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A Retrospective Study of Corneal Endothelial Dystrophy in Dogs (1991–2014)

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Purpose: To retrospectively evaluate the clinical data, diagnostic tests, treatments, and outcomes for dogs with corneal endothelial dystrophy (CED) and determine risk factors for CED when compared with a canine reference population.

Methods: Medical records of 99 dogs (1991–2014) diagnosed with CED at the University of California Davis Veterinary Medical Teaching Hospital were reviewed and compared with 458,680 dogs comprising the general hospital population during the study period. Retrieved data included signalment, examination findings, diagnoses, treatments, and outcomes associated with CED. The exact Pearson χ^2 test or exact Kruskal–Wallis test was used to compare parameters between the groups. Progression of corneal edema was assessed using 3 independent Kaplan–Meier curves, identifying clinically significant changes in corneal opacity.

Results: Boston terriers, German wirehaired pointers, and Dachshunds were overrepresented in the CED-affected group, whereas Labradors were underrepresented. Dogs older than 11 years were overrepresented in the CED-affected group, whereas intact dogs were underrepresented. Surgical intervention was performed (n = 11) based on the severity of disease and secondary complications from CED. Median time to progression of corneal edema was 1) 368 days when an at-risk eye initially without edema developed edema at a subsequent visit, 2) 701 days when there was progression from mild to marked corneal edema, and 3) 340 days when there was progression from focal to diffuse corneal edema.

Conclusions: Many CED-affected dogs progress over months to years without surgical intervention, making dogs with CED a useful model for studying genetic predispositions and development of novel therapeutics for Fuchs endothelial corneal dystrophy.

Key Words: dog, corneal endothelium, Fuchs endothelial corneal dystrophy, corneal endothelial dystrophy

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Fuchs endothelial corneal dystrophy (FECD) is characterized by progressive corneal endothelial cell loss, and affects approximately 4% of humans over 40 years of age in the United States.[1] Corneal endothelial cells have a limited capacity to regenerate, and once a critically low number is reached in patients with FECD, corneal edema can occur concomitantly with loss of vision. The 2016 Eye Banking Statistical Report identified FECD as the most frequent indication for corneal transplantation in the United States. Although several factors have been implicated in the pathogenesis of FECD, including damage from oxidative stress,[2] RNA toxicity,[3] unfolded protein response,[4] abnormal extracellular matrix deposition,[5] and genetic mutations,[6] the fundamental mechanisms responsible for this disease remain poorly understood.

A similar condition to FECD termed corneal endothelial dystrophy (CED) occurs in dogs. This is a bilateral, primary degenerative process of corneal endothelial cells in which affected dogs subsequently develop corneal edema. With chronicity, both FECD and CED can result in bullous keratopathy and ulcerative and/or infectious keratitis, which severely compromise vision and comfort in affected patients.[7] It has been suggested that several canine breeds are predisposed to CED, including Boston Terriers (BTs),[8] Chihuahuas,[9] Dachshunds,[10] German Shorthaired Pointers (GSHPs),[11] and German Wirehaired Pointers (GWHPs),[11] but there are no studies directly comparing the prevalence of CED in affected individuals against a reference population. Furthermore, neither a prospective nor retrospective evaluation of a large population of dogs with CED has been performed to evaluate progression of this disease.

Thus, this study sought to 1) retrospectively evaluate the clinical data, diagnostic test results, treatments, and outcomes for dogs with CED and 2) determine risk factors for CED when compared with a reference population. The results from this study will help inform subsequent analyses designed to determine additional genetic loci associated with CED in the dog and thereby provide additional potential

targets for investigation in humans with FECD and for whom a genetic cause has not yet been identified.

