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SHORT COMMUNICATION

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KRT5 in-frame deletion in a family of German Shepherd dogs with split paw pad disease resembling localized epidermolysis bullosa simplex in human patients

Abstract

Split paw pad disease is a scarcely defined phenotype characterized by skin lesions on the paw pads of dogs. We studied a family of German Shepherd dogs, in which four dogs developed intermittent paw pad lesions and lameness. The paw pads of two of the affected dogs were biopsied and demonstrated cleft formation in the stratum spinosum and stratum corneum, the outermost layers of the epidermis. Whole genome sequencing data from an affected dog revealed a private heterozygous 18 bp in frame deletion in the *KRT5* gene. The deletion NM_001346035.1:c.988_1005del or NP_001332964.1:p.(Asn330_Asp335del) is predicted to lead to a loss of six amino acids in the L12 linker domain of the encoded keratin 5. *KRT5* variants in human patients lead to various subtypes of epidermolysis bullosa simplex (EBS). Localized EBS is the mildest of the *KRT5*-related human diseases and may be caused by variants affecting the L12 linker domain of keratin 5. We therefore think that the detected *KRT5* deletion in dogs represents a candidate causal variant for the observed skin lesions in dogs. However, while the clinical phenotype of *KRT5*-mutant dogs of this study closely resembles human patients with localized EBS, there are differences in the histopathology. EBS is defined by cleft formation within the basal layer of the epidermis while the cleft formation in the dogs described herein occurred in the outermost layers, a hallmark of split paw pad disease. Our study provides a basis for further studies into the exact relation of split paw pad disease and EBS.

Keratins form the largest subgroup of intermediate filaments in vertebrates (Gu & Coulombe, 2007). Humans and dogs possess 55 and 61 functional keratin genes, respectively. Over half of all keratin genes are expressed in adult skin (Balmer et al., 2017; Wang et al., 2016). They are classified into type I for acidic and type II for basic to neutral keratins (Moll et al., 2008). Intermediate filaments share common structural elements comprising a central coiled-coil rod domain with α-helical confirmation and a non-helical head and tail domain. The central rod is divided into subdomains 1A, 1B, 2A, and 2B, which are connected by three linkers named L1, L12, and L2 (Parry et al., 2007). Type I and type II keratin monomers form a heterodimer by antiparallel alignment of their central rod domains. The resulting filaments play a major role in providing mechanical stability to skin (Gu & Coulombe, 2007). Keratinocytes in the basal epidermal layers predominantly express KRT5 (type I) and KRT14 (type II). They form a heterodimer mainly present in the form of loose filament bundles (Wang et al., 2016). The resulting keratin filament network is connected to hemidesmosomes, which attach the basal keratinocytes to the underlying basement membrane (Walko et al., 2015). Genetic variants in *KRT5* and *KRT14* are known to represent the most frequent cause of epidermolysis bullosa simplex (EBS) in humans and animals alike (Bergson et al., 2023; Dettwiler et al., 2020; Kiener et al., 2022; Lane et al., 1992). EBS is the most widespread type of epidermolysis bullosa (Has et al., 2020). The disease is defined by skin blistering within the basal layer of the epidermis due to a higher skin fragility and reduced resilience to mechanical stress. Depending on the severity and localization of the lesions, three subtypes of EBS are defined: localized, intermediate, and severe (Has et al., 2020). EBS is mostly inherited as an autosomal dominant trait (Coulombe et al., 2009). In dogs, so far one case of EBS associated with a *KRT5*:p.E476K missense variant has been described in a Welsh Corgi with skin blistering and erosions on the paw pads, lips, and oral mucosa (Kiener et al., 2022; OMIA 002081-9615). The objective of this study was to investigate clinically, pathologically, and genetically four closely related German Shepherd dogs with skin lesions on their paw pads (Table S1).

A general and dermatologic case history for cases 1–4 was gathered by contacting the general practitioners and

owners via phone by a veterinary dermatological specialist (U.M.) or resident (A.L.).

All affected dogs had their first clinical signs in young adulthood (age 6–12 months) with one or more paws being affected with intermittent, painful lesions on the paw pads leading to lameness (Figure 1a). The clinical signs resembled descriptions of split paw pad disease in the literature (Gross et al., 2005; Miller et al., 2012). In three of the four dogs, the lameness was the first clinical sign before the skin of the paw pads was macroscopically affected with visible erosions. Case 2 had to be euthanized due to bad quality of life. The other three affected dogs showed milder signs of skin detachment on the paw pads that were often noted after walks.

