Short Communication

A frameshift variant in the *COL5A1* gene in a cat with Ehlers-Danlos syndrome

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Running title: *COL5A1* frameshift variant in a cat with EDS

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Summary

Ehlers-Danlos syndrome (EDS) is a group of heritable connective tissue disorders caused by defective collagen synthesis or incorrect assembly of the collagen triple helical structure. EDS is characterised by joint hypermobility, skin hyperextensibility, abnormal scarring, poor wound healing and tissue friability. Human EDS may be caused by variants in several different genes including *COL5A1*, which encodes the collagen type V alpha 1 chain. For the present study we investigated a 1.5-year-old, spayed female, domestic shorthair cat with EDS. The affected cat showed multiple recurrent skin tears, hyperextensibility of the skin and joint abnormalities. We obtained whole genome sequencing data from the affected cat and searched for variants in candidate genes known to cause EDS. We detected a heterozygous single-base pair deletion in exon 43 of the *COL5A1* gene, c.3420delG. The deletion was predicted to result in a frameshift and premature stop codon, p.(Leu1141SerfsTer134). Sanger sequencing confirmed that the variant was present in the affected cat and absent from 103 unaffected cats from different breeds. The variant was also absent from a Burmese cat with EDS. Based on knowledge of the functional impact of *COL5A1* variants in other species, *COL5A1*:c.3420delG represents a compelling candidate causative variant for the observed EDS in the affected cat.

Keywords: felis catus, skin, dermatology, collagen, genodermatosis, whole genome sequencing

Ehlers-Danlos syndrome (EDS) is a clinically and genetically heterogeneous group of heritable connective tissue disorders. EDS is caused by defective collagen synthesis or incorrect assembly of the collagen triple helical structure. The phenotype is characterised by joint hypermobility, skin hyperextensibility, abnormal scarring, poor wound healing and tissue friability (Byers & Murray 2012; De Paepe & Malfait 2012). Collagen provides the connective tissue matrix with shape, strength and the ability to resist deformation, thus being a key structural protein of the connective tissue. Fibrillary collagen proteins consist of three either homo- or heterotrimeric polypeptide chains, designated as α-chains, which together form a triple helical structure (De Paepe & Malfait 2012). As a result of defects in fibrillary collagen, the skin may become more fragile and tear more easily.

According to the 2017 international classification there are 13 recognized subtypes of EDS in humans. Depending on the causative variant, EDS may follow an autosomal dominant or autosomal recessive mode of inheritance (Malfait et al. 2017).

The most common type of EDS, the so called classical EDS (cEDS), formerly categorized as EDS I or EDS II, is caused by genetic variants in the *COL5A1* and *COL5A2* genes (Nicholls et al. 1996; Symoens et al. 2008; Malfait et al. 2010; Bowen et al. 2017). Variants in many other genes are known to have an impact on collagen structure, which may also result in the clinical picture of EDS (Byers & Murray 2012; De Paepe & Malfait 2012; Malfait et al. 2017).

Connective tissue diseases resembling human EDS were observed in many different mammalian species such as e.g. cattle, dogs, minks, horses, rabbits, and sheep (Hegreberg et al. 1969; Harvey et al. 1990; Colige et al. 1999; Sequeira et al. 1999; Paciello et al. 2003; Zhou et al. 2012; Monthoux et al. 2015). A closely related phenotype is the equine regional dermal asthenia (HERDA) in horses, which is caused by a variant in the *PPIB* gene (Tryon et al. 2007). Furthermore, a Holstein calf with a phenotype described as variant form of EDS and a heterozygous missense variant in the *EPYC* was reported (Tajima et al. 1999). This calf had a deficiency of dermatan sulfate proteoglycan, but the causality of the *EPYC* genetic variant was not conclusively proven. So far, neither *PPIB* nor *EPYC* variants were reported in human EDS patients.

In cats, isolated EDS cases have been described as cutaneous asthenia or dermatosparaxis in several individual purebred and crossbred animals (OMIA 000327-9685; Scott, 1974; Patterson & Minor 1977; Counts et al. 1980; Holbrook et al. 1980; Verweij & van Zuylen 1986; Plotnick et al. 1992; Sequeira et al. 1999; Benitah et al. 2004; Smids, 2008; Dokuzeylül et al. 2013; Weingart et al. 2014). An experimental domestic shorthair cat colony with an autosomal dominant form of EDS was established to study the biomechanical properties and would healing characteristics of skin (Freeman et al. 1989a; Freeman et al. 1989b). More recently, EDS has also been observed in several Burmese cats and it was suggested that an autosomal recessive form of EDS may be segregating in this breed (Hansen et al. 2015).

