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SHORT COMMUNICATION

Heterozygous *ATP2A2* missense variant identified in a Shih Tzu with Darier disease

Abstract

Darier disease is caused by heterozygous loss of function variants in the *ATP2A2* gene encoding the endoplasmic/sarcoplasmic reticulum Ca^{2+} pump ATP2A2. Defective intracellular calcium signaling in the epidermis results in a loss of desmosomal adhesion and the development of characteristic skin lesions. In this study, we investigated a Shih Tzu that developed erythematous papules on the ventrum and, over time, the dorsal neck and a nodule in the right ear canal with secondary ear infection. Histopathologic examination demonstrated discrete foci of acantholysis affecting suprabasal layers of the epidermis. Whole genome sequencing of the affected dog identified a heterozygous missense variant, p.N809H, affecting an evolutionarily conserved amino acid residue of the ATP2A2 protein. The highly characteristic clinical and histopathologic findings together with a plausible variant in the only known functional candidate gene establish the diagnosis of canine Darier disease in the studied dog and highlight the potential of genetic analyses as complementary diagnostic approach in veterinary medicine.

Darier disease is a rare autosomal dominantly inherited skin disease in humans with keratotic papules in seborrheic areas, palmo-plantar pits and nail dystrophy (MIM #124200; Beck et al., 1977; Burge & Wilkinson, 1992). Clinical signs result from a separation between keratinocytes as well as abnormal cornification due to loss of desmosomal connections (Foggia & Hovnanian, 2004). The disease is caused by heterozygous variants in the *ATP2A2* gene, encoding the endoplasmic/sarcoplasmic reticulum Ca^{2+}-transporting ATPase 2, also termed SERCA2 (Nellen et al., 2017; Sakuntabhai et al., 1999). This calcium pump plays a key role in intracellular calcium signaling which in turn is central to the regulation of cell-to-cell adhesion, differentiation, and cornification in the epidermis (Foggia & Hovnanian, 2004). A canine form of Darier disease has been reported in an Irish Terrier with a heterozygous intronic SINE insertion in *ATP2A2* that resulted in a near-complete loss of functional transcripts from the mutant allele (OMIA 002265-9615; Linek et al., 2020).

This study investigated a 9-year-old, spayed female Shih Tzu from the USA that was referred to a dermatology specialty clinic. One year prior to referral, the dog was treated for bilateral otitis externa that improved but failed to completely respond to topical medication. The patient subsequently developed intense non-seasonal pruritus that was attributed to atopic skin disease and secondary superficial bacterial infection. Culture-based antibiotics, topical antimicrobials, antifungal medication, oclacitinib (Apoquel, Zoetis), and lokivetmab (anti-canine IL-31 monoclonal antibody, Cytopoint, Zoetis) were unsuccessful.

Dermatologic examination revealed erythematous papules on the ventrum, which frequently had a depressed center that was filled with keratin plugs (Figure 1a). The right external ear canal contained a pink-tan nodule with purulent exudate. Cytologic examination of the papules on the ventrum revealed numerous acantholytic cells with few scattered non-degenerative neutrophils (Figure 1b). Six-millimeter skin punch biopsies obtained under general anesthesia from the ventrum and the otic mass revealed discrete foci of marked epidermal hyperplasia with extensive acantholysis affecting the suprabasal layers of the epidermis. A broad cleft containing acantholytic keratinocytes separated the adherent basal layer from the overlying layers of the epidermis (Figure 1c). The acantholytic keratinocytes were either large, rounded cells with perinuclear halos and eosinophilic cytoplasm ('corps ronds') or small, ovoid cells with pyknotic flattened nuclei and intensely eosinophilic cytoplasm ('corps grains'; Figure 1d).