Biopsies taken from cases 1 and 2 were diagnosed compatible with 'split paw pad disease' by two different commercial laboratory pathologists. Recuts were reviewed by one of the authors (S.S.). Histopathological examination revealed a focal-extensive to complete split/cleft in the epidermal stratum spinosum and stratum corneum, respectively (Figure 1b). In one of the dogs, the split was associated with coagulative necrosis of the epidermis, while in the other it was filled with abundant fibrin, some necrotic debris, and some degenerated neutrophils. Prominent epidermal hyperplasia and hyperkeratosis were also present. One dog presented ulcerated areas with associated superficial dermal neutrophilic infiltrates, while the dermis of the other dog presented in the upper dermis some lymphocytes and plasma cells and rare neutrophils.

A relative to the four described cases presented with healing paw pad lesions to one of the authors (N.T.). A subsequent histopathological examination of a biopsy showed different findings compared to cases 1 and 2. This dog presented irregular epidermal hyperplasia with parakeratotic peaks, and scattered vacuolization and apoptosis of the keratinocytes in the basal cell layer. We therefore hypothesized that this dog was affected by a clinically related, yet different disease.

EDTA blood samples for DNA isolation were obtained from 36 German Shepherd dogs including the four related cases. The genome of case 1 was sequenced at 25.3× coverage on an Illumina Novaseq 6000 instrument. Mapping to the UU_Cfam_GSD_1.0 reference genome assembly and variant calling were performed as described (Jagannathan et al., 2019). Comparison to 1498 control genomes yielded 735 heterozygous and 92 homozygous private variants (Tables S2 and S3). Nine of these variants were predicted to change an encoded protein, eight heterozygous and one hemizygous on the X-chromosome. Only one of these variants was located in a functional candidate gene for a genodermatosis. This was a heterozygous 18-bp deletion in the coding sequence of *KRT5*, a known candidate gene for EBS (Figure 2a). The variant can be designated as chr27:44081942_44091959del (UU_Cfam_GSD_1.0) or NM_001346035.1:c.988_1005del. It is located in the fifth of the nine exons of *KRT5*. The in-frame deletion is predicted to lead to a loss of six amino acids in the encoded protein, NP_001332964.1:p.(Asn330_Asp335del), which are located in the L12 linker domain of the KRT5 protein.

Additional dogs were genotyped by fragment size analysis of PCR amplicons generated with primers GGTACCAGGACTCAGCTTCC and GAATGCCTGACTTCAAATCTCC followed by fragment size analysis on a 5200 Fragment Analyzer system (Figure 2b). All four affected dogs carried the deletion in a heterozygous state. Unexpectedly, the dam of case 1, which had been reported as unaffected by the owner, also carried the deletion (Figure 2c). This dog was not clinically investigated by a veterinarian. This indicates that the clinical phenotype of dogs carrying one copy of the deletion allele might be variable. Based on the limited available data, we cannot rule out incomplete penetrance in some dogs with the heterozygous genotype. This could explain why an allele potentially causing a dominant and early-onset hereditary disease has been propagated over several generations and several litters in the German Shepherd dog population.

The deletion was absent from another 11 close relatives, the related dog described above with clinically similar, but histopathologically different paw pad lesions, and 15 unrelated German Shepherd dogs.

In human EBS patients, the localization of pathogenic variants in the *KRT5* gene correlates with the clinical severity of the phenotype. The most severe phenotypes were described for variants affecting the helix boundary motifs located at the beginning and end of the central rod domain. Variants affecting other regions of the KRT5 protein tend to result in less severe phenotypes of

