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SHORT COMMUNICATION

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SDR9C7 missense variant in a Chihuahua with non-epidermolytic ichthyosis

Abstract

Ichthyoses represent a heterogeneous group of cornification disorders that are associated with skin barrier defects. We investigated a 9-month-old Chihuahua showing excessive scale formation. Clinical and histopathological examinations revealed non-epidermolytic ichthyosis and a genetic defect was suspected. We therefore sequenced the genome of the affected dog and compared the data with 564 genetically diverse control genomes. Filtering for private variants identified a homozygous missense variant in *SDR9C7*, c.454C>T or p.(Arg152Trp). *SDR9C7* is a known candidate gene for ichthyosis in humans and encodes the short-chain dehydrogenase/reductase family 9C member 7. The enzyme is involved in the production of a functional corneocyte lipid envelope (CLE), a crucial component of the epidermal barrier. Pathogenic variants in *SDR9C7* have been described in human patients with autosomal recessive ichthyosis. We assume that the identified missense variant in the affected Chihuahua of this study impairs the normal enzymatic activity of SDR9C7 and thus prevents the formation of a functioning CLE, resulting in a defective skin barrier. To the best of our knowledge, this is the first report of a spontaneous *SDR9C7* variant in domestic animals.

Ichthyoses represent a group of genetic skin disorders that are characterized by dry, thickened and scaly skin. Various forms of ichthyosis have primary causes associated with skin barrier function (Akiyama, 2017; Akiyama & Shimizu, 2008; Oji et al., 2010). The skin barrier is fundamental for protection from environmental insults and maintaining body hydration (Mauldin & Elias, 2021). During epidermal terminal differentiation transglutaminases crosslink protein products (e.g. loricrin, involucrin, envoplacin, periplacin, small proline-rich protein family) at the plasma membrane, resulting in the formation of the cornified cell envelope. Subsequently, the intercellular lipid bilayer, composed of ceramides, free fatty acids, cholesterol, proteases and antimicrobial peptides, forms. This lipid bilayer is connected to the cornified envelope by the corneocyte lipid envelope (CLE), which serves as a bond between these two structures. The CLE is a monolayer mainly composed of acylceramides of the EOS class (cerEOS), a combination of esterified ω-hydroxy ultra-long-chain fatty acids and sphingosines (Akiyama, 2021).

A defective or absent CLE constitutes a prime structural defect in many diseases with impaired skin barrier function (Akiyama, 2017; Elias et al., 2014). In human patients, several genes associated with either biosynthesis or the processing of ceramides that form the CLE have been described to cause different forms of ichthyosis (Akiyama, 2021; Crumrine et al., 2019). Variants in two of these genes, *PNPLA1* and *ABHD5*, have also been reported in dogs with ichthyosis (Grall et al., 2012; Kiener et al., 2022).

Next-generation sequencing technologies have seen huge advances in recent years with concomitant decreases in sequencing costs. Whole genome sequencing, with the ability to identify the underlying genetic defect of inherited diseases, has become more accessible in veterinary medicine (Leeb, Bannasch, et al., 2022a). Together with clinical and histopathological examinations, genetic investigations offer a unique opportunity for a relatively fast and low-invasivity precise diagnosis, which in turn enables a more accurate prognosis and potentially even targeted therapy (Leeb, Roosje, et al., 2022b; Park et al., 2022). The objective of this study was to clinically and histopathologically characterize a cornification disorder in a Chihuahua and to investigate a possible underlying genetic defect.

A 9-month-old Chihuahua was presented owing to progressive abnormal scale formation since adoption at 3 months of age (Figure 1). On the first visit, the dog had scales all over the haircoat, mild to moderate pruritus associated with mild erythema and malodorous skin. A trichogram did not show any *Demodex* spp. and a tape test showed various *Malassezia* yeasts. Antiparasitic treatment (Nexgard®, afoxolaner) and twice weekly shampoos (Sebolytic® Zinc gluconate and Malaseb®, miconazol) were prescribed. Fungal culture results were

