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Association of the *FGF4L2* retrogene with fibrocartilaginous embolic myelopathy in dogs

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Abstract

Background: Fibrocartilaginous embolic myelopathy (FCE) is a well-documented condition in dogs although rarely reported in chondrodystrophic breeds. Genetic associations have not been defined.

Objectives: Define the association of the chondrodystrophy-associated *FGF4L2* retrogene with histopathologically confirmed cases of FCE.

Animals: Ninety-eight dogs with a histopathologic diagnosis of FCE.

Methods: Retrospective multicenter study. Dogs were genotyped for the *FGF4L2* and *FGF4L1* retrogenes using DNA extracted from formalin-fixed, paraffin-embedded tissue. Associations between breed, FCE and retrogene status were investigated with reference to a hospital population and known breed and general population allele frequencies.

Abbreviations: CDDY, chondrodystrophy; CDPA, chondrodysplasia; DNA, deoxyribonucleic acid; FCE, fibrocartilagenous embolism; FFPE, formalin-fixed paraffin-embedded; *FGF4*, fibroblast growth factor 4; *FGF4L1*, fibroblast growth factor 4 retrogene 1; *FGF4L2*, fibroblast growth factor 4 retrogene 2; IVD, intervertebral disc; MPa, mega pascal; TGFb, transforming growth factor beta.

Results: *FGF4L2* genotype was defined in 89 FCE cases. Fibrocartilaginous embolic myelopathy was present in 22 dogs from *FGF4L2*-segregating breeds with allele frequencies of ≥5%; however, all dogs were wild type. Two Labrador retrievers with FCE carried *FGF4L2* alleles. Frequency of the *FGF4L2* allele was significantly ($P < .001$) and negatively associated with FCE relative to predicted hospital-population dogs. FCE was overrepresented in Boxer, Great Dane, Yorkshire Terrier, Bernese Mountain Dog, Miniature Schnauzer, Rottweiler, and Shetland Sheepdog breeds.

Conclusions and Clinical Importance: Study data based on genotypically and histopathologically defined cases support the historical observation that FCE is uncommon in chondrodystrophic dog breeds. FGF4 plays an important role in angiogenesis and vascular integrity; anatomical studies comparing chondrodystrophic and non-chondrodystrophic dogs might provide insight into the pathogenesis of FCE.

KEYWORDS
canine, chondrodystrophy, FCE, fibroblast growth factor

1 | INTRODUCTION

Fibrocartilaginous embolic myelopathy (FCE) is a commonly reported disease in dogs where a presumptive diagnosis based on signalment, history, and variable imaging modalities is made in most cases.[1] The underlying pathogenesis of FCE, in particular how fibrocartilaginous material gains entry to the vascular system, has remained elusive since the condition was first described in humans in the 60's[2,3] and in dogs by Ian Griffiths in 1973.[4] FCE is reported most commonly in giant and large-breed dogs; however, small and medium-breed dogs represent approximately 30% of the FCE population.[1] There is a higher frequency of FCE in specific breeds including Miniature Schnauzer,[5-7] Great Dane,[7-9] Irish Wolfhound,[10] English Staffordshire Bull Terrier,[6,11] Labrador Retriever,[7,12,13] German Shepherd,[7,12] Dobermann Pinscher,[7] Golden Retriever,[7] and Shetland Sheep dogs,[7] but few findings are reported relative to general hospital population data.[11]

