treatment

with tacrolimus resulted in partial healing, however, novel oral lesions occurred. The cat was adopted from a cat rescue center at 1.5 months of age, having already ulcerative lesions on paw pads and ear pinnae, and a diffuse non-scarring alopecia on the dorsal neck.

At presentation, the cat was lethargic and inappetent. Partial to complete sloughing of the paw pad skin affecting multiple pads with multifocal re-epithelization underneath was present (Fig. 1a). The gums, hard palate, buccal mucosa and ventral aspect of the tongue were multifocally ulcerated (Fig. 1c). Lesions were not contiguous with teeth, which appeared normal. Ulcers were also present at the inner aspect of both pinnae (Fig. 1b). The cat tested negative for FIV, FeLV, toxoplasma and Coronavirus.

Biopsy specimens from paw pads and tongue were submitted for histopathological examination. Both tissues displayed a multifocal detachment of the epithelium, without associated interface inflammation (Fig. 2a-c). These blisters were roofed by the epithelial basal layer showing an uneven contour (Fig. 2b). The basement membrane covering the dermis and lamina propria, respectively, was the blister floor, as confirmed by PAS reaction (Fig. 2c). In areas with adherent epithelium, multifocal cytoplasmic vacuolation of basal keratinocytes was seen (Fig. 2b). The paw pad tissue was covered with one to two layers of necrotic detached epidermis alternating with serocellular crusts (Fig. 2a). Paw pad dermis and mucosal connective tissue displayed a mild to moderate mixed inflammation.

The histopathological findings and the early onset of the disease were suggestive of EB, most likely EBS. To probe the genetic basis, EDTA blood was collected and genomic DNA isolated. Subsequently, the genome of this cat was sequenced at 32× coverage. Single nucleotide variants and short indels were called with respect to the

reference genome assembly FelCat 9.0 according to the methodology described previously (Jagannathan *et al.* 2019). The sequencing data were deposited in the European Nucleotide Archive (ENA) under project accession PRJEB7401 and sample accession SAMEA5885922. For variant filtering, we used 38 control genomes, which were either publicly available or produced during other projects of our group. In a scenario for a dominant disease allele, we filtered for private heterozygous protein-changing variants in the affected cat. In a separate analysis for a potentially recessive disease allele, we filtered for private homozygous protein-changing variants in the affected cat. In both analyses, we hypothesized that the mutant allele at the causative variant should be rare in cats and completely absent from the 38 unrelated control cats in our sample set. This analysis yielded 727 heterozygous and 82 homozygous private protein-changing variants in the cat with EBS. Three of these variants were located in one of the nine known EBS candidate genes (*DSP, DST, EXPH5, JUP, KRT5, KRT14, PKP1, PLEC, TGM5*). Two of the three candidate variants represented heterozygous missense variants in *DSP* (p.Arg1508His) and *DST* (p.Glu141Lys). We considered these variants unlikely to be causative, as human EBS forms caused by variants in these genes are recessively inherited (Groves *et al.* 2010), (Jonkman *et al.* 2005). Moreover, the reported human variants in the *DST* gene are nonsense variants, and defects in desmoplakin (*DSP*) manifest with suprabasal split formation, unlike in the present case (Fine *et al.* 2014). The third candidate variant was a nonsense variant in the *KRT14* gene truncating more than 31% of the open reading frame: XM_003996860.5:c.979C>T or XP_003996909.2:p.(Gln327*). Sanger sequencing confirmed the mutant allele being present in a homozygous state in the affected cat and absent from 154 unaffected cats from different breeds.

To investigate the protein expression of keratin 14 and keratin 5 in the tissue, immunohistochemistry using antibodies directed against human keratin 14 (clone LL002, Leica Novocastra), and human keratin 5 and 6 (clone D5/16B4, Dako/Agilent Technologies), respectively, was performed (Fig. 2e-f). Keratin 14 staining in the paw pad epithelium and dermal apocrine glands was completely absent in the patient tissues (Fig. 2e), while keratin 5/6 expression was present (Fig. 2d).

KRT14 encodes the basal keratin 14, which forms a coiled-coil heterodimer with keratin 5, further assembling to tonofilaments to provide strength and flexibility to basal keratinocytes (Coulombe *et al.* 2009). Structural alterations in either keratin 14 or 5 lead to increased fragility of the basal cell layer and laceration thereof upon shearing force effect, causing separation in the basal cell layer (Coulombe *et al.* 2009). In the affected cat, histopathology clearly showed an intrabasal split formation, reflected by a denuded basement membrane on the blister floor and an irregular blister roof formed by shrunken basal keratinocytes. Furthermore, there was multifocal basal cell vacuolation. Both lesions are likely the sequel of the weakened cytoskeleton due to a truncation and/or reduced expression of the keratin 14 protein. Genetic variants in *KRT5* and *KRT14* are the most frequent cause of EBS in humans (Coulombe *et al.* 2009; Bolling *et al.* 2011). In animals, EBS with intrabasal split formation has only been described in cattle and dogs (Medeiros & Riet-Correa 2015; Mauldin *et al.* 2017). In cattle with a dominant form of EBS, a missense variant in the *KRT5* gene has been identified (Ford *et al.* 2005). In a litter of Eurasier dogs with recessive EBS, a nonsense variant in the *PLEC* gene encoding plectin was found (Mauldin *et al.* 2017).