MATERIALS AND METHODS

Retrospective Study

Medical records were electronically searched for dogs diagnosed with CED at the University of California Davis Veterinary Medical Teaching Hospital (UCD-VMTH) between August 22, 1991 and October 23, 2014. The earlier cutoff date corresponds to the establishment of electronic records at UCD-VMTH, and the later cutoff date reflects the beginning of clinical trials where CED patients were specifically recruited to the UCD-VMTH to study their disease process. Each record was carefully reviewed to ensure it met the inclusion criteria: 1) a clinical diagnosis of CED/degeneration by a board-certified veterinary ophthalmologist or resident in training, 2) a supporting description of corneal edema as part of the ophthalmic examination, and/or 3) in vivo confocal microscopy results supporting the diagnosis. Patients with a history of intraocular surgery, chronic anterior uveitis, diabetes mellitus, anterior lens luxation, and/or glaucoma were excluded. For all dogs meeting the inclusion criteria, the signalment (age at presentation, gender, and breed), ophthalmic examination findings, concurrent systemic disease, treatments before presentation, results of all diagnostic testing performed including fluorescein stain to assess for corneal ulceration, and medical treatments instituted were recorded. Vision and light perception were assessed by the presence of a menace response (an elicited blink, retraction of globe, or head turn when a threatening hand motion was made at a single eye) and a dazzle reflex (a partial or complete eyelid blink as a reflex to a very bright light shone in the eye), respectively.[12,13] Central corneal thickness (CCT) was measured using ultrasound pachymetry (USP, Pachette 3; DGH Technology, Inc, Exton, PA) on 6 CED-affected dogs.[8] In vivo confocal microscopy (ConfoScan 4; Nidek Technologies, Gamagori, Japan) of the central cornea was performed on 7 dogs using the previously described methods,[8,14] for which data were obtained from 5 examined dogs because severe corneal edema precluded analysis on 2 animals. Where possible, endothelial cell density was averaged between eyes (3 dogs) or reported from 1 eye only of 2 dogs because of enucleation of the contralateral globe in 1 case and severity of corneal edema in the other case (CCT: 1097 μm). In 2 additional dogs examined, corneal edema severity prevented accurate assessment of endothelial cell density (CCT OD: 1022 μm, OS: 830 μm; CCT OD: 1871 μm, OS: 1029 μm).

Corneal Edema Analysis

At the UCD-VMTH, severity and extent of corneal edema are graded in a subjective but standardized manner by all examining clinicians and routinely recorded in the medical record as "absent," "mild," "moderate," or "severe" for severity and as "none," "focal," or "diffuse" for extent. Owing to the asymmetric progression of CED between each eye of the same dog, corneal edema was analyzed for individual eyes.[8] Animals with surgical intervention [superficial keratectomy and conjunctival advancement hood flap (SKCAHF)] were excluded from this analysis. Progression criteria were defined as follows:

Criterion 1: Eyes recorded as having clear (nonedematous) corneas during the first visit, but showed signs of CED in the contralateral globe, subsequently progressing to any degree of corneal edema in the unaffected globe at later visits.

Criterion 2: Eyes recorded as having mild corneal edema during the first visit and subsequently progressing to severe corneal edema at later visits.

Criterion 3: Eyes recorded as having focal corneal edema during the first visit and subsequently progressing to diffuse corneal edema in later visits.

Kaplan–Meier curves were generated to demonstrate time to edema progression, defined as time from initial examination to each progression criterion.

Statistical Analysis

All dogs presented to the UCD-VMTH for any reason during the same period formed a reference population against which the study population was compared. Proportions of dogs by breed and sex were each compared between the study and reference populations using an exact Pearson χ^2 test. To compare sex status between populations, dogs were assigned to one of the following 4 groups: intact male, neutered male, intact female, and spayed female dogs. To compare age among populations, dogs were assigned to one of the following 5 groups: <1, 1 to 5, 6 to 10, 11 to 15, and >15 years. The exact Kruskal–Wallis test was then used to compare age distributions between the study and reference populations. For all analyses, a P value of <0.05 was considered significant.

RESULTS

Signalment

The hospital-wide reference population consisted of 458,680 dogs of which 11,200 were presented to the UCD-VMTH Ophthalmology Service. A total of 99 dogs diagnosed with CED were included in the study and comprised 0.022% of all dogs presented to the VMTH and 0.94% of dogs presented to the UCD-VMTH Ophthalmology Service. Dogs diagnosed with CED were categorized as belonging to 36 distinct breeds in addition to 18 mixed breed dogs. Of these 36 breeds affected by CED, 10 breeds were significantly overrepresented when compared with the reference population, particularly BTs and Dachshunds, having 8 or more dogs diagnosed during the 23-year study period (Table 1). Labrador Retrievers were significantly underrepresented in the study versus the UCD-VMTH reference population (Table 1). The median (range) age of CED-affected dogs was 12 (5–20) years versus 7 (0–20) years for the reference hospital population with 92% (n = 91) of CED-affected dogs presented at older than or equal to 8 years of age. Dogs older than or equal to 11 years of age were significantly overrepresented in the CED-affected population, whereas dogs younger than or equal to 5 years of age were significantly underrepresented ($P < 0.001$; Fig. 1). Affected intact male