It is noteworthy from a pathophysiological perspective that published FCE cases have rarely been reported in presumed chondrodystrophic dog breeds. Recent definition of the fibroblast growth factor 4 retrogene 2 (*FGF4L2*) as the genetic underpinning of chondrodystrophy and premature degeneration of the intervertebral disc[14-17] has provided a more objective means of assessment of chondrodystrophy-associated disease through genotyping of affected animals, and general breed population allele frequencies can also provide perspective on historical data. The low frequency of FCE in chondrodystrophic breeds might be related to characteristics of the embolized material or the vascular system in which embolization occurs. Leveraging biological and anatomical differences in intervertebral disc (IVD) and spinal cord vascular anatomy in chondrodystrophic and non-chondrodystrophic dogs might provide insight into FCE pathophysiology, however before undertaking such studies validation of anecdotal observations relating to FCE in chondrodystrophic breeds would be advisable. Genotyping and histopathological confirmation of FCE cases is necessary to provide objective data to define the association of FCE and chondrodystrophy. Several disease conditions resulting in intrinsic myelopathy can mimic FCE, most notably acute extrusion of nucleus pulposus (NP) or IVD material, that is accompanied by minimal extradural mass effect.[6,18-20] Even in the era of MRI, diagnostic criteria for FCE have not been validated and coexistence or connectivity of embolic disease and acute non-compressive disc extrusion has not been defined. We hypothesized that presence of the *FGF4L2* retrogene would be negatively associated with FCE cases. The primary objective of this study was to define the association between the *FGF4L2* (chondrodystrophy) retrogene and FCE in histopathologically defined cases. Secondary objectives were (a) to define the association between the Fibroblast growth factor 4 retrogene 1 (*FGF4L1*; chondrodysplasia) and FCE in histopathologically confirmed cases and (b) to compare breed data from the study to historical published data including both histopathologically confirmed cases and suspected cases.

2 | METHODS AND MATERIALS

2.1 | Cases

Cases of FCE were histopathologically diagnosed by board certified veterinary anatomical pathologists from 8 veterinary institutions (Auburn University, University of California Davis, Cornell University, University of Georgia, North Carolina State University, University of Pennsylvania, Texas A&M University and Virginia-Maryland) where paraffin-embedded formalin-fixed tissue was available for deoxyribonucleic acid (DNA) extraction and genotyping. Historical published data relating to FCE breed frequency was obtained from case

series[4-13,21-33] and single case reports[34-45] where owner-defined breed data were available. Historical data were separated into histopathologically confirmed cases and cases with a presumed diagnosis.

2.2 | Genotyping

DNA was extracted from 25 µm sections of formalin-fixed paraffin-embedded (FFPE) tissue samples using a Quick-DNA FFPE miniprep kit according to the manufacturer's instructions (Zymo Research, Irvine, California, USA). Genotyping of the *FGF4L1* and *FGF4L2* retrocopies was performed using a 3 primer PCR approach as previously described,[16] but using novel primers that resulted in smaller amplicons consistent with fragmented FFPE-derived DNA. Forward and reverse primers flanking the respective insert as well as an additional forward primer located within the insert were designed using Primer3[46] (Table 1) and validated with previously genotyped control blood and FFPE-derived DNA. Genotyping PCR products were visualized by agar gel electrophoresis.

2.3 | FGF4 retrogene breed allele frequencies

Breed allele frequencies for *FGF4L2* (Chondrodystrophy, CDDY) and *FGF4L1* (Chondrodysplasia, CDPA) were determined based on combined data from Batcher et al[15] and The Veterinary Genetics Laboratory, UC Davis, Davis CA (May 2023).

2.4 | FCE independence

Independence of FCE and *FGF4L2* and *FGF4L1* genotype status was determined using all study cases where genotyping was successful. FCE retrogene allele frequency was compared to the predicted frequency of the *FGF4L1* and *FGF4L2* alleles in the UC Davis veterinary hospital general population over the duration of the collected samples based on general population reported allele frequencies (15.215% and 11.951% respectively) in 1 054 293 mixed and pure-bred dogs from more than 150 countries.[47,48]

2.5 | Breed predisposition

Breed predisposition for FCE was determined in breeds with greater than 2 cases in the study population and was based on the total hospital reference population (228 763) at 1 institution (UC Davis) during the study period (April 1987-June 2020). Additionally, the same analysis was repeated for UC Davis cases alone (greater than 2 cases) during the period of their collection, April 1987 to May 2019 (reference population 216 657).

2.6 | Statistical analysis

Statistical analysis was done using GraphPad Prism 9.5.0 (GraphPad Software, Boston, Massachusetts). Breed predisposition and independence of *FGF4L2* allele frequency in FCE cases vs total population were determined using a 2-sided Fisher's exact test. Odds ratios were reported with 95% confidence intervals using the Woolf logit interval. Significance was defined as $P < .05$.

