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Precision medicine using whole genome sequencing in a cat identifies a novel COL5A1 variant for classical Ehlers-Danlos syndrome

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Abstract

Background: Ehlers-Danlos syndromes (EDS) are a heterogeneous group of heritable connective tissue disorders occurring in both human and veterinary patients. The genetics of these disorders are poorly described in small animal patients.

Hypothesis/Objectives: Define the clinical manifestations and genetic cause of a suspected form of EDS in a cat.

Animals: A 14-week-old male domestic medium hair cat was presented with skin hyperextensibility and fragility. The classic tragic facial expression was observed as well as chronic pruritus and mild hyperesthesia.

Methods: Blood samples and a skin biopsy sample were collected from the affected cat. Clinical examinations, histology, electron microscopy and whole genome sequencing were conducted to characterize the clinical presentation and identify possible pathogenic DNA variants to support a diagnosis. Criteria defining variant pathogenicity were examined including human disease variant databases.

Results: Histology showed sparse, disorganized collagen and an increase in cutaneous mast cells. Electron microscopy identified ultrastructural defects commonly seen in *collagen type V alpha 1 chain (COL5A1)* variants including flower-like collagen fibrils in cross-section. Whole genome sequencing and comparison with 413 cats in the 99 Lives Cat Genome Sequencing Consortium database identified a novel splice acceptor site variant at exon 4 in *COL5A1* (c.501-2A>C).

Abbreviations: Bp, base pair; cEDS, classical Ehlers-Danlos syndrome; *COL5A1*, *collagen type V alpha 1 chain*; EDS, Ehlers-Danlos syndromes; H&E, hematoxylin & eosin; SAM, systolic anterior motion; SEI, skin extensibility index; TEM, transmission electron microscopy; WGS, whole genome sequencing.

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Conclusions and Clinical Importance: Our report broadens the current understanding of EDS in veterinary patients and supports the use of precision medicine techniques in clinical veterinary practice. The classification of variants for pathogenicity should be considered in companion animals.

KEYWORDS

animal models, cutaneous asthenia, dermatosparaxis, Ehlers-Danlos syndrome, *Felis catus*, precision medicine, whole genome sequencing

1 | INTRODUCTION

Ehlers-Danlos syndromes (EDS) are a heterogeneous group of heritable connective tissue disorders affecting collagen synthesis and processing. The collagen abnormalities lead to variable degrees of joint hypermobility, skin hyperextensibility and fragility, and vascular and visceral fragility. In humans, 13 distinct types of the disorder have been described.¹ In veterinary medicine, EDS has been documented in cats, dogs, horses, cattle, sheep, rabbits, and mink,²⁻⁸ but is likely underdiagnosed.

Ehlers-Danlos syndrome (OMIA 002165-9685) was first described in cats in 1974 and also is referred to as cutaneous asthenia or dermatosparaxis.^{3,9} Both autosomal recessive and dominant modes of inheritance are suspected in cats.⁹⁻¹² Individual DNA variants are identified in the Bengal and Bombay breeds, as well as 2 different variants in random-bred shorthair cats. Unfortunately, commercial genetic testing for EDS is not yet available for cats, requiring a diagnosis based on clinical signs such as skin fragility and hyperextensibility. The skin extensibility index (SEI), which is defined as the length of the dorsal skin fold divided by the length from occiput to tail base \times 100%, is a screening test for EDS in cats, with a score of $\geq 19\%$ suggesting presence of the disorder.^{11,13}

To date, information on the genetics of EDS in cats is increasing but limited. In the 1980s, 2 case reports used transmission electron microscopy (TEM) and Western blotting to describe aberrant processing of the N-terminal propeptide of type I procollagen, as seen in humans, cattle, sheep, and dogs with dermatosparaxis EDS caused by a mutation in a *disintegrin and metalloproteinase with thrombospondin*

motifs 2 (ADAMTS2).^{1,2,8-10,14} Recently, several *collagen type V alpha 1 chain (COL5A1)* mutations have been described in cats, a finding that is associated with classical EDS (cEDS) in humans. A heterozygous deletion, c.3420del in *COL5A1* exon 43 leading to a frameshift mutation and premature stop codon was first described in a domestic shorthair cat in 2018.¹⁵ After the initial discovery, 3 new *COL5A1* mutations were reported in cats.¹⁶ The mutations are a c.112_118+15del a 22-base pair (bp) deletion removing the boundary between exon 1 and intron 1, a c.3514A>T nonsense variant predicted to truncate 36% of the coding sequence, and a 1 bp deletion at c.3066del leading to a frameshift mutation predicted to truncate 44% of the open reading frame.¹⁶

