Short Communication

A frameshift variant in the *EDA* gene in Dachshunds with X-linked hypohidrotic ectodermal dysplasia

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Running title: *EDA* frameshift variant in dogs with XLHED

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Summary

X-linked hypohidrotic ectodermal dysplasia (XLHED) is a genetic disease characterized by hypoplasia or absence of hair, teeth and sweat glands. The *EDA* gene located on the X chromosome encodes the type II transmembrane protein ectodysplasin A. Variants in the *EDA* gene can lead to XLHED in humans, mice, cattle and dogs. In the present study, we investigated a litter of Dachshund puppies, of which four male puppies showed clinical signs of XLHED. We performed a candidate gene analysis in one affected puppy and several non-affected relatives. This analysis revealed a single base-pair deletion in the coding sequence of the *EDA* gene in the affected puppy (NM_001014770.2:c.842delT). The deletion is predicted to cause a frameshift, NP_001014770.1:p.(Leu281HisfsTer22), leading to a premature stop codon which truncates more than one quarter of the EDA protein. Sanger sequencing results confirmed that this variant was inherited from the dam. Based on the knowledge about the functional impact of *EDA* variants in dogs and other species, c.842delT is a convincing candidate causative variant for the observed XLHED in the male puppies.

Keywords: Ectodysplasin A, XHED, Sanger sequencing, development, dermatology, skin, dog, *Canis lupus familiaris*

Ectodermal dysplasia (ED) is a group of genetically heterogeneous diseases characterized by abnormal development of two or more ectodermal structures, such as hair, teeth, nails and sweat glands (Pinheiro and Freire-Maia 1994). Hypohidrotic ectodermal dysplasia can be inherited with autosomal recessive, autosomal dominant, or X-linked modes of inheritance (Pinheiro and Freire-Maia 1994). X-linked hypohidrotic ectodermal dysplasia (XLHED) is one of the most common forms of ED and is caused by variants in the *EDA* gene encoding ectodysplasin A (Kere et al. 1996; OMIM #305100). Ectodysplasin A is a type II transmembrane protein with several alternative isoforms of which the longest isoform (isoform 1) consists of 391 amino acid residues. EDAR and XEDAR are two distinct EDA receptors which bind to isoform 1 and isoform 2 of EDA, respectively (Yan et al. 2000). Once isoform 1 binds to and activates EDAR, it results in translocation of NFκB to the nucleus, where it activates transcription of several target genes. These target genes are required to initiate and differentiate ectoderm derived tissues such as hair, teeth, nails and sweat glands (Cui and Schlessinger 2006). Several spontaneous *EDA* variants have been previously reported in human XLHED patients and the *tabby* mouse mutant (Kere et al. 1996; Srivastava et al. 1997). Several independent *EDA* variants were also described in cattle with XLHED (OMIA 000543-9913; e.g. Drögemüller et al. 2001; Drögemüller et al. 2002). In XLHED affected dogs derived from a German Shepherd, an *EDA* splice site variant was identified (OMIA 000543-9615; Casal et al. 2005). These dogs were bred in a colony and used for clinical trials with recombinant ectodysplasin (Casal et al. 2007). XLHED affected crossbred dogs from Israel were found to express a mutant *EDA* transcript lacking exon 2. However, the genomic sequence of exon 2 and the conserved splice sites were normal in these dogs. The causative genetic variant in these dogs thus remains unknown (Waluk et al. 2016).

In the present study, a litter of 4 male and 3 female Dachshund puppies was investigated. All four male littermates had patches of hairless skin on the head and the back. The affected dogs were euthanized during their first three weeks of age. From one of the affected puppies two excisional skin biopsies were taken from the boundary of hairless skin and a normally appearing skin region. Histological examination of these tissue samples revealed a complete

absence of adnexal structures, including hair follicles, sebaceous glands and sweat glands in the hairless region, whereas few hair follicles were present in the adjacent haired skin (Figure 1). In the second tissue sample, which was taken from haired skin, density of follicular compounds was normal. All hair follicles lacked secondary follicles, which is normal for very young dogs.

