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BRIEF REPORT

Heterozygous deletion of the *NSDHL* gene in an Appenzeller Mountain Dog with verrucous epidermal keratinocytic nevi

BACKGROUND

Dermal mosaicism can result in skin disorders with specific distribution patterns of the lesions. The most common example is X-chromosomal functional mosaicism, in which the distribution pattern results from random X-chromosome inactivation (Lyonization) (Vreeburg & van Steensel, 2012). Three different skin patterns have been described, namely Blaschko lines, the checkerboard pattern and lateralization (Happle, 2006). Verrucous epidermal keratinocytic nevi (OMIA 002117) caused by variants in the X-chromosomal *NSDHL* gene may present with any of these patterns. The encoded NAD(P)H steroid dehydrogenase-like protein is a C4 demethylase involved in post-squalene cholesterol biosynthesis. Pathogenic *NSDHL* variants result in disruption of an essential step in cholesterol biosynthesis with a subsequent aggregation of toxic intermediates and a lack of cholesterol in the skin (Caldas & Herman, 2003; König et al., 2000). In heterozygous female dogs, this presents as a cornification disorder and is inherited as an X-linked semidominant trait (Bauer et al., 2017; Christen et al., 2020; Leuthard et al., 2019). *NSDHL*-associated disorders in humans cause a more severe phenotype involving congenital hemidysplasia with ichthyosiform nevus and limb defects (CHILD syndrome; König et al., 2000). In hemizygous males, such variants have been described as embryonic lethal (Happle et al., 1980).

ANALYSES

A 10-month-old female intact Appenzeller Mountain Dog was presented with an 8 month history of severe, progressive hyperkeratosis of the paw pads causing lameness and a primarily left-sided multifocal hyperkeratosis of the haired skin, causing alopecia and mild pruritus. The lesions began on the left inner pinna and hind paw pads and slowly progressed to involve all four paws, although the right front was only mildly affected. Stripes of alopecia with hyperkeratosis were present on the lateral and caudal left thigh, with multiple smaller areas on the left tarsus, left lateral neck and left hip. Complete blood count, serum biochemistry and skin cytology were within normal limits and a fungal culture was negative. Multiple skin punch biopsies were taken under sedation to further pursue a diagnosis. Based on these results the paw pads were treated topically in an attempt to reduce cholesterol precursors in the skin and therefore hyperkeratosis. Two-percent ketoconazole cream and then 2% simvastatin ointment were tried successively, without improvement. The lesions on the haired skin then cleared completely with oral ketoconazole 5 mg/kg once daily but the paw pads remained quite hyperkeratotic, requiring repeated trimming (Figure 1).

Histopathologically, skin biopsies showed moderate to severe hyperplasia of the epidermis and infundibula of the hair follicles, forming spiked fronds (Figure 2). The infundibula of the hair follicles often showed striking compact parakeratotic hyperkeratosis (with retained nuclei as a sign of delayed maturation of keratinocytes), the epidermis showed orthokeratotic (with loss of nuclei as occurs in normal keratinocyte maturation) to parakeratotic hyperkeratosis. The dermis showed mild perivascular lymphoplasmacytic inflammation. The histopathological alterations resembled the findings described in Labrador Retrievers with *NSDHL*-related congenital cornification disorder (Bauer et al., 2017).

We performed Sanger sequencing of all exons of the *NSDHL* gene. The primer sequences are given in Table S1. However, no variant was detected in the coding sequence of *NSDHL*. We subsequently performed short-read whole-genome sequencing of the affected dog at 20× coverage using Illumina TruSeq PCR-free DNA libraries with ~400 bp insert size. Data processing was performed, with respect to the genome reference assembly UU_Cfam_GSD_1.0, as previously described (Jagannathan et al., 2019). Visual inspection of the short-read alignments at the position of the *NSDHL* gene revealed a large heterozygous deletion of >120 kb comprising the entire *NSDHL* gene (Figure S1). The deletion was not present in the dam of the affected dog as a deletion-specific amplicon could not be generated on genomic DNA isolated from blood leukocytes of the dam (Table S1). Samples from the father were not available for genotyping. However, assuming that hemizygous *NSDHL* variants cause lethality, it can be concluded that the variant arose from a *de novo* mutation event, either in

FIGURE 1 Clinical phenotype of the affected Appenzeller Mountain Dog. Lesions on the haired skin were primarily left sided. (a) Severe multifocal hyperkeratosis on the inner pinna, (b) the tarsus, (c) the paw pads and (d) the lateral hind limb.

