

1 **Identification of an *ADAMTS2* frameshift variant in a cat family with Ehlers-**
2 **Danlos syndrome**

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17 **Running head:** *ADAMTS2* variant in cats with EDS

18 **Keywords:** skin, dermatology, dermatosparaxis, veterinary medicine, *felis catus*, whole genome
19 sequence, precision medicine

20

1 **Abstract**

2 We investigated four European domestic shorthair kittens with skin lesions consistent with the
3 dermatosparaxis type of the Ehlers-Danlos syndrome (EDS), a connective tissue disorder. The
4 kittens were sired by the same tomcat, but were born by three different mothers. The kittens had
5 easily torn skin resulting in non-healing skin wounds. Both clinically and histologically, the skin
6 showed thin epidermis in addition to inflammatory changes. Changes in collagen fibers were
7 visible in electron micrographs. The complete genome of an affected kitten was sequenced. A one
8 base pair duplication leading to a frameshift in the candidate gene *ADAMTS2* was identified,
9 p.(Ser235fs*3). All four affected cats carried the frameshift duplication in a homozygous state.
10 Genotypes at this variant showed perfect co-segregation with the autosomal recessive EDS
11 phenotype in the available family. The mutant allele did not occur in 48 unrelated control cats.
12 *ADAMTS2* loss-of-function variants cause autosomal recessive forms of EDS in humans, mice,
13 dogs, cattle and sheep. The available evidence from our investigation together with the functional
14 knowledge on *ADAMTS2* in other species allow to classify the identified *ADAMTS2* variant as
15 pathogenic and most likely causative variant for the observed EDS.

16

1 Introduction

2 The Ehlers-Danlos syndrome (EDS, *Fibrodysplasia elastica*) represents a group of hereditary
3 disorders associated with defects in collagen biosynthesis (Malfait et al. 2017). This heterogenous
4 group of connective tissue disorders is named after Edvard Ehlers and Henri-Alexandre Danlos
5 who independently described human patients with the syndrome in detail at the beginning of the
6 19th century (Parapia and Jackson 2008).

7 Signs may vary between species and the different types of EDS, e.g. joint hypermobility is
8 primarily observed in humans. However, a universally occurring feature is the hyperelasticity of
9 the skin and the resulting tendency to skin tears. One type of EDS, called dermatosparaxis EDS,
10 was first observed and its biochemical background described in detail in cattle (Lapière et al. 1971)
11 (OMIA 000328-9913). The connective tissue shows alterations of its normal structure due to a
12 deficiency of the enzyme procollagen peptidase, which catalyzes the formation of type 1
13 procollagen (Lapière and Nusgens 1993). The structurally abnormal dermal collagen shows
14 decreased strength, therefore skin wounds can already be caused by minimal trauma like
15 handling or even normal activity (Counts et al. 1980; Crosaz et al. 2013; Hargis and Myers 2017).
16 Histologically, dermatosparaxis skin shows variations in terms of the caliber of collagen fibers,
17 which are irregular, undirected, and loosely arranged (Colige et al. 2004; van Damme et al. 2016).
18 Variants in the *ADAMTS2* gene are known to cause dermatosparaxis in humans (Colige et al. 2004;
19 van Damme et al. 2016) as well as in cattle (Colige et al. 1999), sheep (Zhou et al. 2012;
20 Monteagudo et al. 2015; Joller et al. 2017), and dogs (Jaffey et al. 2019). The *ADAMTS2* gene
21 encodes ADAM metallopeptidase with thrombospondin type 1 motif 2, also termed procollagen
22 I N-proteinase, which cleaves the propeptides of procollagen type I and II (Wang et al. 2003). The
23 role of different ADAMTS proteases for normal collagen biosynthesis and in dermatosparaxis EDS
24 has been reported (Le Goff et al. 2006).

25 Different forms of EDS are known to occur in humans (Malfait et al. 2017; Malfait et al. 2020) as
26 well as in several animal species, including cattle (Hanset and Lapière 1974; Carty et al. 2016;
27 Jacinto et al. 2020), dog (Bauer et al. 2019; Jaffey et al. 2019; Kiener et al. 2022b), sheep (Joller et
28 al. 2017), cat (Counts et al. 1980; Weingart et al. 2014; Spycher et al. 2018), horse (Eßer et al.
29 1999) and mink (Hegreberg 1975). Diagnosis is mainly based on the clinical appearance of the
30 affected animal, histopathological examination of the collagen fibrils, and genetic analyses. In
31 domestic cats, until now, only one gene, *COL5A1*, was reported to be involved in autosomal-
32 dominant EDS (Spycher et al. 2018; Kiener et al. 2022a). Herein we report the results of a
33 comprehensive clinical, pathological and genetic analysis of dermatosparaxis EDS in a cat family.

34 Materials and methods

35 *Ethics statement*

36 All cats in this study were privately owned. The index case, a deceased kitten, was transferred to
37 the Institute of Veterinary Pathology of the Justus Liebig University Giessen for diagnostic
38 purposes. The other three affected kittens were examined and treated at the Small Animal Clinic

1 of the Justus Liebig University Giessen. All animals in this study were examined with the consent
2 of the owner and handled according to good ethical standards. The “Cantonal Committee for
3 Animal Experiments” (Canton of Bern; permit 71/19) and the Regional Council of Gießen
4 (reference number 19 c 20 15 h 02 Gi 19/1 KTV 22/2020) approved the collection of samples from
5 control cats.

6 *Animals*

7 A group of free roaming farm cats (European domestic shorthair) is presented here. Initially, one
8 female kitten with skin lesions resembling the appearance of dermatosparaxis was found dead.
9 Later, three additional affected kittens were observed in two subsequent litters. All three litters
10 that produced affected kittens were apparently from the same sire (tomcat). Subsequently, as
11 many cats as possible (n=27) from this semi-feral population, including mothers, littermates and
12 the tomcat, were captured for sampling and visual inspection. Despite the free roaming lifestyle
13 of the cats, the farmer and owner of the cats was able to provide information about the kinship
14 of the population.