Due to the phenotypic similarity of the affected puppies with earlier described XLHED cases and the fact that only male dogs were affected, we hypothesized that the affected Dachshund puppies also had XLHED and considered *EDA* the top functional candidate gene for this phenotype. We obtained EDTA blood samples from the female littermates, their dam, a brother of the dam, and the maternal granddam. Genomic DNA was extracted from the available affected dog and the unaffected relatives. We designed primer pairs for the amplification of all eight exons of the *EDA* gene (Figure S1; Table S1). PCR products for each *EDA* exon were amplified from genomic DNA using AmpliTaq Gold 360 Master Mix (ThermoFisher). Amplicons were treated with shrimp alkaline phosphatase and endonuclease I, and sequenced on an ABI 3730 capillary sequencer (ThermoFisher). The Sanger sequencing data were then analyzed using the software Sequencher 5.1 (GeneCodes).

The fragment harbouring exon 7 of the *EDA* gene contained a single base deletion, NM_001014770.2:c.842delT or ChrX:54,509,504delT (CanFam 3.1) or NP_001014770.1:p.(Leu281HisfsTer22) in hemizygous state in the affected puppy (Figure 2). This frameshift variant leads to the occurrence of a premature stop codon and the replacement of 106 amino acids (27%) of the predicted open reading frame of the wildtype ectodysplasin A protein by 21 new residues (Figure S1). As the premature stop codon is located very close to the 3'-end of the penultimate exon, it is difficult to predict whether the mutant transcript is likely to be subject to nonsense mediated decay or other cellular quality control mechanisms (Karousis and Mühlemann, 2018).

Genotyping the available family members showed that two female littermates of the affected dog and their dam were heterozygous carriers for the variant. The remaining female littermate, the brother of the dam, and the maternal granddam did not carry this variant (Figure 2). As the

maternal granddam did not carry the variant in her blood leukocytes, it is likely that the variant arose due to a *de novo* mutation in the dam or in the germ line of one of the maternal grandparents. Unfortunately, no data is available from the maternal grandsire. Hence, it is unknown whether the grandsire had any phenotypic signs of the XLHED. It seems unlikely that a male dog with a fully developed, severe XLHED phenotype would have been used as breeding sire, but minimal disease expression in a mosaic dog might have gone undetected.

As of 2015, 82 different *EDA* variants were reported in human XLHED patients, comprising of deletion, missense, nonsense, frameshift and splice variants (Huang et al. 2015). In cattle, at least five independent pathogenic *EDA* variants were described. We now report the third instance of XLHED in dogs and the second instance where a likely causative genomic variant in the canine *EDA* has been identified. Our study nicely illustrates how a genetic investigation in veterinary medicine may help to confirm a suspected clinical diagnosis. The results are important to develop sustainable breeding programs and decrease the prevalence of hereditary diseases. In this study we identified three female carriers, which were removed from breeding in order to prevent the disease allele from spreading throughout the breed and avoid the unintentional breeding of further affected dogs. Simultaneously, a clear female dog was identified that may be used to maintain the particular breeding line.

In summary, we identified a single nucleotide deletion in *EDA* in a male Dachshund with XLHED. The well known functional impact of *EDA* variants in dogs, humans, mice, and cattle suggest that c.842delT is a compelling candidate causative variant for the observed XLHED phenotype.

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References

Casal M.L., Scheidt J.L., Rhodes J.L., Henthorn P.S. & Werner P. (2005) Mutation identification in a canine model of X-linked ectodermal dysplasia. Mammalian Genome 16, 524–31.