Although the late age of onset was unusual, the histopathologic features were typical for Darier disease. Therefore, EDTA whole blood was collected for genetic investigations. Genomic DNA was isolated with the Maxwell RSC Whole Blood Kit on a Maxwell RSC instrument and whole genome sequencing was performed on an Illumina Novaseq 6000 instrument with 2×150-bp paired-end reads at 18× coverage. The data was processed as described in Jagannathan et al. (2019); however, here we used the UU_Cfam_GSD_1.0 reference assembly

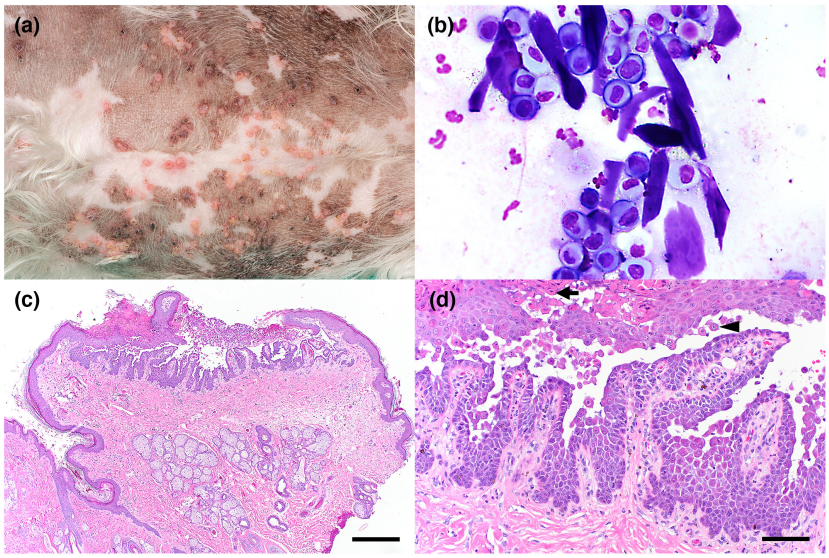

FIGURE 1 Clinical lesions, cytologic findings, and histopathologic features in a 9-year-old Shih Tzu with Darier disease. (a) Erythematous coalescing papules containing central keratin plugs. (b) Cytologic examination of the papules reveals numerous acantholytic cells with scattered non-degenerative neutrophils and keratin. Diff-Quik. (c) On histopathology the papule consists of discrete and marked epidermal hyperplasia with acantholytis of the spinous and granular cell layers. The basal layer remains adhered to the basement membrane zone. Hematoxylin and eosin, scale bar = 500 µm. (d) The corps ronds are acantholytic keratinocytes that are large and round with perinuclear halos and eosinophilic cytoplasm (arrowhead). The corps grains are acantholytic keratinocytes that are small, ovoid cells with pyknotic flattened nuclei and intensely eosinophilic cytoplasm (arrow). Hematoxylin and eosin, scale bar = 100 µm.

(a)

Variants in affected dog	homozygous	heteterozygous
total	2'715'742	3,856,764
↓ *filter against 926 control genomes*		
private	1,224	16,518
↓ *filter SnpEff impact high or moderate*		
private & protein-changing	2	113
↓ *prioritize functional candidate gene*		
in *ATP2A2* candidate gene	0	1

(b)

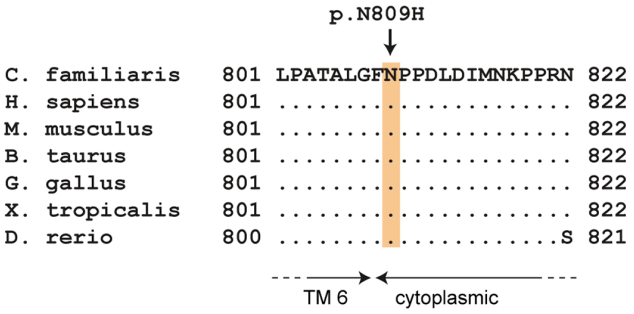

FIGURE 2 Details of the genetic analysis. (a) Workflow to identify the candidate causative *ATP2A2* missense variant from whole genome sequence data of the investigated case. Details of the identified variants are given in Table S2. (b) Multiple-species alignment of ATP2A2 amino acid sequences in the region harboring the p.N809H variant. The variant affects a perfectly conserved asparagine residue in the cytoplasmic domain following the sixth transmembrane helix. Accession numbers: dog (*Canis lupus familiaris*) NP_001003214.1; human (*Homo sapiens*) NP_733765.1; mouse (*Mus musculus*) NP_001103610.1; cattle (*Bos taurus*) XP_024833179.1; chicken (*Gallus gallus*) NP_001258903.1; frog (*Xenopus tropicalis*) XP_004910568.1; zebrafish (*Danio rerio*) NP_957259.1.

