

A Spontaneous *KRT16* Mutation in a Dog Breed: a Model for Human Focal Non-Epidermolytic Palmoplantar Keratoderma (FNEPPK)

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TO THE EDITOR

The keratin 16 gene (*KRT16*) encodes an intermediate filament protein mainly expressed in palmoplantar epidermis. In humans, mutations in *KRT16* are responsible for pachyonychia congenita and focal non-epidermolytic palmoplantar keratoderma (FNEPPK; Smith *et al.*, 2000; McLean and Moore, 2011). One of the main symptoms is a painful thickening of the palms and soles. To understand molecular mechanisms involved in this keratoderma, Krt16 mutant mouse models have been developed, but only one reproduces fully the palmoplantar phenotype (Lessard and Coulombe, 2012). In this study, we present a spontaneous canine model of FNEPPK inherited as an autosomal recessive disorder in the Dogue de Bordeaux breed. Because of its population structure, which features genetic isolates, the purebred dog model has recently proven its utility in understanding the molecular mechanisms of hereditary cornification disorders, notably in humans and dog Autosomal Recessive Congenital Ichthyosis (Grall *et al.*, 2012).

We investigated a family of 130 dogs including 28 affected animals; no sex bias was observed among the 13 males and 15 females analyzed. The onset usually occurred between 10 weeks and 1 year of age. First described by Paradis (1992), affected dogs exhibit a painful thickening of the footpads with severe keratinous proliferations and fissures only at the ground contact locations similar to those observed in FNEPPK patients (Figure 1). Cracks predispose the dogs to secondary infections, leading to lameness, causing the dog to be reluctant to walk. Nails did not seem to be affected, as reported in

some human FNEPPK patients and in Krt16-null mice models (Shamsher *et al.*, 1995; Smith *et al.*, 2000; Liao *et al.*, 2007; Lessard and Coulombe, 2012). Similarly, no other cutaneous sign such as oral leukoplakia, cysts, or follicular keratosis was reported. This is concordant with our results of quantitative reverse transcription PCR of messengerRNA from unaffected dog biopsies, showing strong and specific expression of *KRT16* in the footpad, nose, and keratinocytes but not in body skin, oral mucosa, or other organs (data not shown).

Histopathological examinations of footpad biopsies revealed thick hyperkeratotic digital epidermis that was roughened by marked conical papillae with a prominent “church spire” appearance (Figure 1e). On the top of this, there is a thin compact column of parakeratotic cells with absent or decreased underlying granular layer and cytoplasmic vacuolization of superficial corneocytes at their base (Figure 1f and g). Outside the conical papillae, the epidermis exhibited a well-developed granular layer and compact orthohyperkeratosis. Between the conical papillae, small valleys were observed that presented dyskeratosis, an irregular and prominent granular layer, and light to moderate keratotic plugging (Figure 1h). No epidermolytic changes were noticed. Chronic superficial perivascular dermal infiltrate was sometimes observed.

Immunohistochemistry and immunofluorescence staining were performed on FFPE skin biopsies from four affected and four unaffected dogs. The Ki67 proliferation index showed that, as expected, keratinocytes in affected footpads were not proliferating. Expression

of keratin 1, 6, and 16 was investigated in normal footpad biopsies (Figure 2). As previously described (Bowden *et al.*, 2009), keratin 1, 6, and 16 are co-expressed in the suprabasal layer of the footpad epidermis, with K16 located in the center of the rete ridges. No differences were observed in the expression of K1 and K6 between cases and controls. However, immunostaining revealed an abnormal distribution of K16 in affected samples: although K16 expression was diffuse and suprabasal all over the thickness of the epithelial layer of control dogs, its expression was not detected in affected samples (Figure 2). In affected dogs, discrete aggregates of K16 could be observed in the cornified layer in footpads not in contact with the ground (Supplementary Figure S1a online).