Casal M.L., Lewis J.R., Mauldin E.A., Tardivel A., Ingold K., Favre M., Paradies F., Demotz S., Gaide O. & Schneider P. (2007) Significant correction of disease after postnatal administration of recombinant ectodysplasin A in canine X-linked ectodermal dysplasia. American Journal of Human Genetics 81, 1050-6.

Cui C. & Schlessinger D. (2006) EDA signaling and skin appendage development. Cell Cycle 5, 2477–83.

Drögemüller C., Distl O. & Leeb T. (2001) Partial deletion of the bovine ED1 gene causes anhidrotic ectodermal dysplasia in cattle. Genome Research 11, 1699–705.

Drögemüller C., Peters M., Pohlenz J., Distl O. & Leeb T. (2002) A single point mutation within the ED1 gene disrupts correct splicing at two different splice sites and leads to anhidrotic ectodermal dysplasia in cattle. Journal of Molecular Medicine 80, 319–323.

Huang S.X., Liang J.L., Sui W.G., Lin H., Xue W., Chen J.J., Zhang Y., Gong W.W., Dai Y. & Ou M.L. (2015) EDA mutation as a cause of hypohidrotic ectodermal dysplasia: a case report and review of the literature. Genetics and Molecular Research 14, 10344-51.

Karousis E.D. & Mühlemann O. (2018) Nonsense-mediated mRNA decay begins where translation ends. Cold Spring Harbor Perspectives in Biology doi: 10.1101/cshperspect.a032862.

Kere J., Srivastava A.K., Montonen O., Zonana J., Thomas N., Ferguson B., Munoz F., Morgan D., Clarke A., Baybayan P., Chen E.Y., Ezer S., Saarialho-Kere U., de la Chapelle A. & Schlessinger D. (1996) X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nature Genetics 13, 409–16.

Pinheiro M. & Freire-Maia N. (1994) Ectodermal Dysplasias: A clinical classification and a causal review. American Journal of Medical Genetics 53, 153–62.

Srivastava A.K., Pispa J., Hartung A.J., Du Y., Ezer S., Jenks T., Shimada T., Pekkanen M., Mikkola M.L., Ko M.S., Thesleff I., Kere J. & Schlessinger D. (1997) The Tabby phenotype is caused by mutation in a mouse homologue of the EDA gene that reveals novel mouse and human exons and encodes a protein (ectodysplasin-A) with collagenous domains. Proceedings of the National Academy of Sciences of The United States of America 94, 13069-74.

Waluk D., Zur G., Kaufmann R., Welle M.M., Jagannathan V., Drögemüller C., Müller E.J., Leeb T. & Galichet A. (2016) A splice defect in the *EDA* gene in dogs with an X-linked hypohidrotic ectodermal dysplasia (XLHED) phenotype. G3: (Bethesda) 6, 2949–54.

Yan M., Wang L.C., Hymowitz S.G., Schilbach S., Lee J., Goddard A., de Vos A.M., Gao W.Q. & Dixit V.M. (2000) Two-Amino Acid Molecular Switch in an Epithelial Morphogen That Regulates Binding to Two Distinct Receptors. Science 290, 523–27.