Herein the clinical, histopathological, ultrastructural, and genetic findings of a 4-year-old male castrated domestic medium hair cat presented with clinical signs consistent with cEDS are reported. Using whole genome sequencing (WGS) and comparison to the 99 Lives Cat Genome Sequencing Consortium database, a novel splice site variant in *COL5A1* was implicated in causing EDS in the affected cat.

2 | CASE DESCRIPTION

2.1 | Clinical description

A male domestic medium hair cat was surrendered to a shelter with an unaffected sibling at 12 weeks of age (Figure 1A). The first skin laceration was noted at 14 weeks of age. While at the rescue facility, the



FIGURE 1 Ehlers-Danlos syndrome in a 4-year-old castrated male medium hair cat. (A) Note the characteristic “tragic” facial expression with ptosis and prominent jowls. (B) Laceration on the ventral neck. (C) Demonstration of skin hyperextensibility.



FIGURE 2 Cutaneous self-trauma was significantly mitigated by a declaw procedure in an EDS cat. (A) Self trauma to the skin of the dorsal cervical region indicated by lacerations and eschars at approximately 5-months-of-age. (B) Healthy, intact skin over the dorsal cervical region 8-months post hind declaw procedure.

cat experienced recurrent skin lacerations (Figure 1B), atrophic scarring, and large eschars over the dorsal cervical region. The cat was noted to have thin and hyperextensible skin with an SEI of 27.4% (Figure 1C). In addition, the classic tragic facial expression observed in many EDS cats was present, consisting of ptosis and prominent jowls. A presumptive diagnosis of EDS was made at 6 months of age and the animal then was relinquished for adoption.

The cat was adopted at 7 months of age by a veterinarian and was well managed in a multianimal household. Chronic pruritus was observed and is thought to occur secondary to chronic mast cell activation, a condition observed in up to 66% of human patients with EDS and postural orthostatic tachycardia syndrome.¹⁷ Management has included hind limb declawing to mitigate self-trauma, particularly to the dorsal cervical region and face (Figure 2A,B), and clothing and a soft fabric Elizabethan collar additionally were used to prevent cutaneous trauma. Small lacerations are managed using cyanoacrylate (skin glue) whereas larger lacerations are repaired surgically. Anesthesia is well tolerated.

Clinical signs consistent with mild hyperesthesia, such as twitching of the lumbosacral skin, vocalization, and excessive biting or grooming of skin over the dorsal lumbar spine have been observed. These signs are well managed using amitriptyline (1 mg/kg PO q12h) to prevent mast cell degranulation and gabapentin (10 mg/kg PO q12h) for perceived neuropathic pain. Treatment with corticosteroids and fluoroquinolones is avoided because of the risk of further damage to collagen. Lacerations, seromas, and cutaneous abscesses have decreased in frequency with age and diligent management.

The cat also has been noted to have a mild lower motor neuron paraparesis (Supplementary Video S1), medial patellar luxation, mild dysphagia, and episodic constipation. Echocardiography has been unremarkable apart from mild systolic anterior motion (SAM) of the mitral valve.

2.2 | Histopathology

Histopathology was performed using standard techniques on a skin biopsy specimen obtained during routine wound repair from the affected animal and from a control cat. Staining with hematoxylin & eosin (H&E), Masson's trichrome, and Giemsa was performed.

Staining with H&E identified loosely arranged collagen bundles with shortened, irregularly arranged collagen fibers when compared to a sample from a control cat (Figure 3A,B). Collagen fibrils containing red cores after Masson's trichrome staining were not observed, in contrast to previously described EDS cases in cats.¹⁸ Giemsa staining disclosed an increased number of cutaneous mast cells in the superficial dermis with >25-30 mast cells at 400 \times , which is increased above the published normal for cats of 7-20 mast cells at 400 \times ,¹⁹ a finding repeated in the control sample.