15 *Clinical and pathological examination*

16 Standard clinical and pathological examinations were done. Necropsy was performed on all
17 affected kittens and representative organ samples were fixed in 10% neutral buffered formalin,
18 embedded in paraffin and stained with hematoxylin and eosin (HE). Additionally, histochemical
19 stains were performed on the skin, included periodic acid–Schiff reaction (PAS) and Masson
20 Trichrome stain. The skin of one affected kitten was examined by transmission electron
21 microscopy. For this purpose skin samples were fixed with 1.5% glutaraldehyde and 1.5%
22 formaldehyde (freshly made from paraformaldehyde) in 0.15 M HEPES buffer. For epoxy resin
23 embedding, cells were postfixed in 1 % osmium tetroxide in aqua bidest., stained in half-saturated
24 watery uranyl acetate, dehydrated in an ascending ethanol series and finally embedded in Agar
25 100 (Agar scientific Ltd. UK). Ultrathin sections were cut using an ultramicrotome (Reichert
26 Ultracut E, Leica) and examined with a transmission electron microscope (Zeiss EM 902). Digital
27 images were captured with a slow-scan 2K CCD camera (TRS, Tröndle, Moorenweis, Germany).

28 *DNA extraction*

29 For the purpose of whole genome sequencing, genomic DNA was isolated from muscle tissue of
30 the deceased kitten using a Maxwell RSC Tissue DNA Kit and a Maxwell RSC instrument (Promega,
31 Dübendorf Switzerland). For genotyping, DNA extraction from buccal swabs (sterile transport
32 swabs, COPAN Italia SpA, Brescia Italy) was executed using the Gentra Puregene Tissue Kit
33 (QIAGEN GmbH, Hilden Germany) following the manufacturer’s instructions.

34 *Whole genome sequencing, variant calling and variant filtering*

35 An Illumina TruSeq PCR-free DNA library with ~330 bp insert size of the deceased affected cat was
36 prepared and sequenced on a NovaSeq 6000 instrument with 23× coverage (Illumina, San Diego,
37 CA, USA). The sequence data were submitted to the European Nucleotide Archive with the study
38 accession PRJEB7401 and sample accession SAMEA7376282. Mapping and alignment to the

1 *F.catus* Fca126 mat1.0 reference genome assembly were performed as described (Jagannathan
2 et al. 2019). Variant calling was performed using GATK HaplotypeCaller (McKenna et al. 2010) in
3 gVCF mode as described (Jagannathan et al. 2019). Functional effects of the called variants were
4 predicted with the SnpEff version 4.3t software (Cingolani et al. 2012) together with NCBI
5 annotation release 105 for the *F.catus*_Fca126_mat1.0 genome reference assembly.

6 For variant filtering, we used 77 control genomes (Table S1). A hard filtering strategy was
7 employed, which required either a homozygous alternate (1/1) or heterozygous (0/1) genotype
8 call in the affected kitten while the 77 control cats were required to have either a homozygous
9 reference (0/0) or missing (./.) genotype call in the vcf-file (Table S2). Variants in 20 known
10 functional candidate genes for EDS obtained from Kiener et al. 2022b were prioritized.

11 *Genotyping by Sanger sequencing*

12 The *ADAMTS2* variant was genotyped by Sanger sequencing of PCR amplicons
13 (XM_023254116.2:c.698dup or ChrA1:90,995,621dup (*F.catus*_Fca126_mat1.0 assembly). A
14 forward primer 5'-TTCAATGTACCTGGCAAGCC-3' and a reverse primer 5'-
15 ATGCTGCAGATGGTGACTAC-3' were designed with the software Primer3 (Untergasser et al. 2012)
16 to produce a fragment with a size of 169 bp (wild type) or 170 bp (mutant) with standard PCR
17 conditions. Purified PCR products were sent to LGC Genomics GmbH, Berlin (Germany) for Sanger
18 sequencing, using the reverse primer. A similar approach was used to genotype the
19 *COL1A2*:XM_003982764.6:c.2384G>A variant, using 5'- TCCCTAGAGCTGCCATTGAT-3' and 5'-
20 GAGGCAAGGTTGTTGGCTA-3' as forward and reverse primer, respectively (152 bp fragment
21 size).

22 *Parentage testing*

23 A DNA profile, based on 16 microsatellite markers, for parentage verification was commissioned
24 from Laboklin GmbH & Co KG (Bad Kissingen, Germany). It was carried out with genomic DNA
25 from the three mother cats, the four affected kittens and the presumed father.

26 **Results**

27 *Clinical and pathological findings*

28 The initial case, a deceased female kitten of unknown age was in good body condition (weight: 1
29 kg). Body and tissues were affected by moderate postmortem changes. In addition to moderate
30 anemia, the skin was markedly thin and soft and was easily torn. Large portions of the head, as
31 well as the left side of the neck, exhibited extensive alopecia and severe multifocal ulcerative and
32 purulent dermatitis, occasionally accompanied by partially detachable dark brown crusts up to 1
33 cm thick (Figure 1A). Additionally, there was a prolapse of the rectum (Figure 1B) as well as an
34 invagination in the colon involving 3 cm of the large intestine with venous infarction of the
35 invaginated part (intussusceptum). A diaphragmatic hernia, through which the stomach and large
36 portions of the omentum majus entered the thoracic cavity (Figure 2), were also observed. The
37 urea concentration in the aqueous humor was 20 mmol/l (reference value: 5.0 – 11.3 mmol/l).