3 | RESULTS

3.1 | Signalment

Ninety-eight cases of embolic myelopathy secondary to fibrocartilaginous embolism were included based on histopathological diagnoses from 8 institutions over a period from April 1987 to June 2020 (Data S1). Breeds represented were Labrador Retriever,[14] Boxer, Great Dane (8 each), Rottweiler, Yorkshire Terrier (6 each), German Shepherd dog, Mix breed (5 each), Miniature Schnauzer,[4] Bernese Mountain dog, Shetland Sheep dog (3 each), Airedale Terrier, Belgian Malinois, Chihuahua, Chow, Dobermann Pinscher, Golden Retriever, Irish Wolfhound, Pomeranian (2 each), and 1 each for American Bulldog, American Eskimo, Australian Terrier, Basset Hound, Boston Terrier, Brittany, Bulldog (English), German Short-Haired Pointer, Great Pyrenees, Greyhound, Jack Russell Terrier, Lhasa Apso, Old English Sheepdog, Pitbull Terrier, Pug, Shih Tzu, Standard Poodle, Standard Schnauzer, Swiss Mountain Dog, and Whippet. Median age was 6 years with 46% females (36 neutered, 9 intact) and 54% males (34 neutered, 19 intact).

3.2 | Genotyping

Genotypes for *FGF4L2* and *FGF4L1* were successfully determined in 89 (91%) and 79 (81%) of dogs respectively (Data S1). Genotyping was unsuccessful for both retrogenes in 7 dogs. Genotype data for affected dogs by breed together with general population allele

TABLE 1 *FGF4* retrogene genotyping PCR primers.

	External forward primer	Internal forward primer	External reverse primer	Amplicon size	
FGF4L1 (CDPA)	TGGACCATGAAATAAGTCAGACA	GTCCGTGCGGTGAAATAAAA	CACCAATTTGTTCCCTCCAT	WT	126 bp
				Insert	191 bp
FGF4L2 (CDDY)	TTATGCATTGGGGAGAGTCA	GAACCCCATTGGACTTGATG	TGCTGTAGATTTTGAGGTGTCTTT	WT	121 bp
				Insert	167 bp

frequencies are presented in Data S2. Two dogs tested positive for the *FGF4L2* allele, 1 heterozygous and 1 homozygous. Both were recorded as Labrador Retrievers. The heterozygous dog was also homozygous for the *FGF4L1* allele. The homozygous dog could not be genotyped for *FGF4L1*. The *FGF4L2* allele frequency in the study FCE cases was 1.7%. The *FGF4L1* allele frequency in the study FCE cases was 15.2%. Only 1 FCE case was present in a breed with a *FGF4L2* breed allele frequency greater than 25%. This was a Bassett Hound that carried WT alleles (Table 2). All dogs with FCE from breeds that segregate the *FGF4L2* allele at a frequency of 5% or greater had a WT genotype. When compared to predicted retrogene allele frequencies in the general UC Davis hospital population, the *FGF4L2* allele was significantly underrepresented in the FCE cases ($P < .001$; OR 0.13, 95% CI 0.05 to 0.4). The *FGF4L1* allele frequency in the study FCE cases was not statistically different from the predicted hospital population ($P > .99$; OR 1.0, 95% CI 0.65 to 1.42).

3.3 | Breed predisposition

Nine dog breeds (Table 3) had 3 or more cases and were included in assessment for predisposition relative to the total UC Davis hospital

TABLE 2 *FGF4* retrogene genotyping of FCE cases in common breeds segregating *FGF4L2* at 5% or greater.