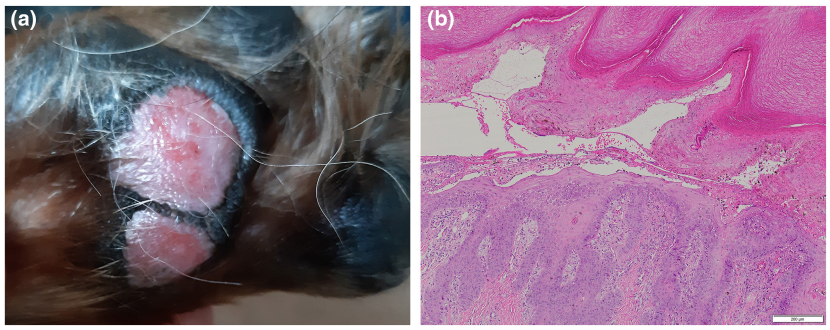

FIGURE 1 Clinical and histopathological phenotype of investigated German Shepherd dogs. (a) Ulcerated lesion on the main paw pad of case 1. (b) The histopathological examination revealed clefting in the stratum spinosum of the epidermis. The cleft is partly filled with fibrin and neutrophils.

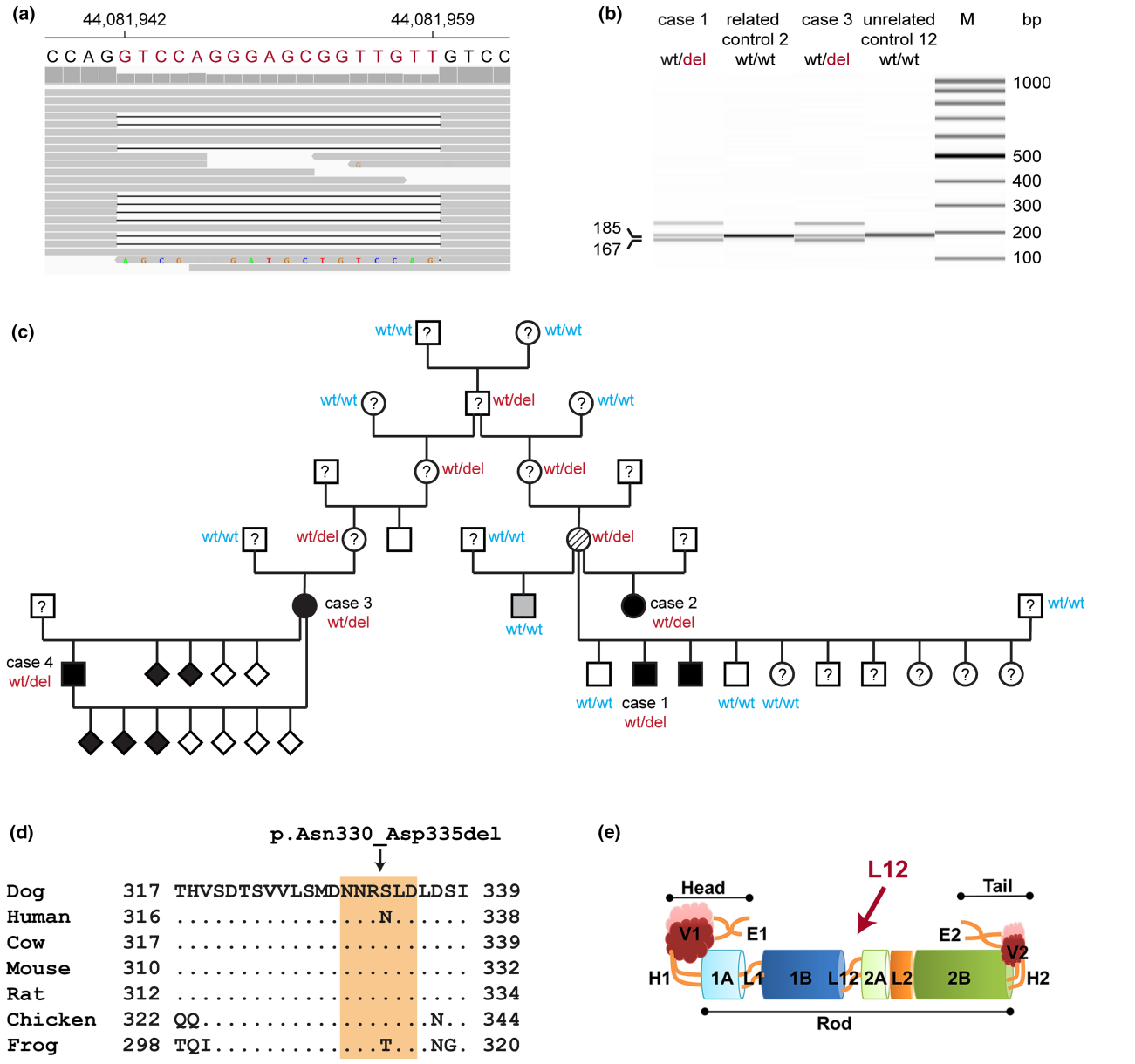

FIGURE 2 Details of the *KRT5*:c.988_1005del variant. (a) Integrative Genomics Viewer screenshot of the whole genome sequence data of case 1 showing the heterozygous deletion. Coordinates refer to chromosome 27 of the UU_Cfam_GSD_1.0 assembly. The *KRT5* gene is transcribed in reverse orientation with respect to the reference genome. (b) Genotyping assay for the 18-bp deletion by fragment size analysis. The wildtype amplicon is 185 bp, the deletion allele is 167 bp. The shorter 167-bp DNA fragment resulting from the *KRT5* deletion is visible in two heterozygous dogs. The additional third band in heterozygous dogs probably represents heteroduplex molecules. (c) Pedigree of dogs with split paw pad disease. Dogs with a diagnosis of split paw pad disease are indicated with solid black symbols. One dog with paw pad lesions that clinically resembled split paw pad disease, but had a different histopathological phenotype, provisionally classified as psorisiaform pododermatitis, is indicated with a gray symbol. The dam of cases 1 and 2 was reported as clinically unaffected by her owner and is indicated with a dashed symbol. Archived samples of ancestors were genotyped in order to identify the presumable founder of the mutant *KRT5* allele. Their phenotypes are unknown and indicated with question marks. Genotypes at *KRT5*:c.988_1005del are indicated for all dogs, from which a DNA sample was available. (d) Multiple species alignment of the L12 linker subdomain of the KRT5 protein. The six amino acids that are predicted to be deleted in affected dogs are highlighted. (e) Schematic representation of the rod domain organization in a keratin heterodimer (modified from Bray et al., 2015). The L12 linker subdomain comprises one of the few non-helical regions of the rod domain, where KRT5 and KRT14 do not form a coiled coil structure. This region is responsible for introducing a kink in the otherwise linear KRT5/KRT14 heterodimers (Bray et al., 2015) and may therefore be slightly more tolerant for variants than the rigid coiled-coil regions.