1 Three additional affected kittens from the following litters showed similar dermatological lesions
2 as the first kitten (Figure 1C-D). During handling, the skin was easily torn and preexisting wounds
3 were exacerbated even by gentle manipulation. Wounds in different stages and sizes were
4 present. The head, ventral neck and front legs and axillar region were most severely affected in
5 all three cats, distribution of the lesions was more or less symmetrical. In addition, these kittens
6 showed significantly reduced growth compared to their unaffected littermates. One of the cats
7 was euthanized at first presentation due to an impaired general condition. In two of the three
8 kittens a symptomatic therapy with topical wound care and systemic anti-infective treatment was
9 attempted, but due to progressive deterioration, both cats were humanely euthanized 5 days and
10 33 days after start of therapy, respectively.

11 Histological examination revealed that the skin of the initial case was multifocally affected by both
12 a mild to severe chronic pyogranulomatous and an acute ulcerative and suppurative dermatitis
13 accompanied by serocellular crust formation, which contained bacteria (Figure 3A). Adjacent to
14 the ulcerative lesions, cleft-formation at the dermo-epithelial junction was often observed (Figure
15 3A). The Periodic acid-Schiff reaction revealed that the basement membrane zone formed the
16 floor of the cleft (Figure 3B). The collagen fibers stained uniformly blue with Masson Trichrome
17 stain (Figure 3C) and showed a loose arrangement around the clefts. In the unaffected skin
18 epidermis, dermis and adnexa were present and the collagen fibers were arranged
19 physiologically. The invagination in the colon was accompanied by a moderate to severe chronic
20 suppurative colitis characterized by a moderate to high amount of mononuclear cells infiltrating
21 the intussusceptum while the part of the colon containing the invaginated part (intussusciens)
22 was infiltrated with macrophages and neutrophil granulocytes.

23 Electron microscopy of the skin of one of the affected kittens showed severe abnormalities in the
24 collagen fibers. The longitudinal section showed electron-loose parts framed by electron-dense
25 filaments suggesting an “empty-tube” appearance. Cross section of collagen fibers showed
26 electron dense ribbon-like structures up to 250 nm in diameter (Figure 4).

27 *Genetic Analyses*

28 The genome of one affected cat was sequenced at 23x coverage. Genome sequences from 77 cats
29 representing 14 breeds and 35 random-bred individuals and one of unknown origin were used as
30 controls. Filtering for private protein-changing variants in the affected cat identified two
31 potentially pathogenic variants in known EDS candidate genes, a heterozygous missense variant
32 in *COL1A2* and a homozygous frameshift variant in the *ADAMTS2* gene (Table 1; Table S2).
33 Genotyping of cats from the pedigree excluded the *COL5A1* variant as the genotypes did not co-
34 segregate with the EDS phenotype and four unaffected cats were homozygous for the mutant
35 allele (Table S3). The *COL1A2* variant was XM_003982764.5:c.2384G>A or
36 XP_003982813.1:p.(Arg795Gln).

37 Visual inspection of the short-read alignments in IGV (Robinson et al. 2011) indicated a
38 homozygous insertion of a single base pair in exon 4 of the 22 annotated exons of the known

1 candidate gene *ADAMTS2* (Figure 5). This variant can be designated as
2 XM_023254116.2:c.698dup or XP_023109884.2:p.(Ser235Glnfs*4). It truncates nearly 80% of the
3 wild type *ADAMTS2* open reading frame. The genomic variant designation is
4 ChrA1:90,995,621dup (*F.catus_Fca126_mat1.0*).

5 The *ADAMTS2* variant was confirmed via PCR and follow-up Sanger sequencing. All available cats
6 (n=31) were genotyped for the variant (Figure 6; Table S3). All four affected kittens were
7 homozygous for the mutant allele. Twenty cats were heterozygous, including the parents of
8 affected kittens as well as some of their littermates. The remaining seven cats were homozygous
9 for the wild type allele. Microsatellite-based parentage testing confirmed the paternity of the
10 suspected tomcat for all three litters, in which the four affected kittens occurred (Table S4).

11 Discussion

12 EDS in humans is known to occur in 13 different subtypes including the autosomal recessive
13 dermatosparaxis EDS (dEDS) caused by *ADAMTS2* variants (Malfait et al. 2017). So far, in domestic
14 cats only classical EDS (cEDS) caused by autosomal dominant *COL5A1* variants has been
15 characterized at the molecular level (Spycher et al. 2018; Kiener et al. 2022a)

16 In this study, we describe a dermatosparaxis EDS phenotype in domestic cats due to autosomal
17 recessive loss of function in the *ADAMTS2* gene by a comprehensive clinical, pathological and
18 genetic analysis in a cat family. *ADAMTS2* loss-of-function variants cause autosomal recessive
19 forms of EDS in humans, mice, dogs, cattle and sheep but have so far not been reported in
20 domestic cats.

21 During the gross and histological examination of the initial case (first deceased kitten), the skin
22 appeared easily torn. Almost the entire head area and the left side of the neck showed focal
23 alopecia and severe ulcerative purulent dermatitis with serocellular crusts. Similar clinical findings
24 were present in the dermatological examination of the other three kittens, with the exception
25 that fresh wounds with less crusting and without secondary pyoderma predominated. In all
26 affected cats, the head, neck and front legs /axilla were most severely affected, which probably
27 resulted from physiological friction and strain to the skin in these body regions. These
28 dermatological findings were consistent with the presence of collagen dysplasia
29 (dermatosparaxia) in other species (overview given by (Vroman et al. 2021). For example, in
30 hereditary equine regional dermal asthenia (HERDA), body sites exposed to stress or pressure are
31 most prone to similar lesions (Rashmir-Raven 2013). Comparable dermatological phenotypes can
32 also be observed when caused by variants in *ADAMTS2*, such as in dogs (Jaffey et al. 2022). In
33 previously reported cats with EDS, in which the molecular cause was not identified, skin fragility
34 and predisposition to skin tears was also described as the main clinical finding (Crosaz et al. 2013;
35 Hansen et al. 2015). Normal handling or even the normal activity of the animal may lead to skin
36 injuries (Counts et al. 1980; Crosaz et al. 2013; Hargis and Myers 2017).