Table 1. Breeds of 99 CED-Affected Dogs That Were Significantly Overrepresented or Underrepresented in Comparison to the Reference Population (n = 458,680 Dogs) Between August 22, 1991 and October 23, 2014

Breed	No. of Dogs With CED	*P* Value	O:E Ratio
German Wirehaired Pointer	2	4.0E-09	19.26042126
Boston Terrier	10	2.5E-23	11.81364783
Bull Terrier	2	1.9E-05	11.02888408
Miniature Dachshund	4	4.2E-06	7.153870754
Brittany Spaniel	3	1.8E-04	6.50884962
Lhasa Apso	4	2.1E-05	6.365003522
Weimaraner	3	3.0E-04	6.187174506
Bassett Hound	3	6.0E-04	5.754200389
Dachshund	8	3.4E-07	5.056221927
German Shorthaired Pointer	3	6.1E-03	4.271870255
Labrador Retriever	3	3.8E-02	0.321393079

Listed are the top 15 breeds overrepresented with CED and the only underrepresented breed (italics).
A Pearson exact χ^2 test was performed.
O:E ratio, observed:expected.

and intact female dogs were significantly underrepresented compared with the hospital population ($P < 0.001$; Fig. 2). The prevalence of CED in spayed female and castrated males dogs was similar to that of the reference population.

Examination Findings

In total, 197 eyes of 99 dogs were included in the study because 1 dog had undergone enucleation OS before the initial visit. Seventy-one dogs (72%) were reported as visual in both eyes with an absent menace response reported OU (n = 3), OD (n = 8), or OS (n = 7) in the remainder; menace response was not reported for 10 dogs. Eighty-two dogs (83%) had light perception OU, evidenced by a positive dazzle reflex. There was an absent dazzle reflex reported in individual eyes of 7 dogs, and a dazzle reflex was not reported as present or absent for 10 dogs. Corneal edema was bilateral in 81 dogs (82%), although affecting only the right eye in 11 dogs (one of which was enucleated OS) and the left eye in 7 dogs. Apparent ocular discomfort (as assessed by blepharospasm) was absent OU in 71 dogs (72%) and present in at least 1 eye of 24 dogs (24%); blepharospasm was not reported as present or absent in 3 dogs. Conjunctival hyperemia was present in at least 1 eye of 68 dogs (69%) and absent OU in 24 dogs (24%); conjunctival hyperemia was not reported as present or absent in 6 dogs. Episcleral congestion was absent OU in 48 dogs (48%) and present in at least 1 eye in 43 dogs (43%); episcleral congestion was not reported as present or absent in 7 dogs.

Diagnostic Test Results

Fluorescein staining was performed in 74 CED-affected animals (75%) and identified a corneal erosion or ulcer in 37 (50%). A Schirmer tear test was performed on 45 CED-affected dogs (45%) with a mean ± SD value of 18.2 ± 4.9 mm/60 seconds (reference range for dogs: 15–25 mm/60 s).[15] Intraocular pressure, estimated by either applanation or rebound tonometry, was performed in 76 CED-affected dogs (77%) with a mean ± SD value of 11.9 ± 4.3 mm Hg (applanation reference range: 7–20 mm Hg; rebound reference range: 12–22 mm Hg).[16] Mean ± SD CCT of 6 (6%) CED-affected dogs (1325 ± 444 μm) was markedly greater than reported values obtained using USP in normal dogs (587.72 ± 32.44 μm).[17] Mean ± SD corneal endothelial cell density was significantly reduced in CED-affected animals (1135 ± 278 cells/mm^2, n = 5, 5%) compared with similarly aged normal dogs from a previous publication (2297 ± 372 cells/mm^2).[8]

Medical Treatment

At initial presentation, 87 dogs (88%) were prescribed topical and/or systemic medications, commonly 5% sodium chloride ophthalmic ointment (n = 58 dogs, 67%), topical antibiotics (n = 49, 56%), topical anti-inflammatories (n = 22, 25%), topical mydriatics (n = 24, 28%), oral anti-inflammatories (n = 15, 17%), or oral antibiotics (n = 6, 7%).