Genotyped cases[a]	Breed	Breed *FGF4L2* freq[a]	Breed *FGF4L1* freq[a]	VMTH population[b]	FCE cases/genotype (98 total)
144	Cavalier King Charles Spaniel	0.97	0.01	1162	0
14	Cocker Spaniel English	0.96	0	480	0
36	Cocker Spaniel, American	0.96	0	4201	0
697	Dachshund (Swiss and UK/US)	0.95	0.98	6130	0
106	Beagle	0.92	0.02	1993	0
29 531	French Bulldog	0.92	0	1596	0
684	Pembroke Welsh Corgi	0.84	0.98	1490	0
14	Basset Hound	0.79	0.89	1110	1 *FGF4L2* **WT (1)**
13	Skye Terrier	0.77	1.00	19	0
29	Cockapoo	0.71	0.05	673	0
717	Poodle, Miniature and Toy	0.60	0.11	1596	0
38	Pekingese	0.59	0.92	762	0
1448	Coton de Tulear	0.42	0.97	133	0
36	English Springer Spaniel	0.38	0	1913	0
4888	Nova Scotia Duck Tolling Retriever	0.35	0	43	0
2	Lhasa Apso	0.25	0.75	1365	1 *FGF4L2* **WT (1)**
41	Schnauzer, Miniature	0.23	0.02	2325	4 *FGF4L2* **WT (4)**
79	Bichon Frise	0.22	0.66	1340	0
173	Shih Tzu	0.21	0.99	3193	1 *FGF4L2* **WT (1)**
8	Schnauzer, Standard	0.19	0	119	1 *FGF4L2* **WT (1)**
940	Portuguese Water Dog	0.16	0	333	0
1015	Bulldog	0.15	0	2438	1 *FGF4L2* **WT (1)**
179	Bernese Mountain Dog	0.11	0.04	1051	3 *FGF4L2* **WT (3)**
226	Chihuahua	0.11	0.79	5849	2 *FGF4L2* **WT (2)**
529	Poodle, Standard	0.10	0	1653	1 *FGF4L2* **WT (1)**
37	American Bulldog	0.08	0	65	1 *FGF4L2* **WT (1)**
156	Golden Retriever	0.08	0	10 396	2 *FGF4L2* **WT (1); NG (1)**
79	Yorkshire Terrier	0.08	0.87	3722	6 *FGF4L2* **WT (6)**
10	Airdale Terrier	0.05	0	635	1 *FGF4L2* **WT (1)**
20	Old English Sheepdog	0.05	0.02	370	1 *FGF4L2* **WT (1)**

Note: Gray shading—FCE study cases in *FGF4L2* segregating breeds showing number of study cases in the breed, FGF4L2 genotype status and number of cases with that status.

Abbreviation: NG, not genotyped.

[a]General population combined genotyping data from Batcher et al[15] and UC Davis Veterinary Genetics Laboratory.

[b]Total breed numbers presenting to UC Davis VMTH during the study period (total population 228 665).

TABLE 3 FCE predisposed breeds.

Breed	FCE cases	Hospital pop %[a]	FCE %	*P* value	OR (95% CI)	FCE UC Davis[b]	*P* value	OR (95% CI)
Boxer	8	1.7	8.2	<.001	5.1 (2.5-10.5)	3	.03	4.8 (1.5-15.7)
Great Dane	8	0.7	8.2	<.001	12.3 (6.0-25.4)	-	-	
Yorkshire Terrier	6	1.6	6.1	<.01	3.9 (1.7-9.0)	3	.02	5.2 (1.6-17.0)
Bernese Mountain Dog	3	0.5	3.1	.02	6.8 (2.2-21.6)	-	-	
Miniature Schnauzer	4	1.0	4.1	.02	4.2 (1.5-11.3)	-	-	
Rottweiler	6	2.5	6.1	.04	2.6 (1.1-5.9)	-	-	
Shetland Sheep Dog	3	0.8	3.1	<.05	3.9 (1.2-12.2)	-	-	
Labrador Retriever	14	8.7	14.3	.07	1.8 (1.0-3.1)	7	<.05	2.3 (1.1-5.2)
German Shepherd Dog	5	4.0	5.1	.6	1.3 (0.5-3.2)	4	.07	2.8 (1.0-7.9)

Note: Blue shading is to highlight/distinguish the subset of data from UC Davis cases vs cases from all institutions. Grey and dark blue shading is to highlight significant findings for All cases and UCD cases respectively.
[a]Total hospital population at UC Davis VMTH during the study period (228 665).
[b]Hospital population for UC Davis cases only (216 657).

TABLE 4 FCE % cases in breeds with 2% or more cases in the study compared to published data.