37 Hypermobility of the joints, as described for examples in humans and dogs with EDS, has not been
38 described in cats (Mauldin and Peters-Kennedy 2015) similar to the present cases. Histologic

1 examination of the skin showed no abnormalities except for focal loose arrangement of collagen
2 fibers and cleft formation. This might result from the severe ulceration and inflammation and has
3 to be differentiated from subepidermal blistering diseases. Lack of joint hypermobility and
4 variation regarding the caliber of the collagen fibers with irregular, undirected, and loose
5 arrangement have been described as typical for dermatosparaxis EDS (Gross et al. 2008). Apart
6 from multifocal loose arrangement of collagen fibers, the skin of the necropsied cat showed a
7 regular anatomical morphology, however, histologic findings may vary in cats with collagen
8 dysplasia ranging from no dermal changes up to a thinner dermis with fine collagen fibers
9 separated by an increased amount of ground substance. Normal collagen fibers stain uniformly
10 blue with Masson Trichrome stain as in this case whereas abnormal fibers have segmental red
11 staining areas that are birefringent under polarized light (Butler 1975; Holbrook et al. 1980;
12 Sequeira et al. 1999; Crosaz et al. 2013; Mauldin and Peters-Kennedy 2015).

13 Due to the postmortem changes in most of the affected kittens, the skin of only one cat was
14 examined by electron microscopy and revealed empty tube appearance of collagen fibers typical
15 for EDS. The inflammatory skin lesions were likely due to secondary infections and not primarily
16 associated with dermatosparaxis EDS. The same applies in all likelihood also for the follicular
17 hyperplasia of the mesenteric lymph nodes. A hernia diaphragmatica (Figure 2), also observed in
18 one of the present cases, has been previously described in cats with collagen dysplasia (Benitah
19 et al. 2004). It is also possible that the rectal prolapse (Figure 1B) represented a consequence of
20 the collagen disturbances due to dermatosparaxis EDS but might also have resulted from the colo-
21 colic intussusception. The accompanied chronic purulent colitis suggested the presence of a
22 bacterial infection and might have been the cause for intussusception (Uzal et al. 2016). Hernia
23 diaphragmatica or rectal prolapse or any other clinical sign except the cutaneous lesions were not
24 present in other kitten affected by EDS.

25 Different variants within the *ADAMTS2* were already proven to be causative for cases of
26 dermatosparaxis EDS in humans (van Damme et al. 2016), sheep (Zhou et al. 2012; Monteagudo
27 et al. 2015; Joller et al. 2017), cattle (Colige et al. 1999), and dogs (Jaffey et al. 2019; Jaffey et al.
28 2022). The human ClinVar database lists NM_014244.4(*ADAMTS2*):c.691del as a pathogenic
29 variant. This variant also introduces a frameshift at a position comparable to the feline c.698dup
30 variant. The feline *ADAMTS2* frameshift variant detected herein therefore represents a highly
31 plausible candidate variant for the EDS phenotype in the affected cats. The causality of the
32 *ADAMTS2* frameshift variant is further supported by the perfect co-segregation of genotypes with
33 phenotypes in an extended pedigree with 31 cats, of which four were affected.

34 When we apply the ACMG/AMP consensus criteria for human diagnostics (Richards et al. 2015)
35 to the feline *ADAMTS2*:c.698dup frameshift variant, we have one very strong evidence for
36 pathogenicity (null variant in a gene, where loss of function is a known mechanism of disease,
37 PVS1), one moderate criterion (mutant allele is absent from 77 control genomes, PM2) and one
38 supporting evidence (co-segregation with disease in multiple affected members, PP1). Taken
39 together, these three lines of evidence allow to classify *ADAMTS2*:c.698dup as pathogenic.

1 The autosomal recessive disorder analyzed herein phenotypically resembles an EDS form that
2 Hansen et al. (2015) already described for a case in Burmese cats (Hansen et al. 2015). No
3 molecular genetic analysis was reported in that case. In contrast, different previously identified
4 variants in the *COL5A1* gene were involved in autosomal dominant classical EDS cases in cats
5 (Spycher et al. 2018; Kiener et al. 2022a). Similar to EDS in humans, there are different types of
6 this syndrome in animals that show locus heterogeneity and different modes of inheritance
7 (Malfait et al. 2017).

8 Our analysis suggests that inbreeding within a population of free-roaming farm cats has provoked
9 the outbreak of a lethal recessive disease. The genome of the sequenced case did not show a
10 particularly high level of homozygous variant calls. Nonetheless, the results of our study are in
11 agreement with a more representative study reporting 19% of UK non-pedigree cats with a higher
12 than expected content of homozygous genome regions due to recent inbreeding events (Irving
13 McGrath et al. 2021). Potential health risks due to inbreeding should be kept in mind when
14 managing free-roaming cat populations.

15 **Conclusion**

16 In summary, we describe the *ADAMTS2:c.698dup* frameshift variant as a highly plausible
17 candidate causative variant for dermatosparaxis EDS, an autosomal recessive form of EDS in cats.
18 Similar *ADAMTS2* variants have been reported in humans, cattle, sheep and dogs with
19 dermatosparaxis EDS. The functional knowledge from other species and the perfect co-
20 segregation of the genotypes with the phenotype in a medium sized cat family support the
21 causality of the detected *ADAMTS2:c.698dup* variant. Our findings enable genetic testing that can
22 be used to detect healthy carriers and to eradicate this potentially lethal disease from the cat
23 population.

24

25 **Data Availability Statement:**

26 The whole genome sequence data from this study is publicly available from ENA (European
27 Nucleotide Archive). The accessions are listed in Table S1. Supplemental Material provided at
28 figshare: <https://doi.org/10.25387/g3.22809347>.

29

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35 infrastructure.

36 **Conflict of interest:**

37 The authors declare no conflict of interest.

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9
 10 **Table 1.** Results of variant filtering in the affected cat against 77 control genomes.