In humans with *KRT14* variants, four clinical subtypes of EBS have been described (Bolling *et al.* 2011). In localized EBS, the lesions first appear in early childhood and are mainly restricted to soles and palms. Oral affection is variable. In generalized severe EBS, generalized skin sloughing relatively sparing palms and soles, but involving the oral mucosa, starts at birth. Generalized intermediate EBS is similar to the latter form, but with variable degree of lesions (Fine *et al.* 2014). In the rare autosomal recessive (AR) form, the severity of the clinical phenotype may correlate with residual expression of mutant or truncated KRT14 protein (Batta *et al.* 2000). The case described here is clinically most similar to the human localized EBS or the AR form. Unfortunately, neither clinical information nor tissue samples of the patient's parents or siblings could be obtained to further characterize the trait, as they were adopted by other owners and became off reach.

By using the anti-keratin 14 antibody reacting with the C-terminus of human keratin 14 homologous with the feline protein, the absence of a conformationally normal keratin 14 protein was demonstrated. In contrast, the expression pattern of keratin 5 was physiological. The complete absence of keratin 14 signal in the whole biopsy is compatible with a loss of function variant on both alleles in a non-mosaic pattern. Immunohistochemistry of skin biopsies may thus be a first diagnostic test to look for altered protein expression due to genetic variants in the keratin 5 and 14 genes. However, with the rapid advances in sequencing technology, genetic analysis may soon become an equally fast and less invasive method, which has the additional advantage to precisely characterize the genetic variant and mode of inheritance ("precision medicine").

To our knowledge, this is the first report of a spontaneously arisen loss of function allele in the *KRT14* gene in a domestic animal with EBS, and the first report of feline epidermolysis bullosa simplex.

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Conflicts of interest statement

The authors have declared no competing interests.

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Figures:

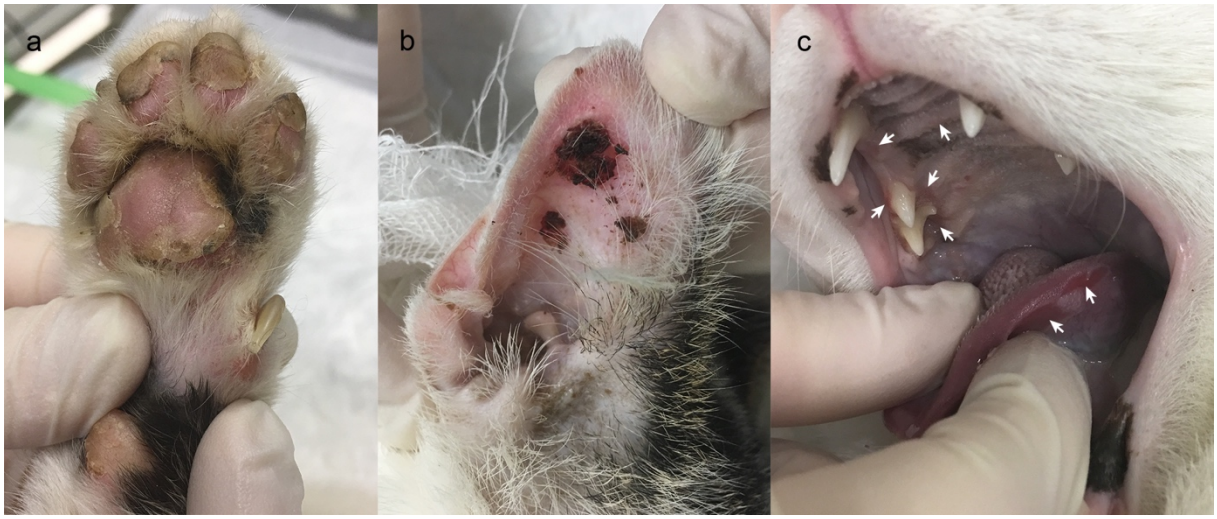

Figure 1:

Photographs of skin and oral lesions in a domestic shorthair cat with epidermolysis bullosa simplex.

a) All paw pads were covered with dry layers of sloughed epidermis, while being re-epithelialized underneath.
b) The inner aspect of the pinnae displayed multifocal ulceration and crusts.
c) In the oral cavity, multifocal ulcerations were evident at the gums, the hard palate and the lower aspect of the tongue (white arrows).