Study breed	% (n = 98)	Published breed confirmed	% (n = 105)	Published breed presumed	% (n = 172)
Labrador Retriever	14.3	Labrador Retriever	8.57	Labrador Retriever	14.61
Boxer	8.2	Boxer	2.86	Boxer	1.83
Great Dane	8.2	Great Dane	14.29	Great Dane	3.65
Rottweiler	6.1			Rottweiler	0.70
Yorkshire Terrier	6.1			Yorkshire Terrier	1.69
German Shepherd Dog	5.1	German Shepherd Dog	8.57	German Shepherd Dog	8.15
Miniature Schnauzer	4.1	Miniature Schnauzer	17.14	Miniature Schnauzer	21.21
Mix Breed	4.1	Mix Breed	8.57	Mix Breed	13.20
Bernese Mountain Dog	3.1	Bernese Mountain Dog	1.90	Bernese Mountain Dog	0.98
Shetland Sheepdog	3.1	Shetland Sheepdog	0.95	Shetland Sheepdog	4.07
Airedale Terrier	2.0				
Belgian Malinois	2.0				
Chihuahua	2.0			Chihuahua	0.70
Chow	2.0			Chow	0.14
Dobermann Pinscher	2.0	Dobermann Pinscher	4.76	Dobermann Pinscher	3.09
Golden Retriever	2.0			Golden Retriever	4.92
Irish Wolfhound	2.0	Irish Wolfhound	5.71	Irish Wolfhound	1.40
Pomeranian	2.0			Pomeranian	0.42

Note: Gray shading—study defined predisposed breeds.

population (228 665) over the study period. Seven breeds were significantly overrepresented: Boxer ($P < .001$), Great Dane ($P < .001$), Yorkshire Terrier ($P < .01$), Bernese Mountain Dog ($P = .02$), Miniature Schnauzer ($P = .02$), Rottweiler ($P = .04$), and Shetland Sheep dog ($P < .05$). Labrador Retriever was the most frequently represented breed in the study and also represented over 14% of the total hospital population with representation approaching defined statistical significance ($P = .07$). Analysis of UC Davis cases only, with reference to the UC Davis population data was done in 4 breeds (Table 3). Similar to total case data, Boxer ($P = .03$) and Yorkshire Terrier ($P = .02$) breeds were statistically overrepresented. Labrador Retrievers were significantly overrepresented in the UC Davis subset ($P < .05$) compared to approaching significance in the total case data set ($P = .07$).

3.4 | Breed frequency comparison to published data

Breed data from published FCE case series and reports (defined in Section 2) are listed in Data S3. There were 105 cases with a

TABLE 5 Rankings of most common breeds with FCE in the study and in published data.

Study breed	% (n = 98)	Published breed confirmed	% (n = 105)	Published breed presumed	% (n = 722)
Labrador Retriever	14.3	Mix Breed	17.14	Mix Breed	21.21
Boxer	8.2	Great Dane	14.29	Labrador Retriever	14.61
Great Dane	8.2	German Shepherd Dog	8.57	Miniature Schnauzer	13.20
Rottweiler	6.1	Labrador Retriever	8.57	German Shepherd Dog	8.15
Yorkshire Terrier	6.1	Miniature Schnauzer	8.57	Golden Retriever	4.92
German Shepherd Dog	5.1	Irish Wolfhound	5.71	Shetland Sheepdog	4.07
Miniature Schnauzer	4.1	Saint Bernard	5.71	Staffordshire Bull Terrier	3.93
Mix Breed	4.1	Dobermann Pinscher	4.76	Great Dane	3.65
Bernese Mountain Dog	3.1	Basset Hound	3.81	Dobermann Pinscher	3.09
Shetland Sheepdog	3.1	Boxer	2.86	Border Collie	2.11
Airedale Terrier	2.0	Miniature Poodle	2.86	Boxer	1.83
Belgian Malinois	2.0	Bernese Mountain Dog	1.90	Yorkshire Terrier	1.69
Chihuahua	2.0	Shih Tzu	1.90	Irish Wolfhound	1.40
Chow	2.0				
Dobermann Pinscher	2.0				
Golden Retriever	2.0				
Irish Wolfhound	2.0				
Pomeranian	2.0				

Note: Gray shading—breeds represented in the study population.

histopathological diagnosis of FCE and 712 cases with a presumed diagnosis of FCE. The percentage of female and male dogs for histopathologically confirmed and presumed cases were 41% female; 59% male and 44% female; 56% male respectively. All of the study predisposed breeds were represented in the published presumed FCE data set, and all study predisposed breeds except Rottweiler and Yorkshire Terrier were represented in the published histopathologically confirmed data set (Table 4). The most frequently reported cases (more than 2% of cases within the groups) for the study group, published histopathologically confirmed group and published presumed groups are shown in Table 5. Of breeds representing greater than 2% of the various group populations, Saint Bernard and Miniature Poodle were present in the published confirmed group but not the study group or published presumed FCE group. Staffordshire Bull Terrier and Border Collie were present in the published presumed group but in neither the study group nor the published confirmed groups (Table 5).