2.3 | Ultrastructure

Transmission electron microscopy (TEM) was performed according to standard procedure using a 2 mm skin biopsy sample. Samples were obtained during routine wound repair in the affected animal and from a control cat. The samples were fixed in glutaraldehyde/paraformaldehyde. The affected sample was embedded in epon-araldite and the control sample was embedded in SpiPon 812 according to standard procedure.^{20,21} Ultrathin sections (80 nm) were cut (Reichert-Jung Ultracut E; Wetzler, Germany; Leica EM UC7; Wetzler, Germany) and poststained with uranyl acetate and lead citrate.

Imaging with TEM identified ultrastructural defects commonly seen in humans and cats with COL5A1 mutations including flower-like collagen fibrils in cross-section, which were characterized by enlarged

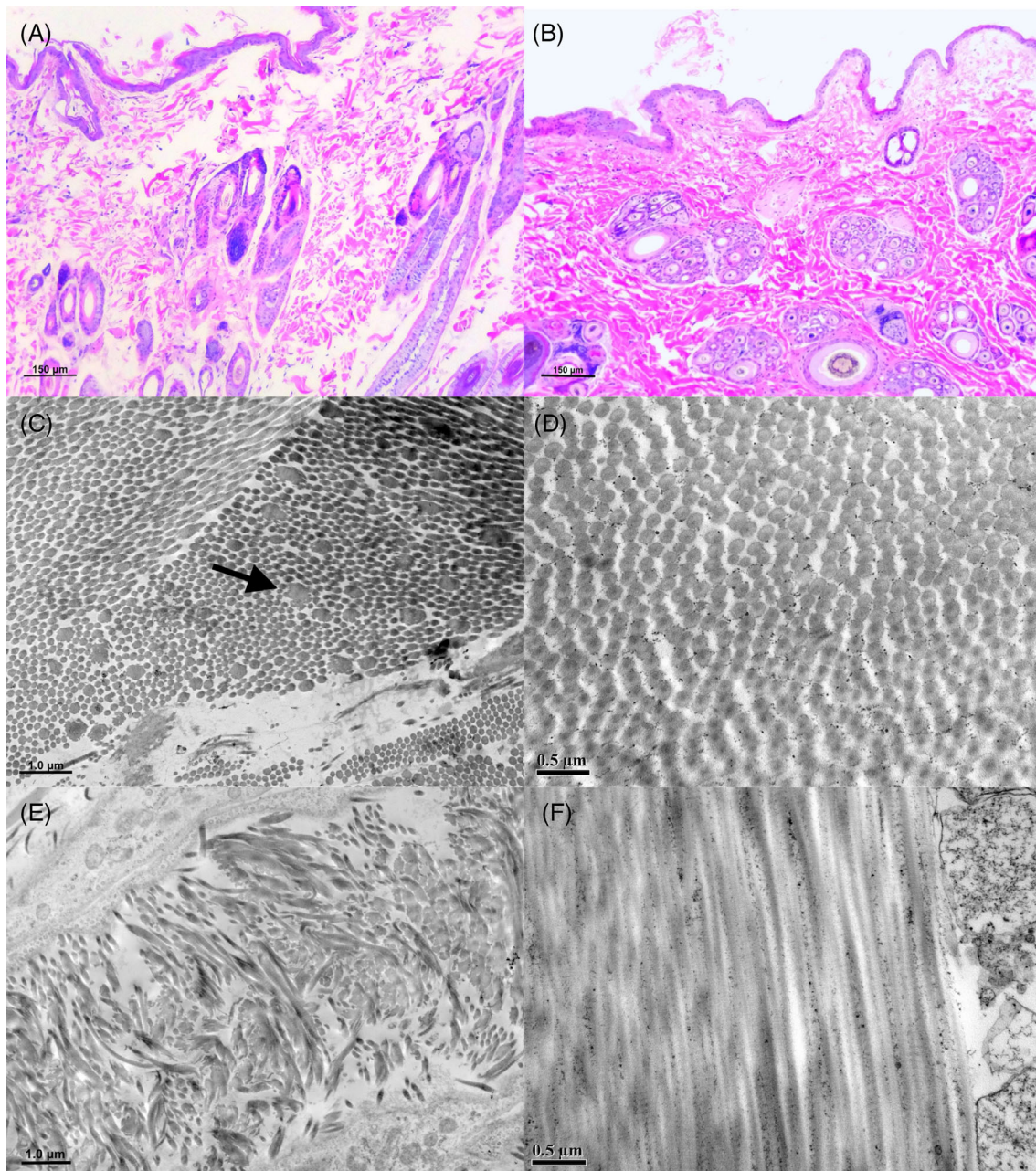


FIGURE 3 Histopathology and ultrastructure of skin in an EDS cat and a control cat. (A) Affected cat, skin biopsy, H&E, 100 \times . Loosely arranged collagen bundles with shortened, irregular fibers are displayed. (B) Control cat, skin biopsy, H&E, 100 \times . Densely packed collagen bundles with regular collagen fiber arrangement are displayed. (C) Affected cat, skin biopsy, TEM, 38 000 \times , flower-like collagen fibrils (arrow) as well as variation in fibril diameter are displayed. (D) Control cat, skin biopsy, TEM, 19 000 \times . Regular fibril packing density and diameter are displayed in cross-section. (E) Affected cat, skin biopsy, TEM, 32 000 \times . Shortened, irregularly arranged fibrils with irregular edges are displayed in longitudinal section. (F) Control cat, skin biopsy, TEM, 19 000 \times . Regular fibril packing density and diameter are displayed in longitudinal section.

fibrils with irregular edges (Figure 3C).^{16,22} The control cat displayed regularly arranged fibrils in cross-section with consistent fibril diameter (Figure 3D). In the affected animal, focal areas of irregular collagen packing density, aberrant fibril arrangement, and shortened fibrils with variable diameter were observed in longitudinal section (Figure 3E), whereas the control cat had densely packed and regularly arranged fibrils (Figure 3F). Normal D-banding was maintained throughout the samples from both animals.

