Short Communication

A missense variant in the *NSDHL* gene in a Chihuahua with a congenital cornification disorder resembling inflammatory linear verrucous epidermal nevi

Fabienne Leuthard[1,2], Georg Lehner[3], Vidhya Jagannathan[1,2], Tosso Leeb[1,2],'Monika Welle[2,4]

[1] Institute of Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

[2] DermFocus, University of Bern, 3001 Bern, Switzerland

[3] Kleintierpraxis Lehner Dermatologie, 89290 Buch, Germany

[4] Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

Running title: *NSDHL* missense variant in a Chihuahua with ILVEN

Address for correspondence

Tosso Leeb
Institute of Genetics
Vetsuisse Faculty
University of Bern
Bremgartenstrasse 109a
3001 Bern
Switzerland

Phone: +41-31-6312326
Fax: +41-31-6312640
E-mail: Tosso.Leeb@vetsuisse.unibe.ch

Summary

CHILD syndrome in humans is a genodermatosis characterized by inflammatory verrucous linear epidermal nevi (ILVEN) often showing a striking lateralisation pattern. It is caused by variants in the *NSDHL* gene encoding a 3beta-hydroxysteroid dehydrogenase involved in the cholesterol biosynthesis pathway. In the present study, we investigated a female Chihuahua, which showed clinical and histological signs of ILVEN. We performed a candidate gene analysis in the affected Chihuahua. This analysis revealed a single missense variant in the *NSDHL* gene in the affected dog (XM_014111859.2:c.700G>A). The variant is predicted to cause a non-conservative amino acid change from glycine to arginine, XP_013967334.1:p.(Gly234Arg). The mutant allele was absent from whole genome sequence date of 594 genetically diverse dogs and 8 wolves. Sanger sequencing confirmed that the variant was heterozygous in the affected dog and absent from 22 control Chihuahuas. Based on the knowledge about the functional impact of *NSDHL* variants in dogs and other species, c.700G>A is likely pathogenic and a convincing candidate causative variant for the observed skin lesions in the affected Chihuahua.

Keywords: Dog, Canis lupus familiaris, skin, dermatology, genodermatosis, ILVEN, Blaschko lines, X-chromosome, candidate gene, precision medicine

Genodermatoses are inherited skin diseases, which can be present at birth or manifest later in life. In humans, congenital hemydysplasia with ichthyosiform nevus and limb defects (CHILD syndrome) characterized by inflammatory linear verrucous epidermal nevi (ILVEN) with striking lateralization represents an X-linked semidominant genodermatosis (Happle et al. 1980). CHILD syndrome is caused by variants in the *NSDHL* gene encoding the NAD(P) dependent steroid dehydrogenase-like protein (König et al. 2000). The NSDHL protein is involved in the cholesterol biosynthesis pathway and its catalytically active site resides within a short-chain dehydrogenase/reductase domain (Filling et al. 2002).

CHILD syndrome is seen almost exclusively in heterozygous females. Their skin lesions are arranged very characteristic, often linear, skin patterns following the lines of Blaschko. Blaschko lines represent borderlines of epidermal malformation induced by functional genetic mosaicism resulting from the random X-chromosome inactivation during early stages of embryogenesis (Happle et al. 2001). A complete loss of NSDHL function in hemizygous males or homozygous females is embryonic lethal (Happle et al. 1980).

Variants in the murine *Nsdhl* gene were shown to cause the phenotype of the *bare patches* (*Bpa*) and *striated* (*Str*) mouse mutants. They show similar linear skin lesions as human CHILD patients, but lack their striking unilateral limb defects (Liu et al. 1999).

In veterinary medicine, comparable phenotypes comprising ILVEN due to variants in the *NSDHL* gene were described in two dogs (Bauer et al. 2018; OMIA 002117-9615) and a cat (De Lucia et al. 2019; OMIA 002185-9685). The affected dogs were a female Labrador Retriever and her crossbred daughter, which were heterozygous for a 14 kb deletion spanning the last three exons of the *NSDHL* gene (Bauer et al. 2018). In this study, we investigated a single Chihuahua with an ILVEN phenotype.

A seven months old female Chihuahua with a congenital cornification disorder was investigated (Figure 1). The cutaneous lesions were mainly distributed on the right side of the dog with the right foreleg being more affected than the right hind leg. The affected skin was characterized by sharply demarcated, alopecic, verrucous, hyperpigmented and lichenified linear lesions covered with brownish-blackish scales. The cutaneous lesions were extremely

painful and the dog was severely lame. According to the clinical examination, haematology results, and the biochemistry parameters, the dog was otherwise healthy. Biopsies were taken from the skin of the right foreleg, the right axilla, and the footpad of the right foreleg. Histological examination of all three biopsies revealed a moderately hyperplastic epidermis and infundibular wall. The epidermis was covered by a thick layer of compact parakeratotic keratin. The lumen of the hair follicle infundibula was distended and equally filled with parakeratotic keratin. The interfollicular dermis was infiltrated with a moderate amount of lymphocytes, plasma cells, neutrophils and fewer histiocytes (Figure 1). The histopathology was compatible with an inflammatory linear verrucous epidermal nevus (ILVEN).