intermediate or localized EBS (Coulombe & Lee, 2012; Lane & McLean, 2004; Lee et al., 2012). Patients in two unrelated human families carrying missense variants affecting the L12 domain of KRT5 have been reported with mild palmar and plantar blistering (Chan et al., 1994). This is in accordance with the observed clinical signs limited to the paw pads of the German Shepherd dogs described in this study.

The histopathological features of cases 1 and 2 carrying the deletion confirm the existence of an intraepidermal split/cleft, as expected for an EBS. However, the split/cleft is not located in the epidermal basal layer, as would be expected for EBS, but in the overlying stratum spinosum/corneum, reminding of the poorly characterized 'split paw pad disease', which has been suggested to represent a cornification disorder (Gross et al., 2005). Although *KRT5* is typically expressed in the epidermal basal layer (Wang et al., 2016), the disease phenotype found in these dogs suggest that a *KRT5* defect in dogs may also be associated with cleft formation in upper parts of the epidermis.

A possible explanation for the histopathological differences between the dogs described in this study and human patients with *KRT5*-related localized EBS might be that dogs have a less restricted expression of KRT5, which may extend beyond the basal layer of the epidermis leading to splitting within outer layers. Alternatively, the impact of the *KRT5* in-frame deletion on the basal layer of the epidermis may extend to the superficial layers as a result of a disrupted cornification process. Additional research is required to clarify the function of KRT5 in different layers of the canine epidermis and its role for the integrity and homeostasis of healthy skin.

In conclusion, we identify a *KRT5* in-frame deletion as a candidate causal variant for intermittent paw pad lesions in German Shepherd dogs with clinical similarities to human-localized EBS. Based on the sample archive of the breed club, we were able to confirm the de novo mutation event giving rise to the *KRT5*:c.988_1005del allele. The presumed founder animal is a common ancestor of all known cases and was born in December 2003. Our findings enable genetic testing to elucidate the penetrance of the mutant *KRT5* allele and to determine how far it is distributed in the German Shepherd dog population. The exact relation between split paw pad disease in dogs, which is characterized by cleft formation in higher layers of the epidermis and EBS with cleft formation at the basal layer remains unclear and warrants further investigation.