In parallel, we performed a genetic linkage study on the Dogue de Bordeaux family using 14 affected and 54 unaffected dogs genotyped on the canine 173,000 SNP array. We identified a 20 Mb locus corresponding to the dog type I keratin cluster. We carried out mutation screening on several keratins in 14 affected dogs and 16 controls and identified a complex mutation in *KRT16* corresponding to an insertion/deletion (indel) of four nucleotides and a separate 1 bp deletion 15 nucleotides downstream in exon 6 (Supplementary Figure S2 online). This complex indel results in an insertion of 1 bp in affected dogs and introduces a frameshift changing the sequence of 10 amino acids and creating a premature stop codon (p.Glu392*) in the open reading frame of the gene. This stop codon located in the 2B domain leads to the loss of the last 85 amino acids of K16, including the helix termination motif

Q3

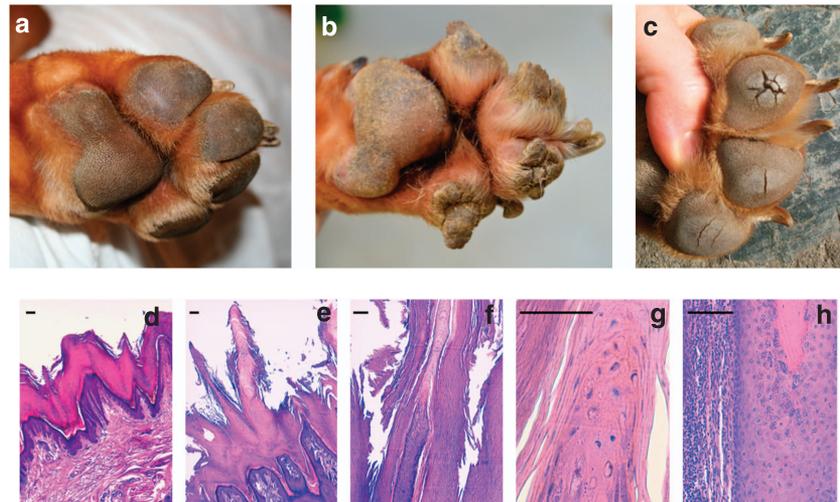


Figure 1. Clinical phenotype and histopathological findings of footpad keratoderma in the Dogue de Bordeaux. (a) Footpad of a 3-year-old unaffected dog. (b) Footpad of an 8-year old and (c) a 1-year old affected dog. (d-h) Hematoxylin and eosin (HE) staining of footpad biopsies. (d) Unaffected dog (scale bar = 100 μm). (e-h) Footpad of an 8-year-old affected dog. (e) Thick hyperkeratotic digital epidermis (scale bar = 100 μm); (f) Thin compact column of parakeratotic cells (scale bar = 100 μm); (g) Higher magnification reveals a thinner or even absent granular layer (scale bar = 50 μm). (h) Irregular prominent granular layer, dyskeratosis, and light keratotic plugging at the bottom of two epidermal valleys (scale bar = 100 μm).