2.4 | Genetics

After obtaining informed consent from the owner, approximately 3 mL of EDTA whole blood was collected by medial saphenous venipuncture and submitted for WGS. Isolated DNA was evaluated for quality and quantity as previously described.²³ Approximately 1 μ g of DNA was submitted to the University of Missouri Genomics Technology Core to construct a 350 bp sequencing library and to produce

approximately 30× sequencing coverage of 150 bp paired reads using an Illumina NovaSeq 6000 (Illumina, San Diego, California) as previously described.²³ Data was processed using a custom Nextflow workflow following best-practices for the Genome Analysis toolkit (GATK) version 4.2.^{24,25} Reads were mapped to *Felis_catus_9.0* (GCF_000181335.3) using Minimap version 2.²⁶ Duplicate reads were marked using Picard version 2.27.²⁷ Specific tools from GATK 4.2 for genotyping, variant database construction, and filtering were performed as previously described.²³ The NCBI RefSeq annotation 104 and GATK Variant Effect Predictor²⁸ were used to characterize the variants. Exonic variants and 10 bp flanking each exon were filtered and visualized using VarSeq software (GoldenHelix, Bozeman, Montana) and included data from 361 additional cat whole genomes and 52 cats with whole exome sequencing data. The WGS data are available in the NCBI short read archive under project accession number PRJNA308208, PRJNA627536, and PRJNA844099 with this EDS case as SAMN35341062.

This case presentation is expected to be rare, and both dominant and recessive modes of inheritance (ie, heterozygous or homozygous) were considered during the variant filtering. The 99 Lives dataset had 2 289 566 variants, the unique variants for the case cat are presented (Supplementary Table S1a). Considering dominant inheritance (ie, heterozygous for the case) and unique variants with a passing VQSRTTranche score²³ that were suspected of altering the gene coding sequences, 151 variants were identified, including a splice variant in *COL5A1* (XM_023242950.1:c.501-2A>C; ENSFCAT00000060880:c.501-2A>C) at position D4:93290016, which disrupts the splice