Filtering step	Heterozygous	Homozygous
All variants in the sequenced cat	6,011,674	5,983,799
Private variants	70,995	20,434
Protein-changing private variants	353	81
Protein-changing private variants in functional candidate genes	1	1

11

1 Figures

2 **Fig. 1.** Gross condition of the deceased affected kittens (A-B initial case; C-D from second and third
3 litter). A) Almost the entire head and the left neck showed extensive alopecia and severe multifocal
4 ulcerative and purulent dermatitis occasionally accompanied by barky, dark-brown crust formation
5 (arrow). The oral mucosa was moderately anemic. B) A rectal prolapse was also present in the initial case
6 (arrow). C) After surgical treatment: severe loss of the epidermis especially in the cranial body regions
7 (head, neck, forelimbs) with severe ulcerative partly crustose dermatitis and fragile skin, that tore at light
8 touch (arrow); D) Kitten with a milder course, gross lesions were found exclusively on the head (temples
9 and mucocutaneous junctions) with mild to moderate ulceration and crusting (arrow).

10 **Fig. 2.** Abdominal cavity (lower and middle part of the figure) with a diaphragmatic hernia (arrows). The
11 stomach and large parts of the omentum majus passed through this defect into the thoracic cavity,
12 which is located behind the diaphragm in the upper part of the figure.

13 **Fig. 3.** Skin of the head of an affected kitten. A) haemotoxylin & eosin stain: Severe chronic
14 pyogranulomatous (arrowhead) and severe acute ulcerative and suppurative dermatitis (black arrows)
15 accompanied by serocellular crusts which contained bacteria (asterisk). Cleft-formation (white arrows)
16 was observed at the dermo-epithelial junction adjacent to the ulcerative lesions. B) Periodic acid-Schiff
17 reaction: Cleft-formation at the dermo-epithelial junction. The basement membrane zone (arrows)
18 formed the floor of the cleft. C) Masson Trichrome stain: Collagen fibers were stained uniformly blue
19 and loosely arranged (asterisks) in the area of the clefts.

20 **Fig. 4.** Longitudinal and cross section of collagen fibers (affected kitten): thin ribbon-like electron-dense
21 fibrils appear disordered with an electron-lucent central area (hollow appearance).

22 **Fig. 5.** The EDS-associated *ADAMTS2* variant on chromosome A1. A) Integrative Genome Viewer
23 screenshot of the affected cat's sequence data indicates a one base pair insertion within a polyC stretch.
24 In the IGV alignment the insertion/duplication is at the left end of this C-stretch. However, according to
25 the 3'-rule of HGVS, the variant is annotated as ChrA1:90,995,621dup. Coordinates refer to the
26 *F.catus_Fca126_mat1.0* assembly. Lower case letters indicate intronic, uppercase letters indicate exonic
27 bases. B) Sanger electropherograms of an unaffected (top), a heterozygous (middle) and an affected cat
28 homozygous for the mutant allele (bottom). Please note that Sanger sequencing was conducted using a
29 reverse primer. Therefore, overlapping electropherogram peaks appear to the left of the heterozygous
30 insertion/duplication. C) Phenotype of a healthy kitten (left) and an EDS-affected sibling showing typical
31 skin lesions and growth retardation (right).

32 **Fig. 6.** Pedigree of cat family comprising three litters with affected kittens, all sired by the same father.
33 Litters 1-3 are consistently numbered in Table S3 and S4. Males are shown as squares and females as
34 circles. Open symbols indicate unaffected cats, which may be heterozygous carriers of the
35 *ADAMTS2:c.698dup* variant as stated in the individuals' genotypes. All affected individuals, homozygous
36 for the *ADAMTS2* frameshift duplication, are deceased and indicated by filled strikethrough symbols.

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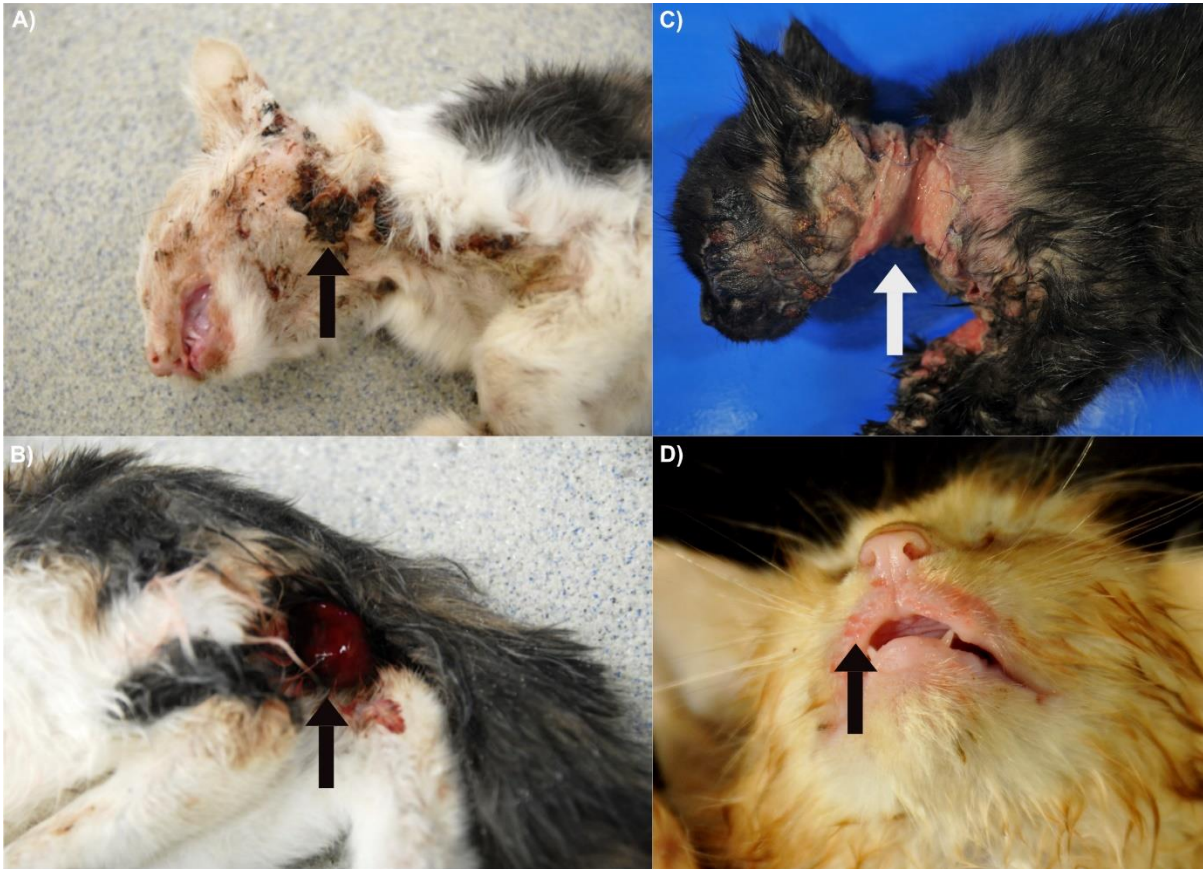