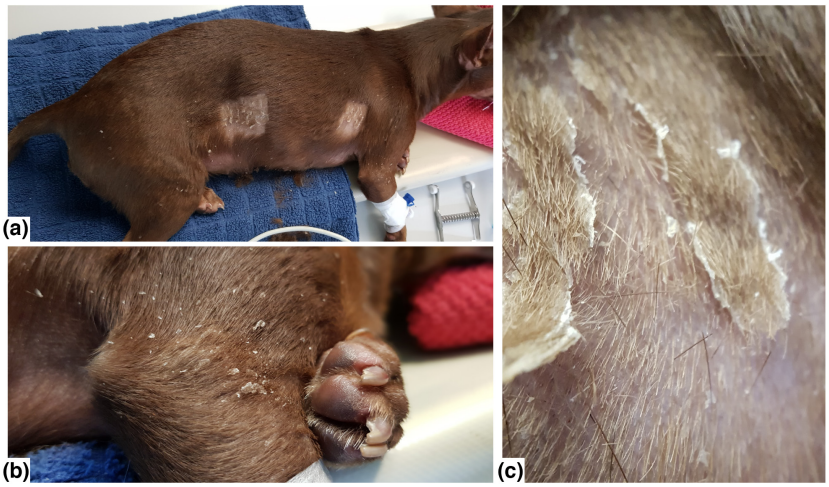

FIGURE 1 Clinical phenotype of the Chihuahua affected with non-epidermolytic ichthyosis. (a) The dog presented with generalized scale formation, clearly visible on the clipped areas. (b) Scales were thick and large and often adherent to the epidermis or the hair shafts. Paw pads and claws were normal. (c) Higher magnification of the clipped area. The scales were white-gray, thick and adhered to the hair coat. The edges were often elevated.

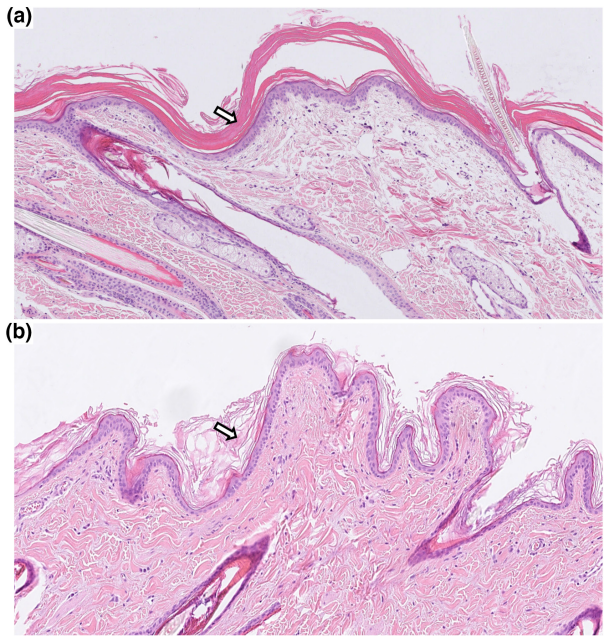

FIGURE 2 Histopathological findings. (a) Skin biopsy of the affected dog. The epidermis is covered by a thick layer of compact orthokeratotic keratin (arrow) which is extending into the follicular ostia. The dermis is mildly edematous, the lymph vessels are dilated and the number of mast cells is mildly increased in the superficial dermis. (b) Skin biopsy of a control Chihuahua. The epidermis is covered by basket-weave orthokeratotic keratin (arrow) which represents the normal stratum corneum.

negative for dermatophytes and positive for *Malassezia pachydermatis* (numerous colonies). A recheck was made 1 month later, when pruritus and erythema were absent, but little improvement was noticed concerning the scale formation.

Two 6 mm skin punch biopsies were taken under general anaesthesia and prepared for histopathological examination. In both biopsies, the epidermis was multifocally mildly hyperplastic and covered by a thick layer of mostly compact orthokeratotic keratin, which was multifocally detaching from the epidermis. The compact keratin was extending into the follicular ostia. In addition, the superficial dermis was oedematous with ectactic lymph vessels and a mildly increased number of mast cells (Figure 2). These findings together with the clinical history led to the diagnosis of a non-epidermolytic ichthyosis.