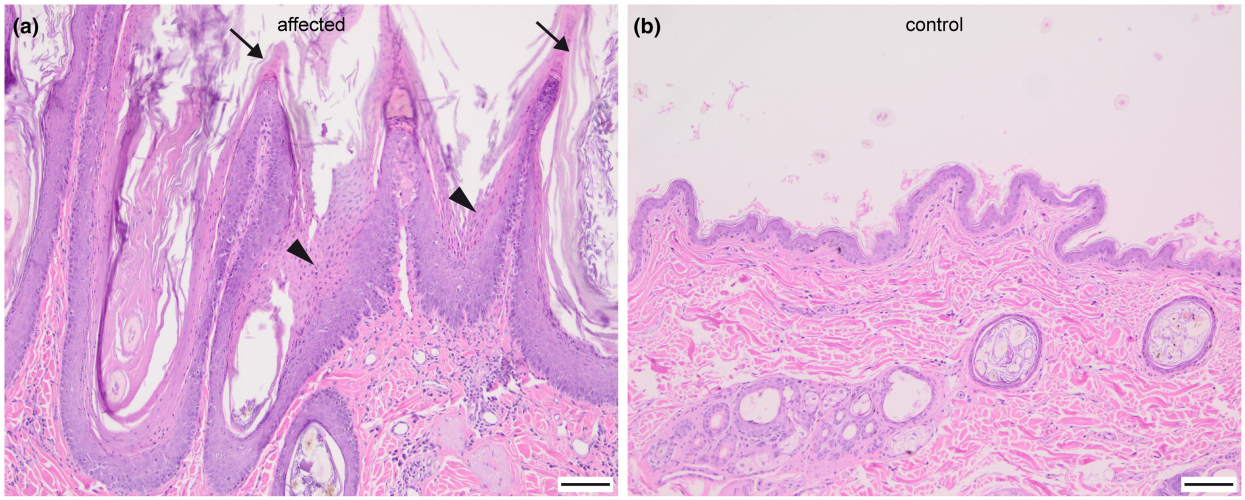

FIGURE 2 Histopathologic findings. (a) Affected dog. Severe hyperplasia of the epidermis, extending to the follicular infundibulum, with severe orthokeratotic (arrows) and parakeratotic (arrowheads) hyperkeratosis forming spiked fronds. (b) Normal control skin from an unaffected dog. Hematoxylin and eosin, scale bars = 100 μm.

the germline of one of the parents or during the early embryonic development of the affected dog.

CONCLUSIONS

We describe an Appenzeller Mountain Dog with clinical signs suggestive of an *NSDHL* defect. This differential diagnosis was further supported by the clear therapeutic success of cholesterol precursor reduction. Genetic investigation revealed a large heterozygous *de novo* deletion spanning the entire *NSDHL* gene. These results highlight the importance of advanced genomic analysis, such as whole genome sequencing, in identifying structural variants that are easily missed by Sanger sequencing of targeted PCR amplicons.

AUTHOR CONTRIBUTIONS

Sarah Kiener: Conceptualization; investigation; visualization; writing – original draft; writing – review and editing. **Brett Wildermuth:** Conceptualization; investigation; visualization; writing – original draft; writing

– review and editing. **Nadine M. Meertens:** Investigation; visualization; writing – original draft; writing – review and editing. **Vidhya Jagannathan:** Data curation; writing – review and editing. **Tosso Leeb:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Primer sequences are given in Table S1. Whole genome sequence data of the affected dog were submitted to the European Nucleotide Archive with project accession PRJEB16012 and sample accession SAMEA110175941.

ETHICS STATEMENT

The diagnostic examinations of the affected dog were conducted during the clinical workup, did not constitute an animal experiment and therefore did not require official or institutional ethical approval. The collection of blood samples from control dogs was approved by the Cantonal Committee for Animal Experiments (Canton of Bern; permit BE94/2022).

CONSENT STATEMENT

Consent for the use of samples and data for research purposes was obtained from the owners of the dogs in this study.

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REFERENCES

Bauer, A., De Lucia, M., Jagannathan, V., Mezzalira, G., Casal, M.L., Welle, M.M. et al. (2017) A large deletion in the *NSDHL* gene in Labrador Retrievers with a congenital cornification disorder. *G3: Genes, Genomes, Genetics*, 7(9), 3115–3121.

Caldas, H. & Herman, G.E. (2003) NSDHL, an enzyme involved in cholesterol biosynthesis, traffics through the Golgi and accumulates on ER membranes and on the surface of lipid droplets. *Human Molecular Genetics*, 12(22), 2981–2991.

Christen, M., Austel, M., Banovic, F., Jagannathan, V. & Leeb, T. (2020) *NSDHL* frameshift deletion in a mixed breed dog with progressive epidermal nevi. *Genes*, 11(11), 1297.

Happle, R. (2006) X-chromosome inactivation: role in skin disease expression. *Acta Paediatrica*, 95, 16–23.

Happle, R., Koch, H. & Lenz, W. (1980) The CHILD syndrome. *European Journal of Pediatrics*, 134(1), 27–33.

Jagannathan, V., Drögemüller, C., Leeb, T. & Dog Biomedical Variant Database Consortium (DBVDC). (2019) A comprehensive biomedical variant catalogue based on whole genome sequences of 582 dogs and eight wolves. *Animal Genetics*, 50, 695–704.

König, A., Happle, R., Bornholdt, D., Engel, H. & Grzeschik, K.H. (2000) Mutations in the NSDHL gene, encoding a 3beta-hydroxysteroid dehydrogenase, cause CHILD syndrome. *American Journal of Medical Genetics*, 90(4), 339–346.

Leuthard, F., Lehner, G., Jagannathan, V., Leeb, T. & Welle, M. (2019) A missense variant in the *NSDHL* gene in a Chihuahua with a congenital cornification disorder resembling inflammatory linear verrucous epidermal nevi. *Animal Genetics*, 50(6), 768–771.

Vreeburg, M. & van Steensel, M.A.M. (2012) Genodermatoses caused by genetic mosaicism. *European Journal of Pediatrics*, 171(12), 1725–1735.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.