Figure 1
160x115 mm (x DPI)

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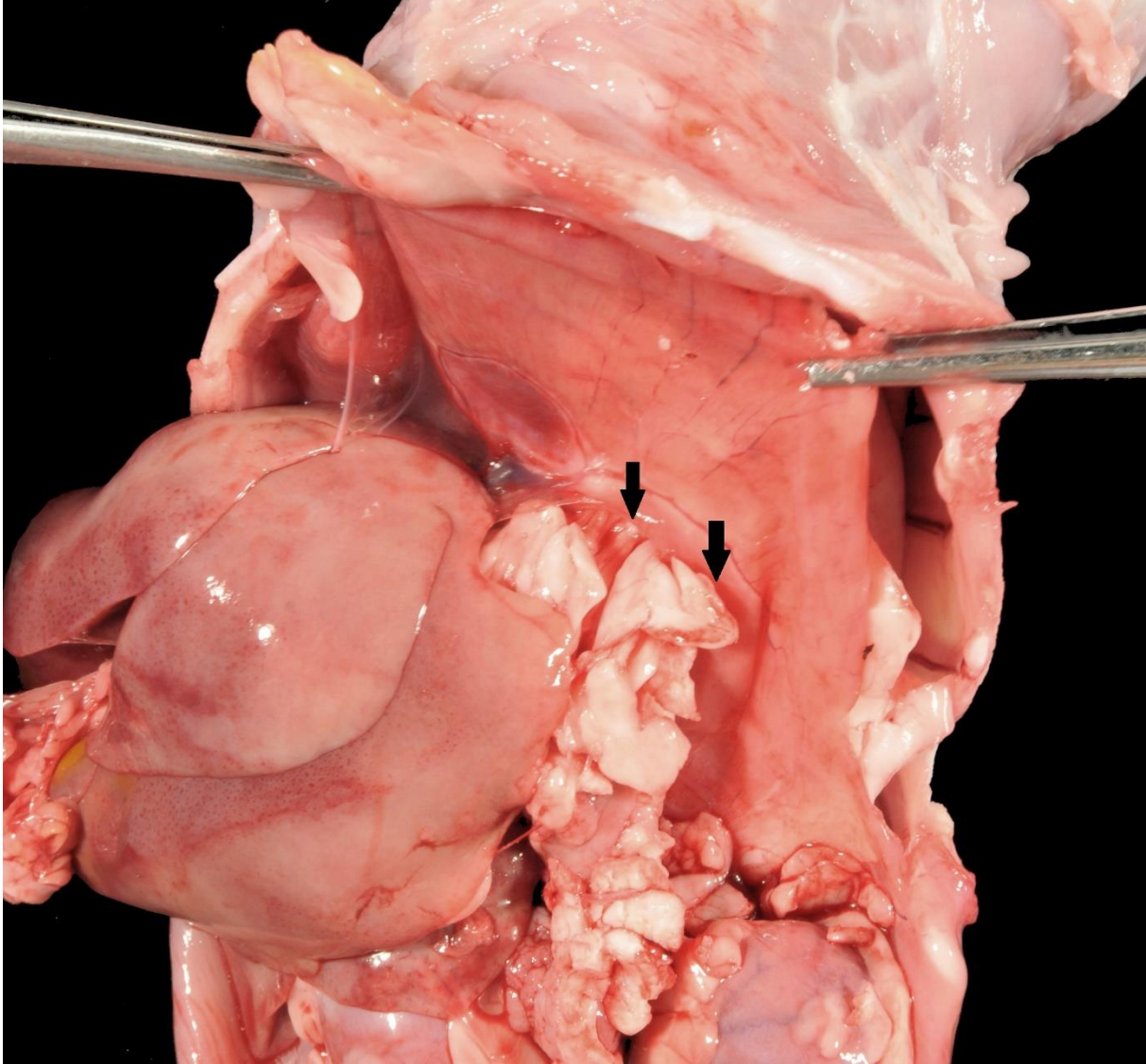


Figure 2
160x149 mm (x DPI)