KEYWORDS

Canis lupus familiaris, dermatology, dog, keratin, precision medicine, skin

AUTHOR CONTRIBUTIONS

Stefan J. Rietmann: Investigation; visualization; writing – original draft; writing – review and editing. **Anja Lange:** Investigation; visualization; writing – original draft; writing – review and editing. **Sara Soto:** Investigation; visualization; writing – original draft; writing – review and editing. **Nina Thom:** Investigation; writing – review and editing. **Eberhard Manz:** Investigation; resources; writing – review and editing. **Vidhya Jagannathan:** Data curation; writing – review and editing. **Ursula Mayer:** Conceptualization; investigation; supervision; writing – original draft; writing – review and editing. **Tosso Leeb:** Conceptualization; funding acquisition; project administration; supervision; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

E.M. is affiliated with a commercial laboratory marketing genetic tests. The other authors declare no conflicts of interests.

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ETHICS STATEMENT

The dogs in this study were privately owned and samples were collected with the consent of their owners. The collection of blood samples from control dogs was approved by the 'Cantonal Committee For Animal Experiments' (Canton of Bern; permit 94/2022; Approval date: 30-11-2022). The collection of samples from affected dogs was performed for diagnostic or therapeutic reasons and did not constitute an animal experiment in the legal sense.

DATA AVAILABILITY STATEMENT

All data are freely available. Accessions for the whole genome sequence data are given in Table S2.

Stefan J. Rietmann[1,2]
Anja Lange[3]
Sara Soto[2,4]
Nina Thom[5]
Eberhard Manz[6]
Vidhya Jagannathan[1]
Ursula Mayer[3]
Tosso Leeb[1,2]

[1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[2]*DermFocus, University of Bern, Bern, Switzerland*
[3]*Anicura Kleintierspezialisten Augsburg GmbH, Augsburg, Germany*
[4]*Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[5]*Small Animal Clinic, Justus-Liebig-University of Giessen, Giessen, Germany*
[6]*Generatio GmbH, Heidelberg, Germany*

Correspondence
Tosso Leeb, Institute of Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland.
Email: tosso.leeb@unibe.ch

Stefan J. Rietmann and Anja Lange contributed equally to this study.
Ursula Mayer and Tosso Leeb contributed equally to this study.

ORCID

Stefan J. Rietmann https://orcid.org/0009-0004-0932-552X
Anja Lange https://orcid.org/0009-0000-7785-9372
Sara Soto https://orcid.org/0000-0003-3777-8724
Eberhard Manz https://orcid.org/0000-0003-1445-0486
Vidhya Jagannathan https://orcid.org/0000-0002-8155-0041
Ursula Mayer https://orcid.org/0000-0001-9028-7604
Tosso Leeb https://orcid.org/0000-0003-0553-4880

REFERENCES

Balmer, P., Bauer, A., Pujar, S., McGarvey, K.M., Welle, M., Galichet, A. et al. (2017) A curated catalog of canine and equine keratin genes. *PLoS One*, 12, e0180359. Available from: https://doi.org/10.1371/journal.pone.0180359

Bergson, S., Daniely, D., Bomze, D., Mohamad, J., Malovitski, K., Meijers, O. et al. (2023) Clinical and molecular features in a cohort of middle eastern patients with epidermolysis bullosa. *Pediatric Dermatology*, 40, 1021–1027. Available from: https://doi.org/10.1111/PDE.15440

Bray, D.J., Walsh, T.R., Noro, M.G. & Notman, R. (2015) Complete structure of an epithelial keratin dimer: implications for intermediate filament assembly. *PLoS One*, 10, e0132706. Available from: https://doi.org/10.1371/journal.pone.0132706

Chan, Y.M., Yu, Q.C., LeBlanc-Straceski, J., Christiano, A., Pulkkinen, L., Kucherlapati, R.S. et al. (1994) Mutations in the non-helical linker segment L1-2 of keratin 5 in patients with weber-Cockayne epidermolysis bullosa simplex. *Journal of Cell Science*, 107, 765–774. Available from: https://doi.org/10.1242/JCS.107.4.765