Initial antimicrobial and anti-inflammatory treatment consisted of fusidic acid cream (Fucidine) twice and mometasone fuorat ointment (Momegalen) once daily in addition to chlorhexidine 2% and miconazole shampoo (Malaseb) every other day. As the lesions did not improve after two months, treatment was replaced by a combination of lovastatin 2% with cholesterol 2% cream, which was applied daily and a glycolic acid 20% cream applied to effect, which had been reported to be effective in human patients (Estapé et al. 2015; Bergqvist et al. 2018; Khalil et al. 2018). This resulted in almost complete resolution of the cutaneous lesions after six weeks. The dog became able to move normal at this time.

Due to the phenotypic similarity of the affected dog with earlier described cases (Bauer et al. 2018; De Lucia et al. 2019), we hypothesized that the affected Chihuahua had a defect in the *NSDHL* gene and considered *NSDHL* the top functional candidate gene for this phenotype. We obtained EDTA blood samples from the affected Chihuahua and extracted genomic DNA. Primer pairs for the amplification of all ten exons of the *NSDHL* gene were designed (Table S1). PCR products for each *NSDHL* exon were amplified using AmpliTaq Gold 360 Master Mix (ThermoFisher), treated with shrimp alkaline phosphatase and exonuclease I, and sequenced on an ABI 3730 capillary sequencer (ThermoFisher). The Sanger sequencing data were analyzed using the Sequencher 5.1 software (GeneCodes).

The affected Chihuahua was heterozygous for a missense variant, XM_014111859.2:c.700G>A or XP_013967334.1:p.(Gly234Arg), in exon 9 of the *NSDHL*

gene (Figure 2). The genomic variant designation is ChrX:120,752,468G>A (CanFam 3.1 assembly). The variant was absent from 22 additional unrelated non-affected Chihuahuas as demonstrated by Sanger Sequencing. It was also absent from whole genome sequence data of 594 genetically diverse dogs and 8 wolves (Table S2). The variant is predicted to affect a highly conserved residue within the catalytic short-chain dehydrogenase/reductase domain and may thus lead to functional inactivation of the NSDHL protein (Figure 2). The human ClinVar database lists a related missense variant, p.Val243Met. Two diagnostic laboratories reported this variant as likely pathogenic and one reported it as uncertain significance. The human position 243 in the NSDHL protein corresponds to canine position 231 and is only three residues away from the p.Gly234Arg missense variant detected in the affected Chihuahua.

We additionally analysed the functional effect of the canine p.Gly234Arg variant *in silico* with SIFT (Sim et al. 2012) and Polyphen-2 (Adzhubei et al. 2010). Both software tools predicted this variant to “affect protein function” (SIFT, score 0.02) or “probably damaging” (Polyphen-2; score 0.999).

To the authors’ knowledge, this is the second time a likely pathogenic *NSDHL* variant has been identified in a dog with skin disorder involving ILVEN. The clinical and histopathological changes were very similar as in the earlier described Labrador Retrievers carrying a large deletion in the *NSDHL* gene (Bauer et al. 2018). It must be cautioned that our results are based on the investigation of a single case. Definitive proof of pathogenicity of the p.Gly234Arg variant and its causality for the investigated case would require additional experiments (e.g. on the protein level) or the identification of additional cases with the same mutant allele.

The histopathology of the Chihuahua described in this report was related, but not identical to the four Cocker Spaniels with cutaneous lesions similar to human ILVEN and multiple congenital defects in five Rottweilers (White et al. 1993; Lewis et al. 1998). However, the underlying genetic cause in those dogs has never been reported. Our results indicate that the skin lesions could be due to inactivation of the NSDHL protein resulting in a lack of cholesterol or other sterols downstream of the block in biosynthesis and the accumulation of toxic

intermediates in the cutis. This observed clinical improvement after topical therapy with cholesterol and glycolic acid in agreement with the hypothesized molecular aetiology.

In humans, approximately 21 different pathogenic *NSDHL* variants have been reported. They compromise deletions, missense, nonsense, frameshift and splice variants throughout the entire *NSDHL* gene (Bornholdt et al. 2005; Du Souich et al. 2011). Most human CHILD patients have severe limb defects in addition to the skin lesions. In contrast, all reported mice, cats, and dogs with *NSDHL* variants show only the characteristic skin lesions (ILVEN). They are thus more comparable to the so-called mild CHILD syndrome in humans, in which only cutaneous lesions are noticeable (Gantner et al. 2014).