4 | DISCUSSION

Results of our study, based on histopathologically confirmed cases and *FGF4L2* genotyping, support the published data that suggested a low incidence of FCE in presumed chondrodystrophic dogs. The signalment of the dogs in the current study was broadly similar to that reported in a recent retrospective review of published cases including presumed and confirmed cases,[1] and review of breed definable published cases (Tables 4 and 5; Data S3). Similar to published data, study cases had a median age of approximately 6 years, a broad age range (3 months-13 years), and a moderate predominance of male dogs (54%). Commonly affected breeds were also similar including a predominance of larger breed dogs, but a substantial number of small and medium breed dogs were also represented (Data S1).

Gene retrocopies are formed by mRNA-mediated gene duplication of processed mRNA and multiple *FGF4* retrocopies have recently been reported in Canids.[49] Two functional *FGF4* retrogenes have been described in dogs located on chromosome 18 (*FGF4L1*) and chromosome 12 (*FGF4L2*), both associated with disproportionate dwarfism.[16,50] The *FGF4L1* retrogene has been associated with marked limb shortening (termed chondrodysplasia)[50] whereas the *FGF4L2* retrogene has been associated with more moderate limb shortening and premature degeneration of the intervertebral disc (termed chondrodystrophy).[14-17,51,52] No additional genetic loci have been identified associated with premature intervertebral disc degeneration, however additional risk loci might be present associated with premature disc degeneration in the absence of *FGF4L2* or as modifiers of the phenotype.[15,53] Other than anecdotally reported breed overrepresentations, there have been no genetically defined associations with FCE in dogs or in humans.

As population data for breed associated *FGF4L1* and *FGF4L2* retrogene frequency in dogs has matured, it has become apparent that some dog breeds previously thought to have chondrodystrophic features do not carry the *FGF4L2* retrogene, and some larger dog breeds and breeds not presenting with typically recognized chondrodystrophic features can carry the *FGF4L2* retrogene at low frequencies.[15] Published, histopathologically confirmed, reports of FCE in small breed, presumed chondrodystrophic breeds are, retrospectively, associated with breeds that have very low *FGF4L2* general population

allele frequencies or segregate the allele at lower frequencies as defined in Section 2. Historically reported breeds include Shih Tzu[42,45] (freq 0.22), West Highland White Terrier[9] (freq 0), Wire Fox Terrier[9] (freq 0), Maltese[24] (freq 0.05), and a Kerry Blue Terrier[24] (freq 0). A small number of confirmed cases are reported in breeds that segregate the *FGF4L2* allele at greater than 50% including 1 Pekingese[28] (freq 0.59), 3 Miniature Poodles[7] (freq 0.6), and 4 Basset Hounds[7,24] (freq 0.79). As with our study, however, one cannot assume the genotypes of dogs from segregating breeds, even when allele frequency is high (Table 2). Similar findings are present in published presumptive cases with small numbers of West Highland White Terriers, Border Terriers, Jack Russell Terriers, Brussels Griffon, Shih Tzus, Chihuahuas, Yorkshire Terriers, Pomeranians, Maltese, Tibetan Terriers, Lhasa Apso, and English Bulldogs where breed allele frequencies are less than 0.25.[6,9,11-13,28,29,31,32,38,44] The Miniature Schnauzer is one of the most commonly reported small breed dogs with FCE, although not typically viewed as chondrodystrophic, and also segregates the *FGF4L2* allele at a lower frequency (0.23).[5,7] Reports of FCE in a small number of dogs from breeds with high, borderline fixed, *FGF4L2* allele frequencies are all in presumptive cases and include 2 Dachshunds[32] (freq 0.95), 2 Cocker Spaniels[9,43] (freq 0.96), 4 French Bulldogs[31,33] (freq 0.92), and 2 Beagles[13] (freq 0.92). Limitations of presumptive FCE diagnoses based on myelography, clinical signs and MRI are considerable, and it is notable that neither our study nor historical confirmed cases included these breeds.