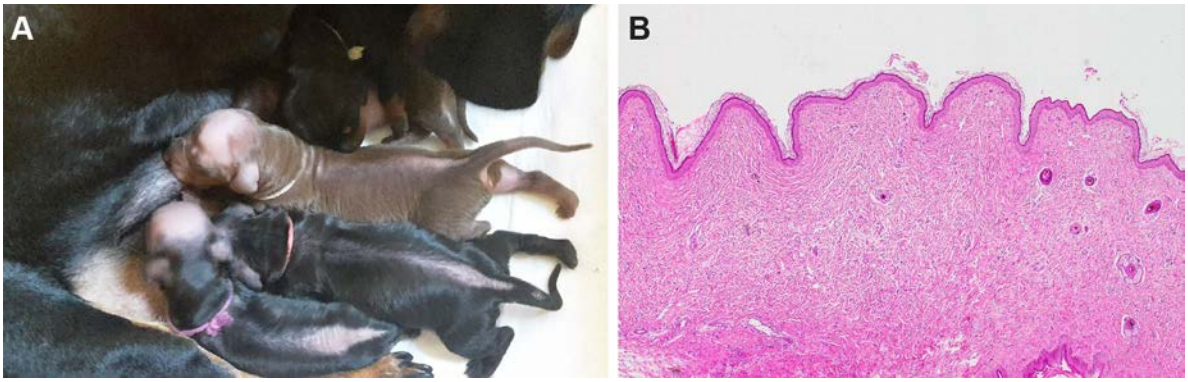

Figure 1: XLHED phenotype in male Dachshund puppies. (A) Patches of hairless skin on the head and back of affected puppies. (B) Histology of a skin sample taken at the boundary of a hairless area (left side – hairless, right side – haired). Adnexal structures (e.g. hair follicles or sebaceous glands) are completely missing on the left side of the sample. Sparse hair follicles are present on the right side. Hematoxylin and Eosin 250X.

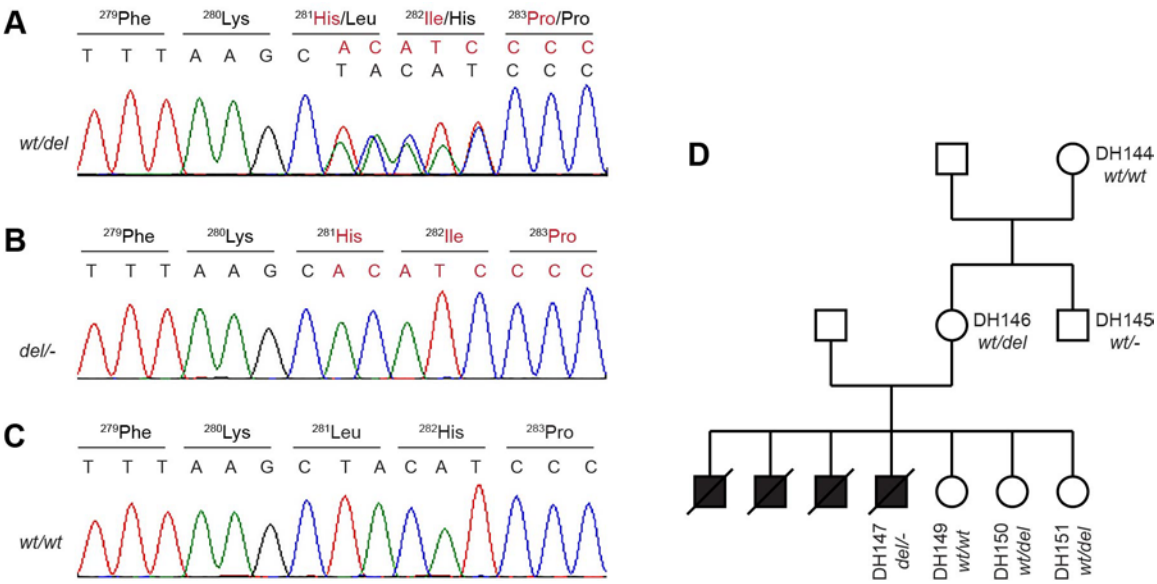

Figure 2: Details of the *EDA*:c.842delT frameshift variant. Wildtype sequences are shown in black, mutant sequences in red. (A) Heterozygous genotype in the dam of the affected puppy. (B) Hemizygous deletion in the affected male puppy. (C) Wildtype sequence in a female littermate of the affected puppy. (D) The pedigree of the family is indicative for an X-linked recessive mode of inheritance and shows co-segregation of the deletion with the phenotype.

Supplementary Material

Figure S1. Genomic organization and annotation of the canine *EDA* gene.

Table S1. Primer sequences.