Given the clinical and histopathological findings together with the early age of the onset, an underlying genetic defect was suspected. We therefore took EDTA blood samples from the affected dog and its unaffected full sibling and extracted genomic DNA with the Maxwell RSC Whole Blood DNA Kit using a Maxwell RSC instrument (Promega). The affected dog's genome was sequenced at 25× coverage on an Illumina Novaseq 6000 instrument. Mapping and variant calling with respect to the UU_Cfam_GSD_1.0 reference genome assembly were performed as described (Jagannathan et al., 2019). Comparing the sequencing data with 564 canine control genomes resulted in 143 heterozygous and eight homozygous private protein changing variants (Tables S1, S2). Among these was a homozygous missense variant in *SDR9C7*, which is a known functional candidate gene for ichthyosis (Shigehara et al., 2016). The single nucleotide variant, Chr10:1471341G>A (UU_Cfam_GSD_1.0) or XM_038549505.1:c.454C>T, is predicted to change a conserved arginine to a tryptophan, XP_038405433.1:p.(Arg152Trp), removing a positive charge from the surface of the protein (Figure S1). The amino acid exchange was categorized as deleterious by the variant impact predictors PROVEAN (Choi & Chan, 2015) and PREDICTSNP (Bendl et al., 2014).

The genomic variant was located in a ~14 Mb homozygous segment (Chr10:30014-13939246). Sanger sequencing confirmed the homozygous genotype in the affected dog. The unaffected brother carried the mutant allele in a heterozygous state and 38 control Chihuahuas from the Vetsuisse Biobank were all homozygous wildtype.

Only recently, *SDR9C7* has been identified as functional candidate gene for ichthyosis owing to its essential role in the formation of the CLE. Several causative variants in *SDR9C7* have been reported in

human patients with autosomal recessive congenital ichthyosis (ARCI13; OMIM #617574; Hotz et al., 2018; Karim et al., 2017; Mohamad et al., 2017; Mazereeuw-Hautier et al., 2019; Seidl-Philipp et al., 2019; Shigehara et al., 2016; Takeichi et al., 2017; Youssefian et al., 2019). The clinical features described in these human patients were characterized by silvery white to brownish scales covering the entire body and in some cases palmoplantar hyperkeratosis. In some cases, the severity of the phenotype decreased with age. Human patients with *SDR9C7* variants were described to frequently suffer from recurrent fungal infections and it was suggested that the dysfunctional skin barrier facilitates this type of infection (Takeichi et al., 2017).

The affected Chihuahua initially presented with a *Malassezia* dermatitis, which could be successfully controlled by antifungal topical therapy. The dermal edema and the increased number of mast cells in the superficial dermis were also compatible with an impaired skin barrier function.

SDR9C7 is encoding the short-chain dehydrogenase/reductase family 9C member 7, which is involved in the production of a functional CLE. The SDR9C7 enzyme generates a highly reactive epoxy-enone that facilitates covalent binding of oxidized acylceramide to cornified cell envelope proteins (Takeichi et al., 2020). Studies in human patients demonstrated that a missense variant, Arg276Cys, resulted either in lower transcription or in an unstable protein that was degraded rapidly in the differentiated keratinocytes (Takeichi et al., 2020). Expression of SDR9C7 in the skin of patients with another missense variant, Ile200Thr, was significantly decreased compared with normal skin (Shigehara et al., 2016). We therefore hypothesize that the identified missense variant in the affected Chihuahua, Arg152Trp, also impairs the proper enzymatic activity of SDR9C7 and thus prevents the formation of a functioning CLE, resulting in a defective skin barrier. Such defects are known to play an important role in the pathogenesis of various types of ichthyosis (Akiyama, 2017).

The unaffected brother of the affected Chihuahua carried the mutant allele in a heterozygous state and was clinically completely normal. These results are consistent with an autosomal recessive mode of inheritance and suggestive of a recent inbreeding event.

In conclusion, this study describes the clinical, histopathological and genetic details of a Chihuahua with ichthyosis. The identified homozygous missense variant in *SDR9C7* represents a plausible candidate causative variant. To the best of our knowledge, this is the first report of a spontaneous *SDR9C7* variant in a domestic animal.

KEYWORDS

animal model, *Canis lupus familiaris*, dermatology, dog, genodermatosis, precision medicine, skin, veterinary medicine

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CONFLICT OF INTEREST STATEMENT

The 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 71/19; approval date 9 September 2019). The collection of samples from the affected dog was performed for diagnostic or therapeutic reasons and did not constitute an animal experiment in the legal sense. The biopsy for the control dog also represented a diagnostic sample from the tissue archive of the Institute of Animal Pathology, Vetsuisse Faculty, University of Bern.

DATA AVAILABILITY STATEMENT

All data are freely available. Accessions for the whole genome sequence data are given in Table S1.

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