Embolization of fibrocartilaginous material can be either arterial or venous and proposed mechanisms for embolization are numerous. Since the intervertebral disc is relatively avascular, access to the vascular system has been suggested variably to occur via direct injection into radicular or other arterial branches or venous plexi following herniation, via persistent vessels within the NP[3] or potentially via revascularized intervertebral disc following aging or degeneration.[54] Fibrocartilage could also originate from herniated fibrocartilaginous material within the vertebral body (Schmorl's nodes) with entry into vertebral sinusoids, arteries or veins.[2,3,55] Whether by arterial or venous routes, fibrocartilage would have to travel retrograde to the normal circulation at some point to reach the spinal cord.[2] Retrograde flow in the vertebral venous system has been demonstrated in the context of increased intrathoracic or intra-abdominal pressures,[56] and injection of dyes into the vertebral body under pressure has resulted in material entering the spinal cord venous system.[57] Movement of dye from the NP into the vertebral body has also been demonstrated following compressive loading of vertebrae resulting in micro-trauma of the endplate.[58] Intradiscal pressures in dogs and humans have been reported in the 0.5-1.0 mega pascal (MPa) range, significantly higher than typical arterial blood pressure (120 mm Hg = 0.016 MPa), potentially providing sufficient force to facilitate penetration of nuclear material.[59,60] Although not the only potential mechanism for elevated pressure within the disc/vascular system, a history of exertion is frequently, but not always, associated with suspected and confirmed human FCE cases in humans,[61-63] and was recorded frequently, but in less than 50% of reported cases in dogs.[1] Different levels of pressure, variable arteriovenous vascular shunts, or potentially different primary mechanisms have been postulated to explain arterial, venous or combined lesions.[2,3,61] and arterio-venous shunts have been specifically described in the epidural and nerve root circulation in dogs.[64] However, hemorrhage that would be likely to occur with direct arterial "injection" is rarely reported, and Schmorl's nodes are only reported in about 30% of human FCE cases[62] and are relatively uncommon in dogs.[65]

In the absence of a defined pathogenesis, leveraging biological differences in genotypically defined dog breeds that appear to have a particularly low incidence of FCE might be as informative as studying breeds that are predisposed. At a simplistic level, assuming that the fibrocartilaginous emboli originate from the NP, biological differences in the fibrocartilaginous nuclear material, the vessels that are vulnerable to entry and propagation of material to clinically significant locations, or both, are likely to explain the low incidence of FCE in chondrodystrophic breeds. As postulated previously by several investigators, premature degeneration of nuclear material in chondrodystrophic breeds might make it non-permissive to entry into the vascular system. Overexpression of *FGF4* via the *FGF4L2* retrogene is implicated in premature intervertebral disc degeneration in chondrodystrophic dogs[15,16,51] and degeneration of the NP is already apparent in chondrodystrophic, *FGF4L2* retrogene carrying dogs as early as 10 weeks.[17,52] The exact mechanism relating *FGF4* overexpression to premature IVDD is not defined, however recent single cell RNASeq studies defining the heterogenous cellular populations in the embryonic and adult intervertebral disc might provide a framework for potential mechanisms.[66-69] Whatever the mechanism is determined to entail, premature degeneration of the IVD in chondrodystrophic dogs does not preclude other mechanisms for FCE pathogenesis since FCE is frequently documented in older non chondrodystrophic breeds where similar age-related NP degeneration is also likely to be present.[70,71]

Developmental differences in vascular anatomy of the disc, vertebra, and spinal cord in chondrodystrophic dogs might also be non-permissive to entry of fibrocartilage, and/or subsequent passage of the material to clinically relevant areas. Interrogation of the nuclear degeneration hypothesis is challenging; however, defining potential differences in vascular anatomy in low-risk breeds could be more tractable. FGF4 has been shown to have diverse functions during embryonic and adult stages and plays a key role in embryonic development, tumorigenesis, and regulation of tissue (and embryonic) stem cells (reviewed in Refs.[72,73]).