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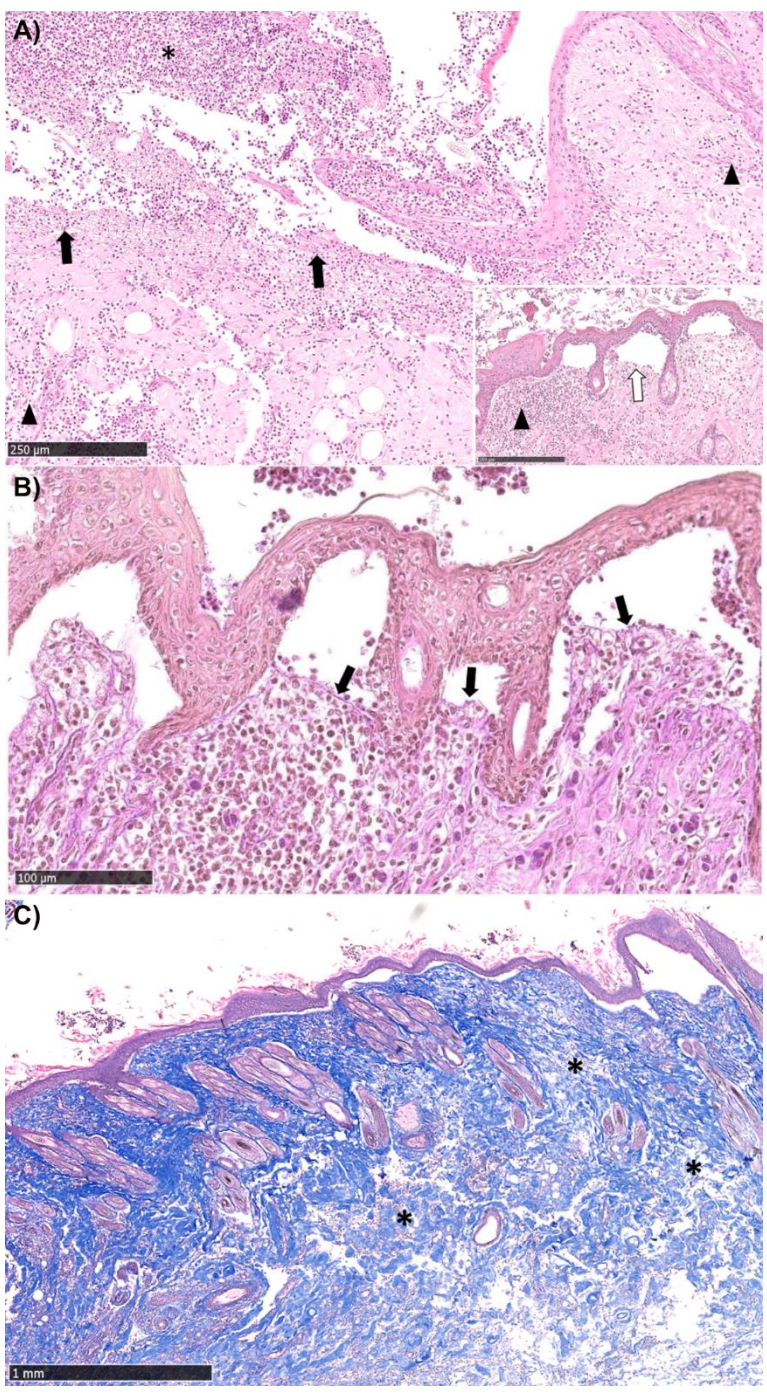
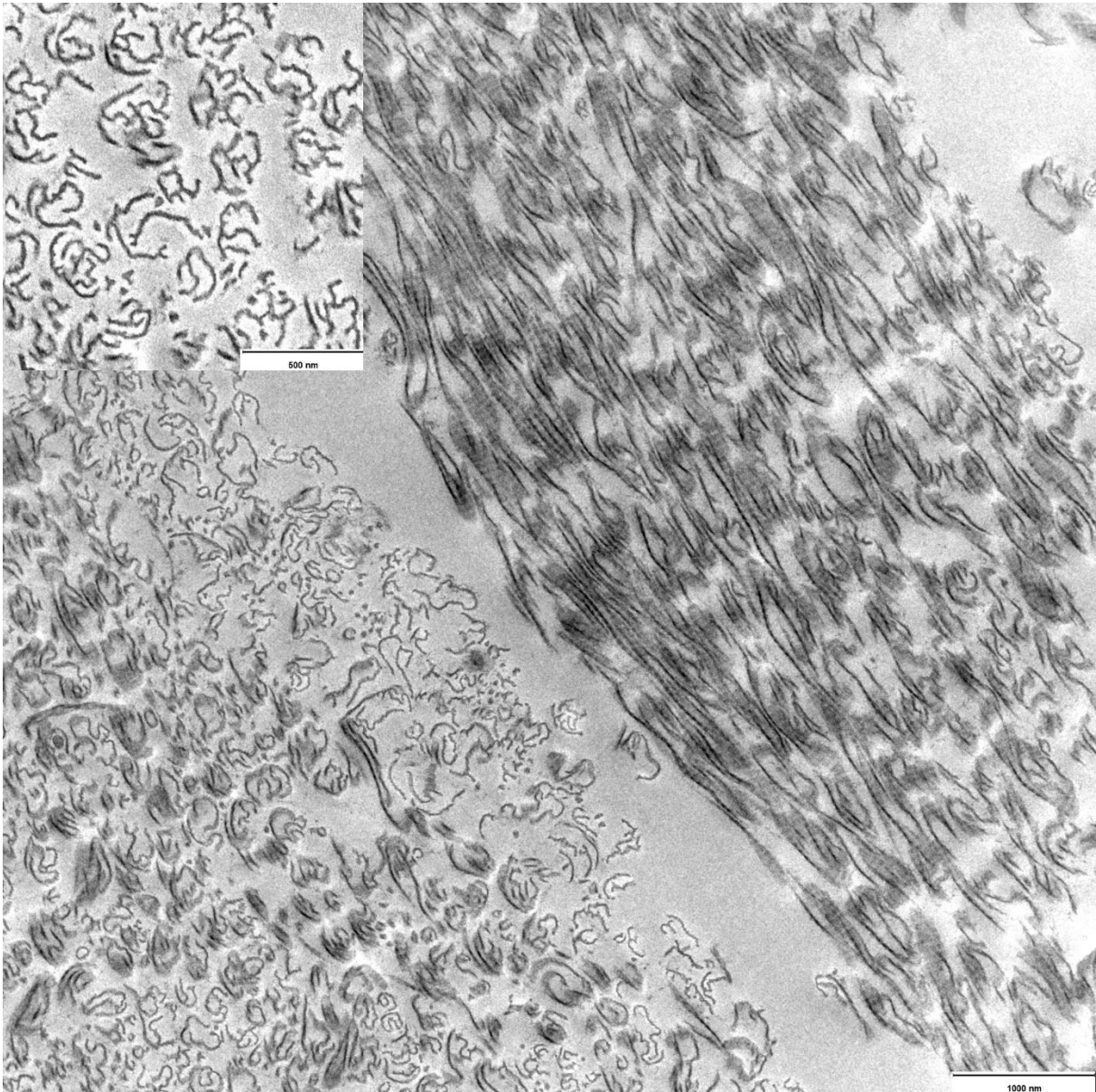