Our study nicely illustrates how a genetic investigation in a precision medicine approach may help to confirm a suspected clinical diagnosis. The results are important to develop better breeding programs in dogs and decrease the prevalence of hereditary diseases. Furthermore, this case report provides an opportunity for a pathogenesis-directed therapy in dogs with similar skin lesions due to proven inactivating *NSDHL* genetic variants.

In conclusion, we identified a missense variant, NSDHL:c.700G>A, as likely candidate causative variant in a single female Chihuahua affected by a cornification disorder resembling ILVEN.

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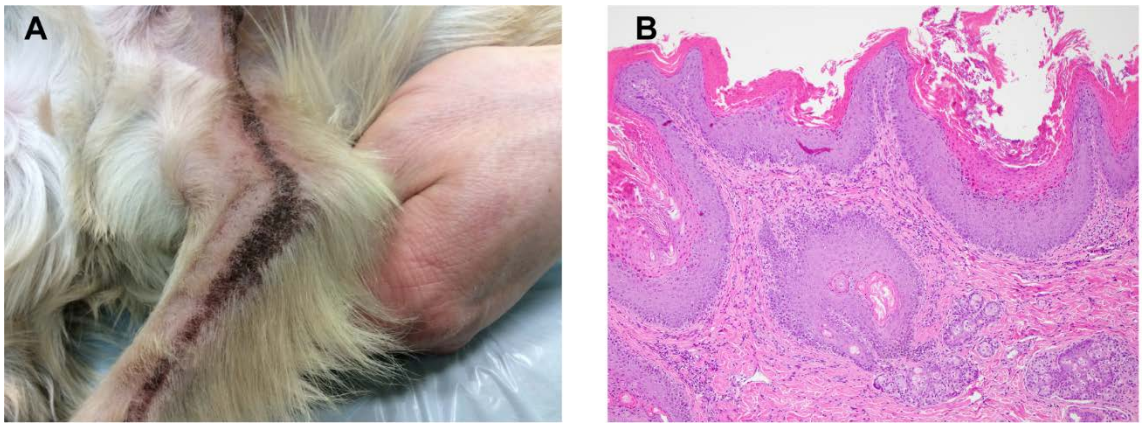

Figure 1. Phenotype. (A) Right foreleg of the affected Chihuahua. (B) Histology of a skin sample taken from the right foreleg. The epidermis and the infundibular wall is severely hyperplastic and the epidermis if covered by a thick layer of compact parakeratotic keratin. The infundibula are distended and equally filled with parakeratotic keratin. The interfollicular dermis is infiltrated with lymphocytes, plasma cells, neutrophils and histiocytes.

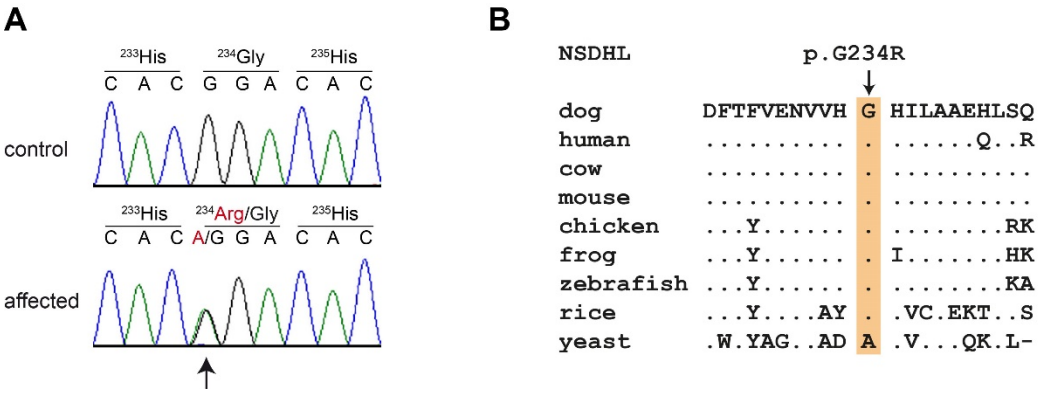

Figure 2. Details of the detected *NSDHL* variant. (A) Sanger electropherograms from a control and the affected dog. The position of the identified c.700G>A variant is indicated by an arrow. (B) Multiple species alignment of NSDHL protein sequences. The variant affects the highly conserved short-chain dehydrogenase/reductase domain. The NSDHL protein is conserved throughout all eukaryotes. The glycine residue at position 234 is strictly conserved across all vertebrates and some plant species. Unicellular eukaryotes and some plants have an alanine at the corresponding position. While glycine and alanine both represent small neutral amino acids, the mutant arginine in the affected Chihuahua has a large positively charged side chain.

Supplementary Material

Table S1. Primer sequences.

Table S2. Accession numbers of 594 dogs and 8 wolf genome sequences.