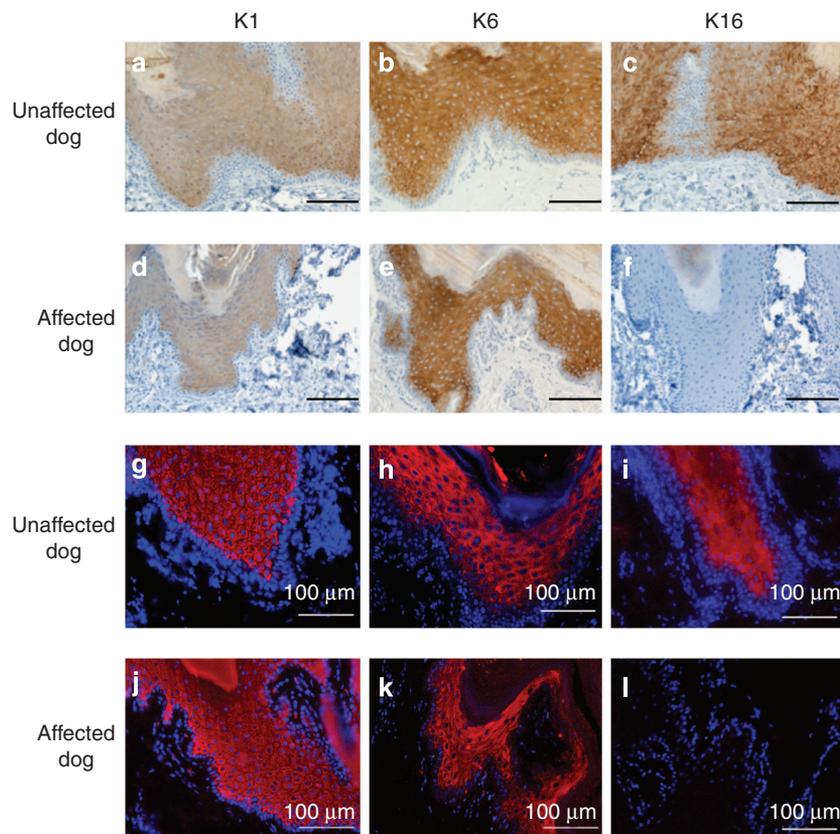


Figure 2. Immunoperoxidase and immunofluorescence staining of unaffected and affected footpad biopsies with Pyl-K1, Pyl-K6, and Pyl-K16. Keratin 1 (a, d, g, j) and keratin 6 (b, e, h, k) have an expected diffuse and suprabasal expression in the epidermis in both in unaffected and affected dogs. (c, i) For keratin 16, a diffuse and suprabasal expression is observed in the epidermis in unaffected dogs (f, l), whereas keratin 16 is weakly or not detected in the epidermis of affected dogs. scale bar = 100 μm.

(Supplementary Figure S2 online). This sequence is the most highly conserved motif in keratins involved in the end-to-

end interactions in keratin assembly. Mutations removing or changing the sequence of the tail domain leading to

the production of truncated or mutated keratins have already been described for keratin 10 in humans and dog patients

(Credille *et al.*, 2005; Müller *et al.*, 2006; Gutierrez *et al.*, 2013) and for *KRT16* in human patients (Smith *et al.*, 2000). It has been suggested that the disruption of the filament assembly could potentially lead to an abnormal distribution of the protein and the creation of aggregates (Smith *et al.*, 2000; Müller *et al.*, 2006) similar to what we observed in Dogues de Bordeaux (Supplementary Figure S1a online).

To confirm that this mutation is causative and specific to the Dogue de Bordeaux, we sequenced a set of 334 Dogues de Bordeaux with known clinical status. All affected dogs were homozygous for the complex mutation, and all unaffected dogs were either homozygous for the wild-type alleles or heterozygous (245/306 and 61/306, respectively). Furthermore, the mutated allele was never detected in a panel of 344 unaffected dogs from 80 different breeds.

Interestingly, heterozygous dogs do not present symptoms that is similar to the situation observed in Krt16-null mice (Lessard and Coulombe, 2012). This feature is also observed in other mutated keratin recessive disorders, such as the epidermolytic palmoplantar keratoderma *Krt9*^{-/-} mouse model (Fu *et al.*, 2014), *KRT10* mutations in humans and dog epidermolytic hyperkeratosis (Credille *et al.*, 2005; Müller *et al.*, 2006; Gutierrez *et al.*, 2013), or *KRT14* mutations in epidermolysis bullosa simplex patients (Titeux *et al.*, 2011). These examples of rare recessive forms show that, in the carrier population, the healthy allele could compensate for the loss of expression of the mutated allele, suggesting a mechanism different from haploinsufficiency usually reported in dominant inheritance.

qRT-PCR analysis of *KRT16* mRNA expression demonstrated a strong reduction (80%) in affected footpads (Supplementary Figure S1b online). This observation is concordant with the absence of the K16 protein as observed by immunostaining (Figure 2). As suggested by other studies, loss of one keratin alters the expression patterns of other plantar keratins, notably in response to skin injury (DePianto and

Coulombe, 2003; Lessard *et al.*, 2013). These keratins could form an atypical heterodimer between the partner of the mutated keratin (here, K6a), and other type I keratins, leading to the hyperproliferative phenotype (Müller *et al.*, 2006). In the Dogue de Bordeaux keratoderma, *KRT14* mRNA levels and K6 distribution, as observed by immunostaining, were not significantly altered and other keratins are under investigation.

Spontaneous models such as dog breeds that fully reproduce human diseases prove useful in understanding the genetics and the physiopathology of these diseases and can lead to the development of efficient new therapies.

ETHICS STATEMENT

The work with dog samples was approved by the CNRS ethical board, France (35-238-13), for UMR 6290. Blood and tissue biopsy samples and pedigrees from dogs were collected by a network of veterinarians through the CaniDNA biobank developed by CNRS in 2000 (<http://dog-genetics.genouest.org>). The dog owners consented to the use of data for research purposes anonymously.

CONFLICT OF INTEREST

The authors CK, CDdC, AT, and GQ are employees of Antagene, a private company selling diagnostic tests in dogs. The remaining authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

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