acceptor at exon 4 (Figure 4). Seventeen of 28 sequencing reads for the case had the variant, suggesting good sequencing coverage of the variant region. *COL5A1* (ENSFCAG00000040298) is at position D4:93 185 154-93 332 157 in the *Felis Catus 9.0* reference assembly using Ensembl annotation 104 and at position D4:93 685 188-93 707 502 in the *Felis Catus Fca126* assembly. The *COL5A1* (transcripts: ENSFCAT00000060880:c.501-2A>C; XM_023242950.1:c.501-2A>C) splice acceptor variant is at position D4:93 290 016 in *Felis_Catus 9.0* using Ensembl annotation 104 and on cat chromosome D4 (NC_058380.1) at position 93 694 436 in *Felis_Catus Fca126* using ReqSeq annotation 105. The variant was validated in the case cat by PCR and Sanger sequencing as described in the [Supplementary Methods](#) using the PCR forward primer 5'-TGAGAACTTCACACAGGCC-3' and reverse primer 5'-GTATC-CAGCAGTGACGACCC-3'. Polymerase chain reaction was performed using ChoiceTaq DNA Polymerase (Denville Scientific, Inc., Metuchen, New Jersey) according to manufacturer protocol with the addition of dimethylsulfoxide (5% v/v) and a primer annealing temperature of 61°C.

Three other unique heterozygous variants in collagen genes were identified in the case as well as a heterozygous missense variant in exon 29 of *COL5A1* (XM_023242950.1:c.2479C>T, ENSFCAT00000060880:c.2479C>T; p.Arg827Trp) at position D4:93231853 in *Felis_Catus 9.0*.²³ The human ClinVar database (www.ncbi.nlm.nih.gov/clinvar) and the gnomAD v2.2.1 allele databases (gnomad.broadinstitute.org) were examined to consider their significance. In humans, a NM_000093.5(*COL5A1*):c.2482C>T