Before genetic analyses became widely available, a detailed biochemical analysis in a single affected Himalayan cat revealed a defect in ADAMTS2, the procollagen N-endopeptidase (Counts et al. 1980). Thus, this case corresponded to the human dermatosparaxis EDS (dEDS or formerly EDS VIIC). To the best of our knowledge, no causative genetic variant in a cat with EDS has yet been reported in the scientific literature.

In the present study, we investigated a 1.5-year-old, spayed female domestic shorthair cat with characteristics of EDS. The affected cat showed multiple recurrent skin tears with little or no bleeding, mainly located on the dorsal neck and the shoulders, and hyperextensibility of the skin (Figure 1). The skin extensibility index according to Hansen et al. 2015 was 27%. Some of the previous lacerations had slowly healed leaving shiny alopecic scars. Other clinical findings included bilateral hip subluxation with positive Ortolani sign even in the awake patient, bilateral carpal hyperextension with plantigrade appearance, pain and laxity during palpation of all joints, and bilateral perineal hernias. The index cat was found on the street when she was a kitten, together with a female littermate, which appeared to be normal at the clinical examination. Information about the parents was not available.

To confirm our hypothesis of a genetic defect related to collagen, we obtained an EDTA blood sample of the affected cat, isolated genomic DNA, and performed whole genome re-sequencing at 20x coverage using 2 x 150 bp reads on an illumina HiSeq 3000 instrument. Private variants were identified by comparing the sequence from the affected cat to the feline

reference genome assembly FelCat 9.0 and to genome re-sequencing data from 11 genetically diverse control cats obtained during other projects (database accessions given in Table S1). The methodology was previously described (Bauer et al. 2017). We identified 93 private homozygous and 2339 private heterozygous protein-changing variants (Table S2). These variants included a heterozygous frameshifting single-base deletion in exon 43 of the *COL5A1* gene (XM_023242950.1:c.3420delG or Chr13:93,210,344delC or XP_023098718.1:p.(Leu1141SerfsTer134)). None of the other private protein-changing variants were located in a known EDS candidate gene.

We confirmed the *COL5A1*:c.3420delG variant in the affected cat using Sanger sequencing and genotyped a sample of 104 genetically diverse cats including the unaffected littermate of the affected cat and a Burmese cat affected by EDS. Primers COL5A1_F1, AAGCTGGCTGAAACCCATC and COL5A1_R1, CGAGCACTCCAGAGATGTCA, were used to amplify a 418 bp amplicon containing the *COL5A1*:c.3420delG variant. Both primers were individually used as sequencing primers to obtain sequences in both orientations on an ABI 3730 capillary sequencer. This experiment confirmed that the variant was exclusively present in the affected cat and did not occur in the other 104 genotyped cats including the Burmese cat with EDS (Figure 2).

The identified *COL5A1*:c.3420delG variant in the affected cat causes a shift in the open reading frame resulting in a premature stop codon and is predicted to truncate approximately one third of the 1837 amino acids of the wildtype *COL5A1* protein. The human and feline proteins share 96% amino acid identity. Heterozygous variants in the *COL5A1* gene may cause EDS in humans and mice (Wenstrup et al. 2006; Malfait et al. 2017). In human EDS patients, many *COL5A1* nonsense, frameshift or splice site variants were reported (Symoens et al. 2008; Malfait et al. 2010; Byers & Murray 2012). The ClinVar database lists human pathogenic *COL5A1* frameshift variants, which are comparable to the identified feline variant and lead to relatively mild clinical forms of cEDS (formerly EDS II), e.g. NM_001278074.1:c.3206dup (p.Ala1070Serfs) and NM_000093.4:c.3752delC (p.Pro1251Argfs). The knowledge on the

functional impact of *COL5A1* frameshift variants in humans suggests a causal role for the detected feline *COL5A1*:c.3420delG variant in the cat with EDS.