Coulombe, P.A., Kerns, M.L. & Fuchs, E. (2009) Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility. *The Journal of Clinical Investigation*, 119, 1784–1793. Available from: https://doi.org/10.1172/JCI38177

Coulombe, P.A. & Lee, C.H. (2012) Defining keratin protein function in skin epithelia: epidermolysis bullosa simplex and its aftermath. *The Journal of Clinical Investigation*, 132, 763–775. Available from: https://doi.org/10.1038/JID.2011.450

Dettwiler, M., Leuthard, F., Bauer, A., Jagannathan, V., Lourenço, A.M., Pereira, H. et al. (2020) A nonsense variant in the KRT14 gene in a domestic shorthair cat with epidermolysis bullosa simplex. *Animal Genetics*, 51, 829–832. Available from: https://doi.org/10.1111/AGE.12979

Gross, T.L., Ihrke, P.J., Waldner, E.J. & Affolter, V.K. (2005) *Skin diseases of the dog and cat: clinical and histopathological diagnosis*, 2nd edition, Oxford: Blackwell Science Ltd. Available from: https://doi.org/10.1002/9780470752487

Gu, L.H. & Coulombe, P.A. (2007) Keratin function in skin epithelia: a broadening palette with surprising shades. *Current Opinion in Cell Biology*, 19, 13–23. Available from: https://doi.org/10.1016/J.CEB.2006.12.007

Has, C., Bauer, J.W., Bodemer, C., Bolling, M.C., Bruckner-Tuderman, L., Diem, A. et al. (2020) Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility. *British Journal of Dermatology*, 183, 614–627. Available from: https://doi.org/10.1111/BJD.18921

Jagannathan, V., Drögemüller, C., Leeb, T., Aguirre, G., André, C. et al. (2019) A comprehensive biomedical variant catalogue based on whole genome sequences of 582 dogs and eight wolves. *Animal Genetics*, 50, 695–704. Available from: https://doi.org/10.1111/AGE.12834

Kiener, S., Mauldin, E.A., Jagannathan, V., Casal, M.L. & Leeb, T. (2022) KRT5 missense variant in a Cardigan Welsh corgi with epidermolysis bullosa simplex. *Animal Genetics*, 53, 892–896. Available from: https://doi.org/10.1111/AGE.13257

Lane, E.B. & McLean, W.H.I. (2004) Keratins and skin disorders. *Journal of Pathology*, 204, 355–366. Available from: https://doi.org/10.1002/PATH.1643

Lane, E.B., Rugg, E.L., Navsaria, H., Leigh, I.M., Heagerty, A.H.M., Ishida-Yamamoto, A. et al. (1992) A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature*, 356, 244–246. Available from: https://doi.org/10.1038/356244a0

Lee, C.H., Kim, M.S., Chung, B.M., Leahy, D.J. & Coulombe, P.A. (2012) Structural basis for heteromeric assembly and perinuclear organization of keratin filaments. *Nature Structural and Molecular Biology*, 19, 707–715. Available from: https://doi.org/10.1038/nsmb.2330

Miller, W.H., Griffin, C.E. & Campbell, K.L. (2012) *Muller and Kirk's small animal dermatology*, 7th edition, St. Louis: Saunders.

Moll, R., Divo, M. & Langbein, L. (2008) The human keratins: biology and pathology. *Histochemistry and Cell Biology*, 129, 705–733. Available from: https://doi.org/10.1007/S00418-008-0435-6

Parry, D.A.D., Strelkov, S.V., Burkhard, P., Aebi, U. & Herrmann, H. (2007) Towards a molecular description of intermediate filament structure and assembly. *Experimental Cell Research*, 313, 2204–2216. Available from: https://doi.org/10.1016/J.YEXCR.2007.04.009

Walko, G., Castañón, M.J. & Wiche, G. (2015) Molecular architecture and function of the hemidesmosome. *Cell and Tissue Research*, 360, 529–544. Available from: https://doi.org/10.1007/S00441-015-2216-6

Wang, F., Zieman, A. & Coulombe, P.A. (2016) Skin keratins. *Methods in Enzymology*, 568, 303–350. Available from: https://doi.org/10.1016/BS.MIE.2015.09.032

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