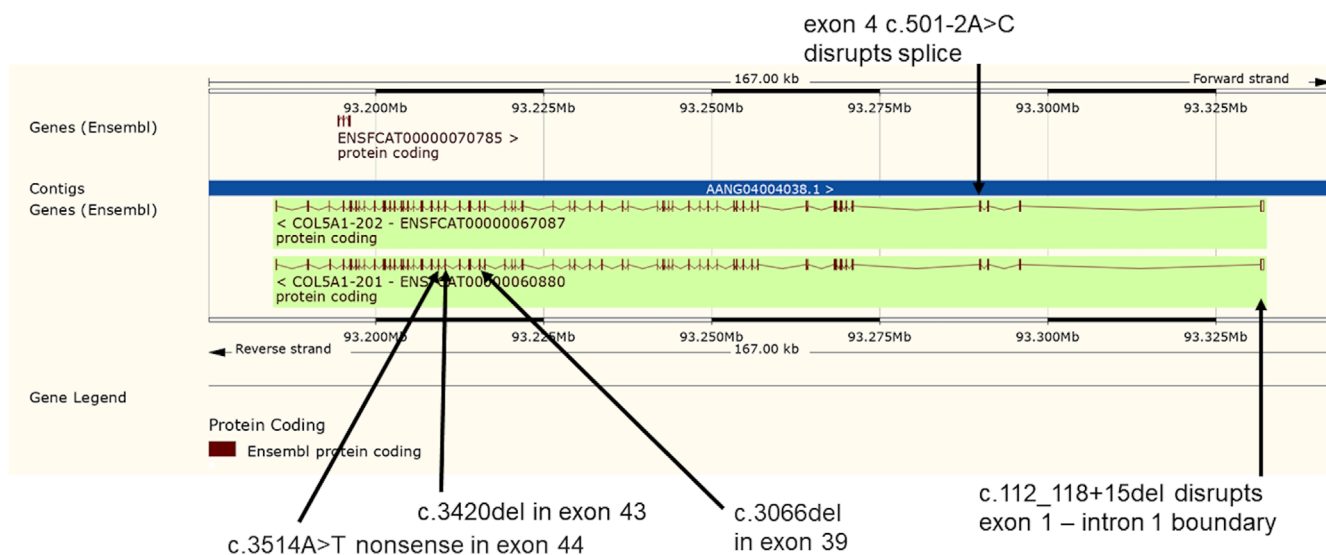


FIGURE 4 Physical positions of DNA variants in cat *COL5A1* associated with Ehlers-Danlos syndrome. Base image exported from Ensembl 109 cat genome assembly *Felis_catus v9.0* for *COL5A1* at positions D4:93 185 154-93 332 157. Cat assembly *Fca126* is not yet available in Ensembl. Arrays denote the approximate positions of the DNA variants associated with Ehlers-Danlos syndrome in the domestic cat, including c.3420del in exon 43 leading to a frameshift mutation and a premature stop codon described in a domestic shorthair cat in 2018¹⁵; a c.112_118+15del a 22-base pair (bp) deletion removing the boundary between exon 1 and intron 1, a c.3514A>T nonsense variant predicted to truncate 36% of the coding sequence, and a 1 bp deletion at c.3066del leading to a frameshift mutation predicted to truncate 44% of the open reading frame.¹⁶ The variant for the presented case is above the image, the c.501-2A>C variant at the end of exon 4, which could lead to exon skipping or a frameshift in the cat.

(p.Arg828Trp) variant is documented in a cEDS case but was determined to be likely benign. A *COL6A1* variant (XM_023238595.1: c.1478G>A; p.Arg493Gln) was identified in the case cat and in the ClinVar database, a *COL6A1/2* c.1468A>G (p.Arg490Gly) variant with uncertain significance has been identified in a Bethlem myopathy 1 patient, whereas a single allele of a p.Arg490Ser variant is present in the gNomeAD dataset with 210 938 total alleles, implying a very rare alteration. A *COL17A1* variant (XM_006938156.4: c.4228G>A; p.Gly1410Ser) was not present in the ClinVar database, but in gnomAD v2.1.1 (p.Gly1411Ser) has an allele count of 5 in 261 896, and thus is very rare. Finally, a variant in *COL22A1* (XR_002739417.1:n.3368G>A) also was identified as unique to the case in the heterozygous state, but also not identified in the human variant databases. The ClinVar data for the collagen genes are presented in Supplementary Table S2a-d.

Only 9 variants were identified assuming a recessive model (ie, case as homozygous), read coverages were lower and none were in a candidate gene for EDS and were not further analyzed (Supplementary Table S1b). All variants detected in 99 Lives Cat Genome Sequencing Consortium dataset for *COL5A1* are presented in (Supplementary Table S1c) including 2 previously published variants as those EDS cats in the 99 Lives dataset.¹⁶

This nucleotide position for the splice-acceptor variant is highly conserved in mammalian species and the protein homology is high for exon 4 in mammals, including 98% homology between humans and cats (Supplementary Figures S1 and S2). Using 2 different splice site prediction tools, this alteration is predicted by the Berkeley *Drosophila* Genome Project²⁹ and ESEfinder to abolish the native splice acceptor site.³⁰⁻³² No other related cats were available to support segregation and correlation with disease. The allele frequency in the 412 cat 99 Lives dataset is 0.001, which includes the 1 case cat.

3 | DISCUSSION

In humans, cEDS is caused by autosomal dominant variants in several genes but most commonly in *COL5A1*. *COL5A1* encodes the $\alpha 1$ chain of type V collagen, a minor fibrillar collagen widely distributed across body tissues and composed mainly of $[\alpha 1(V)]_2\alpha 2(V)$ heterotrimers. These collagen V heterotrimers co-assemble with type I collagen to form heterotypic type I/V collagen fibrils, and type V collagen is believed to negatively regulate the diameter of these fibrils. Genes associated with EDS affect the synthesis of different subtypes of collagen (*COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2*, and *COL12A1*) as well as other regulatory genes in the collagen pathway. In human patients, most variants in *COL5A1* result in haploinsufficiency, whereas a small number are associated with structural variants leading to a dominant-negative effect.^{33,34} Whereas type V collagen accounts for only 5% of total collagen mass, cell culture experiments demonstrate a 50% reduction in total collagen fibrils in haploinsufficient *COL5A1* cell lines, suggesting type V collagen has a nucleating function in the assembly of type I/V heterotypic collagen fibrils.³⁵

In humans, the clinical diagnosis is confirmed by WGS, whole exome sequencing, or by sequencing of a panel including *COL1A1*, *COL1A2*, *COL5A1*, and *COL5A2*, because 90% of cEDS patients have a heterozygous mutation in either *COL5A1* or *COL5A2*. Skin biopsy followed by TEM can be suggestive of a cEDS diagnosis but is not confirmatory.¹