Follow-Up

Most dogs (n = 63 dogs/125 eyes, 64%) had follow-up visits with a median (range) time from diagnosis with CED to the last follow-up visit of 94 (7–2230) days. The predominant surgical intervention performed for advanced CED was a SKCAHF (otherwise known as a modified Gundersen flap; n = 9).[18] Thermal keratoplasty[19] was also performed in 1 patient with progressive corneal edema. Dependent on the

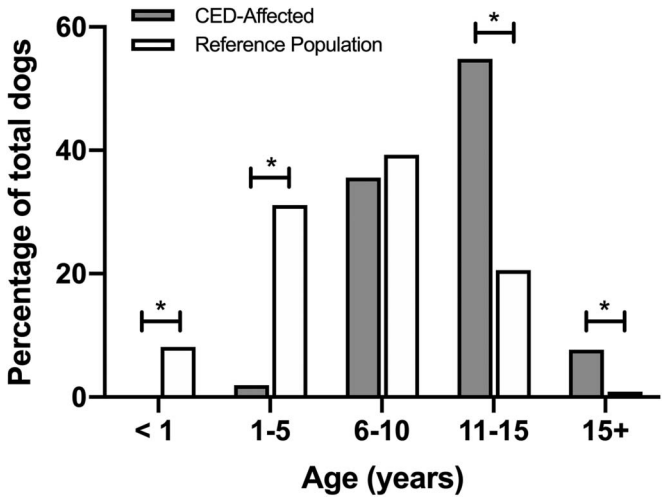

FIGURE 1. In comparison to the reference population (n = 458,680), older dogs in the CED-affected population (n = 99) were significantly overrepresented and younger dogs were significantly underrepresented. All dogs in both populations were examined between August 22, 1991 and October 23, 2014. A Kruskal–Wallis test was performed. * = $P < 0.05$.

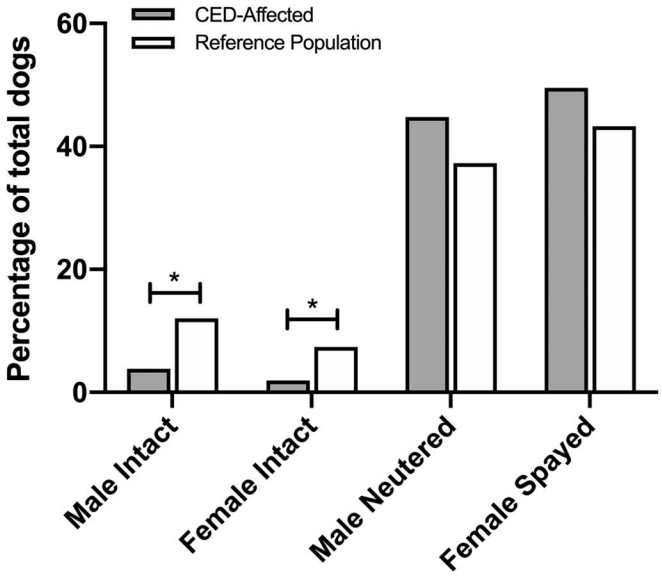

FIGURE 2. In comparison to the reference population (n = 458,680), intact male and female dogs in the CED-affected population (n = 99) were significantly underrepresented. All dogs in both populations were examined between August 22, 1991 and October 23, 2014. A Pearson exact χ^2 test was performed. * = $P < 0.05$.

type and severity of their ophthalmic disease, other CED-affected dogs were treated with grid keratotomy (fine linear scoring of the superficial anterior stroma to promote healing of canine recurrent erosions,[20] n = 3), enucleation (n = 3), or penetrating keratoplasty (n = 1).