Figure 3
100x182 mm (x DPI)

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Figure 4
160x160 mm (x DPI)

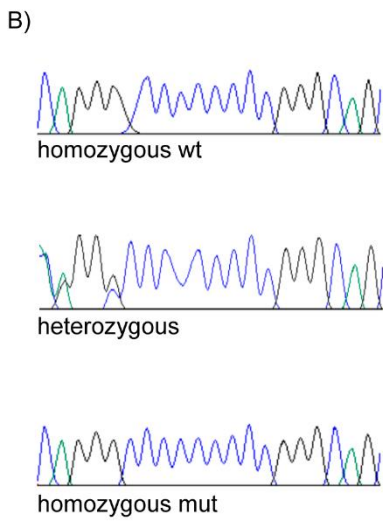
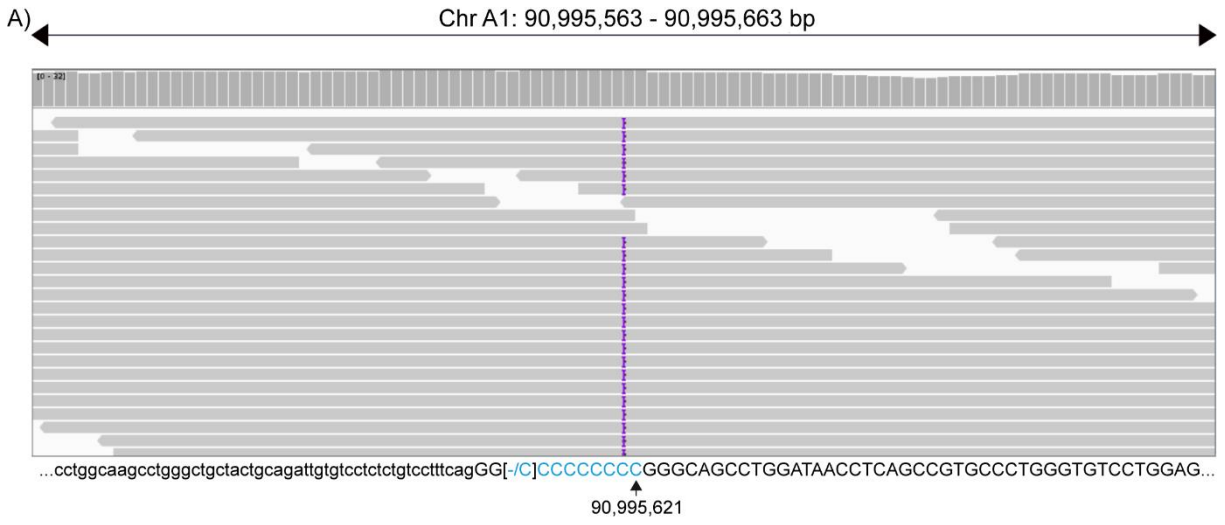


Figure 5
160x141 mm (x DPI)

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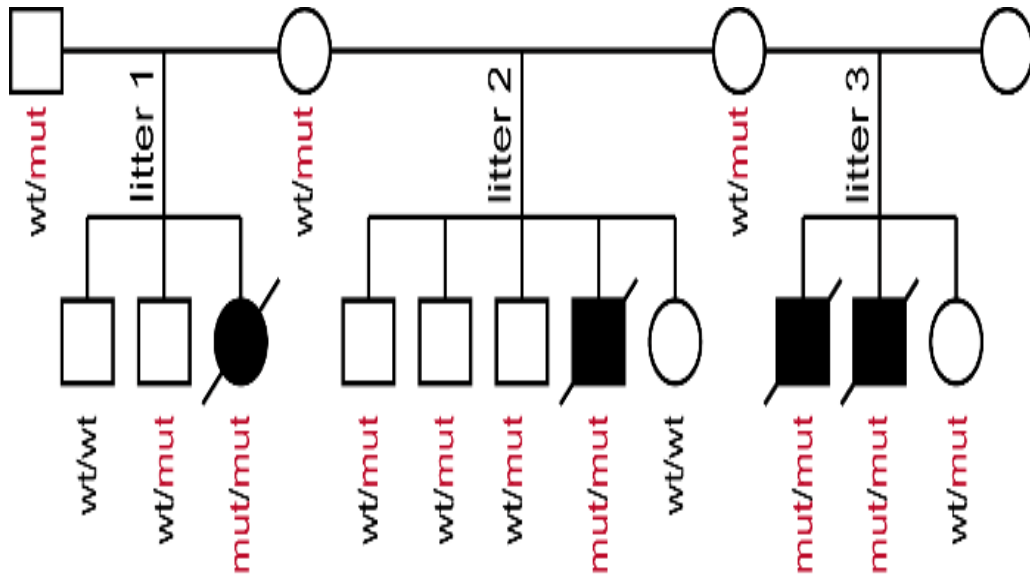


Figure 6
80x27 mm (x DPI)