Major criteria for the diagnosis of cEDS in humans are skin hyperextensibility and atrophic scarring as well as generalized joint hypermobility. Easy bruising, soft or doughy skin, skin fragility, molluscoid pseudotumors, hernias, SC spheroids, epicanthal folds, complications of joint hypermobility, and a family history of a first-degree relative meeting the clinical criteria for cEDS are minor criteria for diagnosis. The presence of both major criteria or of skin hyperextensibility and atrophic scarring plus 3 minor criteria are suggestive of cEDS. Prolonged bleeding in the face of normal coagulation status may be observed. Recurrent hernias including inguinal, incisional, umbilical, and hiatal hernias frequently are described in this population as well as rectal prolapse. Although neurological complications such as Chiari malformation, syringomyelia, and tethered cord syndrome are more common in hypermobile EDS, muscular hypotonia as well as cerebrospinal leaks have been reported in cEDS.^{1,33,36} Cardiovascular manifestations are uncommon in cEDS although mitral valve prolapse, tricuspid valve prolapse, and aortic root dilatation may occur.^{1,33} Numerous gastrointestinal complications have been described in humans with cEDS such as chronic constipation, nausea, vomiting, dysphagia, gastroesophageal reflux, diarrhea, abdominal pain, and irritable bowel-like symptoms.³⁷

Clinically, cats with *COL5A1* variants have been described as having thin, hyperextensible skin, skin fragility, atrophic scarring, and chronic pruritus. Reported SEIs ranged from 19% to 27%.^{15,16} Both perineal and diaphragmatic hernias have been described in EDS cats.¹³ Wound healing in EDS animals is reportedly normal, and these animals may safely undergo procedures such as onychectomy, routine castration and ovariohysterectomy if perioperative precautions are taken.³⁸ In fact, onychectomy or the use of plastic nail caps or regular nail clipping may substantially decrease morbidity in these animals by limiting self-trauma. The proliferation of mast cells in skin in our case is of unclear relevance, but combined with the chronic pruritus experienced by the cat suggests a similar phenotype to the mast cell activation syndrome phenotype experienced by many human EDS patients.¹⁷ Corticosteroids and fluoroquinolones should be avoided in these animals because of potential damage to collagen. Orthopedic abnormalities such as patellar luxation, hip subluxation, and carpal hyperextension have been noted in this population and care should be taken to minimize stress on joints such as limiting excessive jumping from heights and restricting affected animals to an indoor environment. Neurological signs associated with EDS are poorly described in animals, but seizures and flattened cerebral gyri were noted in a previous report and hyperesthesia and mild lower motor neuron paraparesis were noted in our case.^{15,16} Cardiovascular signs are also poorly described in cats. Although mild SAM was observed in our case, we also have observed sudden death, presumably secondary to aneurysm or other vascular events, in cats with

EDS. Routine serial echocardiographic monitoring should be considered in affected animals. Gastrointestinal signs are also poorly described in cats, but our cat presented with dysphagia and chronic constipation, as has been described in humans.

Overall, EDS in cats is often misdiagnosed in the private practice setting, in part because of a lack of availability of genetic testing for the general practitioner. Most cases of cEDS are caused by variants in *COL5A1*, which is comprised of 66 exons and encodes a protein of 1838 amino acids. In humans, approximately 180 variants in *COL5A1* are considered pathogenic or likely pathogenic, and most are associated with cEDS.³⁹ The variants are identified throughout the gene and across many different exons, thus, genotyping for causal variants in *COL5A1* is a complicated process. Other random-bred cats will most likely not share this novel variant, suggesting routine genotyping has limited value when a variant is discovered in random-bred cats, but genotyping can be supportive in suspected clinical cases and to support a diagnosis. Precision medicine techniques using WGS and comparisons to moderately large variant databases, such as the 99 Lives dataset, can effectively support clinical diagnoses by identifying novel *de novo* causal variants.