CED Progression

Of the 197 eyes diagnosed with CED, 95 were excluded from progression analysis because they were immediately lost to follow-up. Therefore, a Kaplan–Meier curve for progression of corneal edema was generated using data from all remaining eyes (n = 102). Of the 102 eyes, 69 were censored and documented as not having edema progression over all examinations up until the last follow-up visit. In accordance with criterion 1, 13 eyes started with no edema at initial presentation (all contralateral eyes were edematous). Six of these eyes subsequently developed corneal edema within the follow-up period. The remaining 7 were censored at the time of the last follow-up visit. The median time to progression from clear cornea to various degrees of edema was 368 days after the initial visit (Fig. 3A). Regarding criterion 2, 30 eyes started with mild edema at initial presentation. Ten of these eyes progressed to have marked edema within the follow-up period. The remaining 20 were censored at the time of the last follow-up visit. The median time to progression from mild edema to severe edema was 701 days after the initial visit (Fig. 3B). Finally, of the patients assessed using criterion 3, 34 eyes had focal edema at initial presentation. Nineteen of these eyes developed diffuse edema within the follow-up period. The remaining 15 eyes were censored at the time of the last follow-up visit. The median time to progression from focal to diffuse edema was 340 days after the initial visit (Fig. 3C).

DISCUSSION

The goals of the current study were to identify the age, sex, and breed of dogs diagnosed with CED at a single institution and compare these results with a reference population presented during the same time interval. We were able to conclude that older dogs and multiple breeds (particularly, BTs and Dachshunds) were overrepresented in the CED-affected group when compared with the reference population, whereas Labrador Retrievers were considered underrepresented. The CCT of CED-affected dogs was greater, and the corneal endothelial cell density was lower when each were compared with normative values found in the literature.[8,17] Despite numerous medical interventions, including topical hypertonic saline and anti-inflammatory medications, there was visible progression of the corneal edema in 32% of these eyes within 2 years of diagnosis.

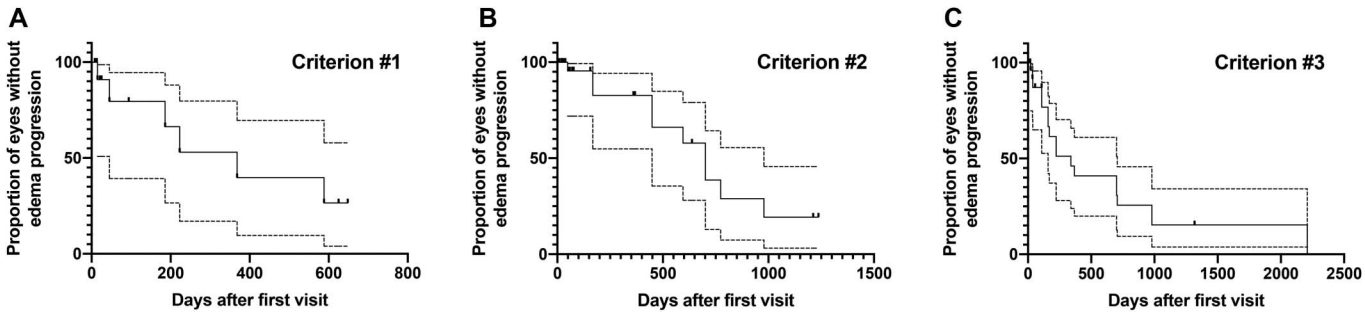

FIGURE 3. Kaplan–Meier curves of eyes demonstrating progression of corneal edema during follow-up visits in dogs diagnosed with CED. A, Criterion 1: eyes without corneal edema at their first visit and progressing to any degree of corneal edema (n = 13). The median time to progression was 368 days. B, Criterion 2: eyes recorded as having mild corneal edema during their first visit progressing to severe corneal edema in later visits (n = 30). The median time to progression was 701 days. C, Criterion 3: eyes recorded as having focal corneal edema during the first visit progressing to diffuse corneal edema in later visits (n = 34). The median time to progression was 340 days. Tick marks indicate censored subjects, and the dotted lines indicate 95% confidence intervals.

Much like human patients with FECD, dogs diagnosed with CED in our population typically were presented to an ophthalmologist during the later years of life (median: 12 yrs, range: 5–20 yrs). Recent studies focused on CED in BTs, GSHPs, and GWHPs determined that CED-affected animals were often older than 10 years of age.[8,11] The age-related progression of FECD and CED is one of the defining characteristics of the disease process in both species.