Formation of the vascular system involves de novo formation of blood vessels (vasculogenesis) followed by angiogenic remodeling.[74] Several signaling pathways have been implicated in these processes including VEGF, NOTCH, TGFb, and FGFs.[74-77] Signaling through the FGF receptor is important for development and maintenance of a mature vascular network in the embryo,[78] and the FGF system has a key role in regulating vascular integrity.[79] FGF4 specifically has been shown to stimulate endothelial cell proliferation and has potent angiogenic activity both in vitro and in vivo,[80-84] and FGF4 maintains Hes7 levels, a downstream target of NOTCH signaling, during somite development.[76,85] With such a diverse involvement in vascular development and maintenance in the normal animal, it is not illogical to

hypothesize that overexpression of *FGF4* through an active retrogene, previously shown to be expressed in the neonatal IVD,[16] might have implications for development of a vascular system that could affect predisposition to embolization in chondrodystrophic dogs.

Study limitations primarily relate to a low number of cases and use of surrogate population data for assessment of breed predisposition and independence of FCE and *FGF4* retrogene status. Assessment of breed predisposition across 8 institutions by integrating all hospital populations was not feasible and a single institution's population data over the period of case selection was used as a surrogate. The specific institution population data used was chosen based on the highest number of samples from that institution. Parallel assessment of breed predisposition based on that institution's more limited data showed very similar findings (generally with lower power) where analysis was possible. Similarly, use of a single institution's population data combined with general dog population allele frequency data limited to a 2-year period[47,48] has limitations. However, this is somewhat mitigated by the high sample size (>1 million dogs) and sampling across pure and mix-breed populations from 150 countries. The striking difference between *FGF4L2* dependence ($P < .001$) and *FGF4L1* independence ($P > .99$) would suggest this was likely not a major issue. Both *FGF4* retrogenes are generally associated with smaller breed dogs, and the marked difference in frequency of the 2 alleles in the case population also suggests that size alone is unlikely to explain the low incidence of FCE in chondrodystrophic (*FGF4L2*-carrying) breeds. Breed data for FCE cases, breed allele frequencies and general population frequencies are based on owner-reported breed status and are likely to contain erroneous data; however, it is not unreasonable to assume that the frequency of incorrect reporting would be relatively consistent across all breeds in the populations.

The only *FGF4L2* positive FCE cases were seen in 2 Labrador Retrievers. The *FGFL2* retrogene is seen at a relatively low frequency in some larger breed dogs, but the *FGF4L1* retrogene, which has a more profound effect on limb length[14] was not found previously in over 200 genotyped Labrador Retrievers. In this context, the finding of an FCE Labrador Retriever heterozygous for *FGF4L2* and homozygous for *FGF4L1* is somewhat anomalous and could be related to misreporting or sample processing, although genotyping was repeated and confirmed. Breed frequency data from our study and historical published data were broadly comparable. The differences between the confirmed FCE cases in our study and confirmed published cases (Tables 3 and 4) might relate to small sample sizes. Interestingly, Staffordshire Bull Terriers and Border Collies were the only breeds representing greater than 2% of the non-confirmed published cases that were not present at similar frequency in either of the confirmed groups. A single histopathologically confirmed case of FCE has been reported in a Border Collie[22] with no confirmed cases in Staffordshire Bull Terriers. Border Collies are reported at high frequencies in cases of presumed acute NP extrusion, whereas Staffordshire Bull Terriers have been reported in 2 United Kingdom studies to occur at high frequency in both presumed FCE and acute NP extrusion cases.[6,11] Border Collies and Staffordshire Bull Terriers have very low *FGF4L2* allele breed frequencies, 0.03 and 0 respectively. Whether the discordance between confirmed and presumptive FCE cases in these breeds represents misclassification or anomalies related to geographical differences in breed popularity remains to be clarified.

The data presented support previous historical observations that FCE occurs at a low frequency in chondrodystrophic breeds, as defined by the presence of the *FGF4L2* retrogene. Comparative vascular studies of the IVD and spinal cord in this defined genetic group appear to be warranted to clarify vascular anatomy that might be relevant to the pathogenesis of FCE.

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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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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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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