As we did not have access to RNA or protein samples from the affected cat, we could not investigate the functional consequences of the genomic variant. It seems likely that transcripts from the mutant allele are degraded by nonsense mediated decay or other quality control mechanisms, which might lead to a reduced amount of synthesized collagen type V alpha 1 chains and cause the clinical phenotype due to haploinsufficiency, similar to what has been observed in *Col5a1*$^{+/-}$ mice (Wenstrup et al. 2006). An alternative pathomechanism, in which at least some of the mutant protein is expressed and incorporated into defective collagen triple helices can also not be ruled out. The triple-helical structure of collagen makes it particularly sensitive to genetic variants as each mutant protein molecule can potentially oligomerize with up to two wildtype protein molecules, thereby exerting a pronounced dominant negative effect. No information on the parents of the affected cat was available. It is therefore impossible to investigate whether the *COL5A1*:c.3420delG variant was due to a *de novo* mutation event or whether it was actually transmitted by one of the cat's parents. Given the autosomal dominant mode of inheritance, we speculate that this variant is probably limited to the observed case.

In summary, we identified a heterozygous single nucleotide deletion in *COL5A1* in a cat with EDS. The variant was not present in 103 unaffected control cats or a Burmese cat with EDS. The known functional impact of *COL5A1* frameshift variants in humans suggest that the detected feline *COL5A1*:c.3420delG variant is an excellent candidate causative variant for the EDS phenotype in the investigated cat.

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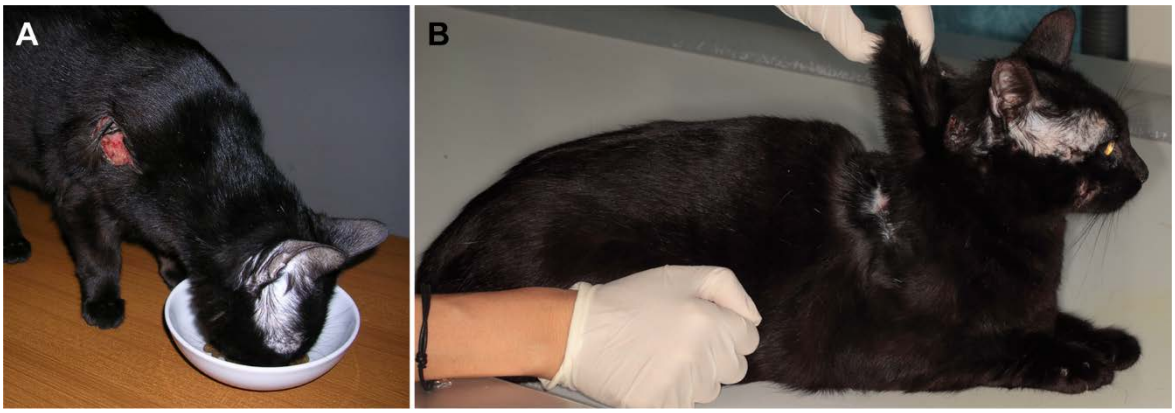

Figure 1. Ehlers-Danlos syndrome in a cat. (A) Skin tear on the right shoulder. (B) Hyperextensibility of the skin.

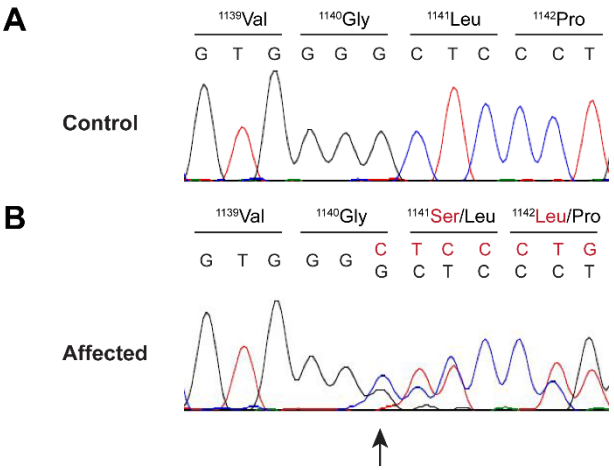

Figure 2. Electropherograms showing the *COL5A1*:c.3420delG frameshift variant. (A) Wildtype sequence of an unaffected cat. (B) Mutant sequence in the affected cat. The heterozygous deletion of a single guanine leading to a frameshift is indicated by an arrow. Wildtype sequences are shown in black, mutant sequences in red.

Supplementary Material

Table S1. Accession numbers of 12 cat genome sequences.

Table S2. Private protein-changing variants in the sequenced cat.