When compared with the general UCD-VMTH population, sexually intact dogs were underrepresented in the CED-affected population whereas a significant difference between populations was not detected for altered dogs. The main difference between these 2 populations is the presence or absence of gonad-derived sex hormones. Sex steroid hormone receptors have been identified in the nucleus of human corneal endothelial cells, suggesting that specific hormones may influence the biological functions of these cells. However, it is unclear how the expression and activation of these hormone receptors relate to endothelial cell survival and function.[21] Sex differences have been noted in human patients with late-onset FECD, with women being more affected than men, and more advanced disease in women who have smoked.[22,23] Previous studies of canine CED have identified female dogs as overrepresented among CED-affected dogs.[8,9,24] The shared predisposition of female subjects to developing FECD and CED in humans and dogs, respectively, furthers the utility of a spontaneous canine model of FECD for understanding the etiopathogenesis of this condition in both species.

In the examined population, 10 breeds with CED were considered overrepresented when compared with the general UCD-VMTH population. BTs, GSHP, GWHP, and Dachshunds are reported to be predisposed to development of CED.[8–11] In humans with FECD, multiple cosegregating genetic loci have been identified, including *COL8A2*, *TCF4*, *ZEB1*, *SLC4A11*, and *AGBL1*; however, other FECD patients do not have abnormalities in these genetic loci.[25–29] Therefore, identification of novel genetic predispositions in the dog,[30] as seen in other genetic association studies associated with spontaneous disease,[31] would identify candidate genes in FECD patients while also establishing dogs as a naturally occurring model of FECD. In addition, as the underlying genetic predispositions of CED in the dog are determined, genetic tests can be developed to guide breeding programs, thus eliminating this condition from the canine population. We are actively recruiting CED-affected dogs of multiple breeds, including BTs, GSHP, and GWHP to perform genome-wide association studies to identify genetic loci that may be responsible for the development of CED in these breeds.

Median time to corneal edema progression in CED-affected animals of the present study ranged from 1 to 2 years depending on the severity of edema at initial presentation. Criteria of corneal edema progression used in the current study were selected so as to reduce bias introduced by interobserver variation in assessment of edema severity. Criterion 1 and criterion 3 required only the assessment of whether edema was present versus absent or focal versus diffuse, which were less subjective evaluations than mild versus moderate edema, for example. Criterion 2 recorded an eye as progressed only if mildly edematous corneas became severely edematous. Although the evaluation of corneal edema in the medical record was inherently subjective, this more conservative criterion of progression prevented eyes with edema characterized by 1 observer as mild and by another observer as moderate from being designated as progressed in our analysis. Despite these methods of reducing the effect of interobserver variability in progression analysis, the retrospective nature of this study and subjectivity in the assessment of corneal edema is a limitation of the present study. Future studies will use more objective measurements of disease severity such as in vivo confocal microscopy, pachymetry, and optical coherence tomography.

The progression criteria in the current study also permitted us to focus on clinically relevant changes. Our results demonstrate that many CED-affected dogs presenting with unilateral edema developed edema in the previously nonedematous contralateral eye. This finding is not only clinically relevant information for owners but indicates that CED-affected eyes may be studied before the development of edema, which may be more applicable to early stages of FECD. In addition, we have characterized progression of corneal edema in mildly or focally affected eyes, demonstrating clinically relevant progression over the course of 1 to 2 years. The progressive nature of CED in dogs is analogous to the more severe clinical signs seen in advanced FECD that may take years to develop.[8] Therefore, based on the similarities of the 2 conditions and the more rapid progression of CED, the dog has the potential to serve as an important spontaneous model of FECD, including evaluation of the clinical utility of novel topical (ie, Rho kinase inhibitors [ROCK inhibitors]) or cell-based therapies.[32,33] Current therapies for canine patients with CED which has progressed to bullous keratopathy include the use of topical hypertonic saline, with minimal efficacy, and surgical intervention with a SKCAHF or, more recently, Descemet stripping endothelial keratoplasty.[18,34] In addition, there are current veterinary studies focused on evaluating the role of Rho kinase inhibitors for treatment of CED in the dog.[35]

The current study identified important breed predispositions in the development of CED in the dog. These data will serve as the foundation for further investigations into the genetic factors that underlie CED within each breed, and which have the potential to uncover additional loci in humans with FECD. Similarities in demographic and phenotypic characteristics of patients with CED and FECD, as well as parallel disease progression, highlight the potential relevance of the dog as a large animal model of FECD, likely to be useful in understanding disease etiopathogenesis, and development and testing of novel therapeutics.

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