Precision medicine techniques were used to identify a likely causal variant for this cat's form of EDS. The identified cat p.Arg827Trp variant is analogous to a p.Arg827Trp missense variant in humans (NM_000093.5(*COL5A1*):c.2482C>T) (p.Arg828Trp), which is considered likely benign, but no publication or function information is associated with this variant.³⁹ *In silico* predictions of this variant in humans have already suggested its lack of pathogenicity. Four different splice acceptor variants (c.655-2A>G, c.655-2A>T, c.655-1G>A, and c.655-1G>C) are associated with disease in humans and exon skipping has been identified in these variants. The *COL5A1* c.655-2A>G variant in humans has been supported by functional studies of the human protein as being pathogenic. Similar to the c.655-2A>T in humans that is considered likely pathogenic by disruption of a splice acceptor site leading to a 2-exon skip, the cat c.501-2A>C, which is also a splice site mutation, is a nucleotide transversion of a purine to a pyrimidine involving the exchange of a 2-ring structure for a 1-ring structure and is also likely pathogenic.⁴⁰ Demonstration of the variant segregating in a family and/or demonstrating the altered mRNA transcript would further support pathogenicity, but neither opportunity was available for our case.

Collagens play important roles in maintaining extracellular matrix structure and function. Members of the collagen family form distinct networks of microfibrils in connective tissue and interact with other extracellular matrix components (See Online Mendelian Inheritance [OMIM] for review).⁴¹ Although variants in other genes were detected in this case, the disease presentations for variants in *COL6A2*, *COL17A1*, and *COL22A1* are different and distinguishable from cEDS. *COL6A1/2* has been associated with several forms of Bethlem myopathy (BTHLM1; OMIM:158810) and Ullrich congenital muscular dystrophy (UCMD1; OMIM:254090). In addition, similar variants in humans to the cat p.Arg493Gln variant detected in our case are suggested as benign or have uncertain clinical relevance. The arginine residue in humans is only moderately conserved. Advanced modeling of protein

sequence and biophysical properties (such as structural, functional, and spatial information, amino acid conservation, physicochemical variation, residue mobility, and thermodynamic stability) indicates this human missense variant is not expected to disrupt *COL6A1/2* protein function. *COL17A1* encodes for a type II transmembrane protein and is a component of hemidesmosomes, which mediate the adhesion of keratinocytes and other epithelial cells to the underlying basement membrane. Associated disorders in humans include non-Herlitz (intermediate) junctional epidermolysis bullosa (JEB4; OMIM:619787) and several forms of epithelial recurrent erosion dystrophy (ERED; OMIM:122400). *COL22A1* is a member of the FACIT (fibrillar-associated collagens with interrupted triple helices) subgroup of the collagen family and specifically localizes to tissue junctions. Variants in this gene are less defined than those of other collagens and are mainly associated with several gene deletions. Overall, these additional collagen variants do not have support as causal for disease or are not present in the human datasets.

The rarity of the cat *COL5A1* variant in a common candidate gene for EDS, the strong conservation of splice acceptor nucleotides and positions, the conservation of the site across species, the clinically relevant disease pathology, and the similarity to known pathological variants in humans strongly support the cat c.501-2A>C variant in *COL5A1* as likely pathogenic and a strong candidate variant for an autosomal dominant form of EDS in cats. Standards for the classification of pathogenic variants have been defined by the American College of Medical Genetics.⁴² Classification criteria include the type of mutation, conservation of the site across species, evidence of inheritance, population, and disease databases, *in silico* predictions of altered protein function, and functional data. Loss of function variants are considered very strong evidence for pathogenicity, however, splice variants, such as the cat variant, leading to potential exon skipping, should be considered with caution. This variant could be a *de novo* mutation in the case cat; genotyping of parents, which were unavailable, would have clarified how recently the variant occurred in the cat population. The guidelines in humans fail to recognize evidence found in other species, except for conservation of the nucleotides and amino acids across diverse species. Thus, as variant classifications for animals move toward developing similar criteria for pathogenicity, cross-species comparisons may need to be evaluated more thoroughly. Other regional feral cats from the area of adoption could be anticipated to have a similar presentation and carry the variant. Precision medicine, such as that which resulted from this cat's skin hyperextensibility and fragility diagnosis as a form of EDS, is becoming increasingly common in veterinary genetic diseases.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The affected cat in this study was privately owned and examined with the consent of the owner. The control cat tissues were obtained post-mortem and did not require IACUC approval.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

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

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