as described in Kiener et al. (2022). The sequencing data were submitted to the European nucleotide archive under the accession number SAMEA110175951. Private variants were filtered by comparing the sequencing data of the affected dog to 926 canine control genomes from diverse dog breeds including three Shih Tzus (Table S1). This resulted in 113 heterozygous and two homozygous private protein changing variants (Figure 2; Table S2). Only one of these variants was in the functional candidate gene for Darier disease, *ATP2A2*. This heterozygous missense variant, Chr26:8434781A>C or NM_001003214.1:c.2425A>C, is predicted to change a neutral asparagine to a positively charged histidine, NP_001003214.1:p.(Asn809His). The predicted amino acid exchange is located in a short cytoplasmic domain of the ATP2A2 protein, immediately after the sixth of 10 transmembrane domains. This region of the protein is evolutionarily conserved among vertebrates (Figure 2). In silico predictors, such as PredictSNP (Bendl et al., 2014), Provean (Choi & Chan, 2015), and MutPred2 (Pejaver et al., 2020), all classify this amino acid exchange as deleterious or pathogenic.

The clinical and histopathological findings in the studied Shih Tzu largely resembled previous descriptions of confirmed or suspected Darier disease in dogs (Linek et al., 2020; Müller et al., 2006; Olivry & Linder, 2009; Sueki et al., 1997). In humans, Darier disease is characterized by waxing-and-waning development of warty papules and plaques in seborrheic areas (central trunk, flexures, scalp, and forehead) associated with distinctive nail abnormalities. Skin lesions may be triggered by environmental factors such as heat, sweating, sunlight, and stress. Disease onset in human patients is highly variable with reports ranging from age 3 to 75 years, but most frequently starts around puberty and usually before the third decade (Foggia & Hovnanian, 2004; Li et al., 2017; Nellen et al., 2017). Clinical signs in the previously reported Irish Terrier with Darier disease started with a lesion in the ear canal and subsequent ear infection at age 4 months (Linek et al., 2020). The present case also started with ear infection but had a much later age of onset, with the 8-year-old dog resembling the huge variability in disease onset seen in human patients. While warty dyskeratoma described in dogs may have similar histopathologic changes (Gross et al., 2005), the multifocality of lesions and identification of an *ATP2A2* variant supported the diagnosis of Darier disease with a later onset. Therefore, genetic analysis in addition to histopathology was required for accurate diagnosis of Darier disease.

The genetic analysis of the affected Shih Tzu identified a heterozygous missense variant, p.N809H, of a conserved amino acid of the ATP2A2 protein. Darier disease is inherited as an autosomal dominant trait caused by haploinsufficiency of *ATP2A2* (Foggia & Hovnanian, 2004). We speculate that the variant has arisen by a de novo mutation event in the germline of the parents or during early embryonic development of the affected dog. Unfortunately, no phenotype information or samples of the parents were available to confirm this hypothesis. We did not experimentally assess the functional impact of the detected variant and, so far, no human patient with the homologous variant has been found. However, the ATP2A2 protein is highly conserved across vertebrates and missense variants in the same region of the protein have been reported in human patients with Darier disease, e.g. p.N796S or p.A838P (Li et al., 2017; Nellen et al., 2017). The highly characteristic phenotype and the finding of a missense variant in the only known functional candidate gene mutually support the diagnosis of Darier disease in the studied dog.

In conclusion, this study describes clinical, histopathologic and genetic findings in a Shih Tzu with a late-onset canine form of Darier disease. The identified heterozygous missense variant in *ATP2A2* represents a plausible candidate causative variant for the observed phenotype and corroborates the clinical diagnosis.

KEYWORDS

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

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All 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: 09-09-2019). 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.

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