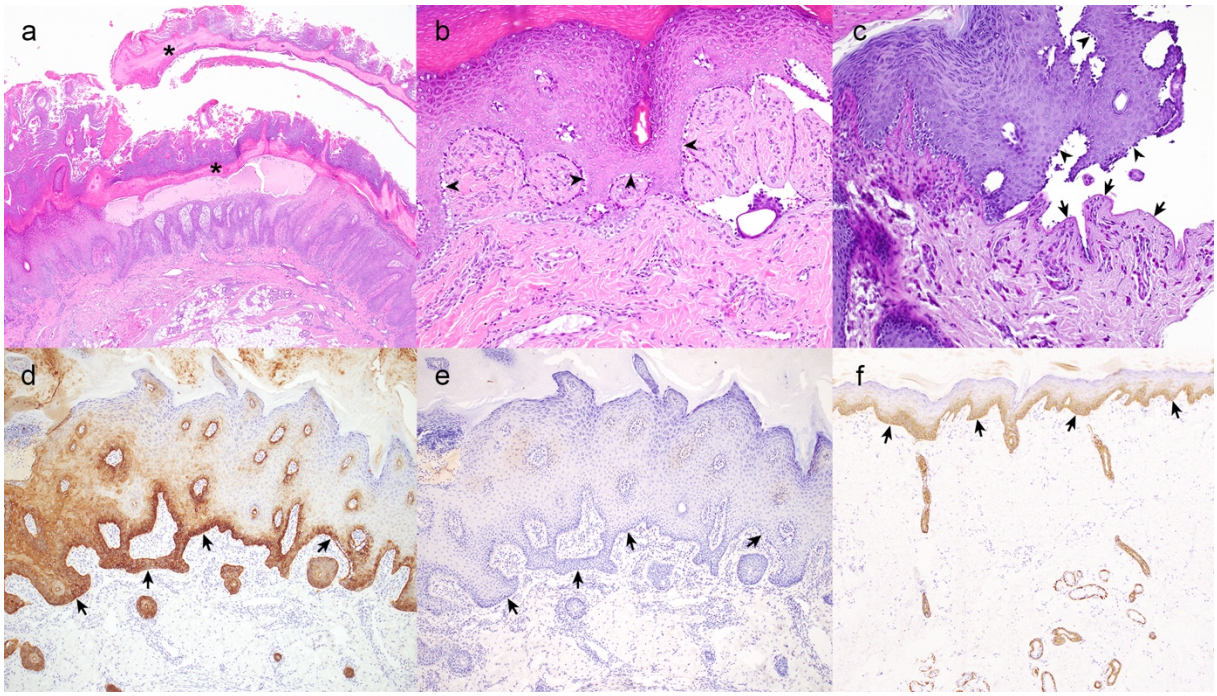

Figure 2:

Histopathological and immunohistochemical findings in the paw pad skin from a domestic shorthair cat with epidermolysis bullosa simplex (EBS) (a-e), and an unrelated domestic shorthair cat euthanized due to non-skin disease (control) (f).

a) The paw pad epidermis is covered by two layers of sloughed necrotic epidermis (asterisk). In the center, there is re-epithelialization underneath a blister evident. Hematoxylin and eosin, 4x.

b) The basal keratinocytes of the paw pad epidermis show vacuolation and loss of adherence to the underlying tissues (arrow heads). In the right parts of the photo, a full-blown blister is evident. Hematoxylin and eosin, 20x.

c) The blister floor is formed by the basement membrane (arrows), while the roof is built by shrunken basal keratinocytes, appearing like a row of teeth (arrow heads). Period acid Schiff reaction, 20x.

d) IHC for keratin 5 and 6 shows a basal to transepidermal protein expression. Positive signal is also evident in the adnexal gland epithelium in the dermis. Arrows indicate the unstained basement membrane of the epidermis. Anti-

keratin 5/6 immunohistochemistry with hematoxylin counterstaining, 10x.

e) IHC for keratin 14 shows complete absence of signal in the paw pad epidermis and the adnexal gland epithelium. Arrows indicate the unstained basement membrane of the epidermis. Anti-keratin 14 immunohistochemistry with hematoxylin counterstaining, 10x.

f) IHC for keratin 14 in normal control feline paw pad tissue shows basal epidermal and glandular protein expression. Arrows indicate the unstained basement membrane of the epidermis. Note also the significantly reduced thickness of epidermis in the normal paw pad compared to the EBS-affected paw pad. Anti-keratin 14 immunohistochemistry with hematoxylin counterstaining, 10x.