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Presence and impact of the exercise-induced collapse associated *DNM1* mutation in Labrador retrievers and other breeds

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ABSTRACT

The impact of the mutation causing *dynamin 1* (*DNM1*)-associated exercise-induced collapse (d-EIC) was determined in a retrospective genetic survey. The frequency of *DNM1* mutant allele carriers in Labrador retrievers from conformation show, field trial/hunt test, pet or service lines ranged from 17.9% to 38.0% and the frequency of homozygous mutant (EE genotype) individuals ranged from 1.8% to 13.6%; 83.6% of these EE Labradors were reported to have collapsed by 4 years of age.

DNM1 mutation carriers and EE dogs with a collapse phenotype were also detected in Chesapeake Bay retrievers, Curly-coated retrievers, Boykin spaniels, Pembroke Welsh corgis and mixed breed dogs thought to be Labrador retriever crosses. The *DNM1* mutation was not identified in Golden, Flat-coated, or Nova Scotia duck tolling retrievers, or 15 other non-retrieving breeds. Veterinarians and breeders should be aware that the *DNM1* EE genotype is not completely penetrant and that d-EIC is a widespread health concern in several very popular breeds, as well as breeds whose genetic similarity to retrievers is not obvious.

Introduction

There are many potential underlying physical, genetic, environmental and nutritional causes of weakness and collapse associated with exercise in dogs (Cosford and Taylor, 2010). Labrador retrievers with the disorder traditionally known as exercise-induced collapse (EIC) are normal at rest but, following 5–20 min of intense exercise, typically develop hind limb incoordination and non-painful flaccid paraparesis, progressing to collapse (Taylor, 2007; Taylor et al., 2009). Repetitive fun retrieves, field training, upland hunting and excited play are most likely to induce collapse. Loss of the patellar reflex during collapse is a consistent finding and signs can sometimes progress to include the thoracic limbs (Taylor et al., 2009). Most dogs fully recover after 15–30 min rest, but there are reports of fatalities during or after exercise (Taylor, 2007; Taylor et al., 2008). Most affected dogs are unable to continue participating in strenuous trigger activities, but can continue with mild to moderate intensity exercises. The condition is heritable (Taylor et al., 2008) and has been recognized in other retrieving breeds (Patterson et al., 2008).

The dynamin gene family encodes enzymes that participate in cellular endocytosis, including the supply of synaptic vesicles necessary for sustained neurotransmission (Noakes et al., 1999; Ferguson et al., 2007). Dynamin 1, encoded by the *DNM1* gene, appears to be particularly important in synaptic vesicle recycling at nerve terminals during high frequency neurological stimulation (Ferguson et al., 2007).

Our recent discovery of a *G767T* (R256L) *DNM1* mutation provides a molecular explanation for a disorder now called *DNM1*-asssociated EIC (d-EIC) (Patterson et al., 2008). Most Labrador retrievers that have multiple collapse episodes with exercise, but are normal at rest, are homozygous (EE) for the *DNM1* mutation (Patterson et al., 2008), while heterozygotes (EN) do not appear to have an increased risk of collapse, consistent with an autosomal recessive mode of inheritance (Patterson et al., 2008; Taylor et al., 2008).

We hypothesize that the mutant dynamin 1 protein retains sufficient enzymatic activity to maintain synaptic transmission at rest and during moderate exercise; however, in homozygotes it cannot keep pace with the need for synaptic vesicles during intense excitement or strenuous exercise, resulting in a loss of neural activity, incoordination and ultimately collapse. We further hypothesize that the elevated body temperature that occurs during strenuous exercise (Matwichuk et al., 1999; Taylor et al., 2009)

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contributes to dysfunction of the mutant dynamin protein, as it does in a *Drosophila melanogaster* model with a mutation in the fly homolog of *DNM1* (Patterson et al., 2008).

Despite this improved understanding of the underlying basis for collapse with exercise in dogs with d-EIC, a number of issues remain. The aim of the present study was to determine the presence and impact of the mutant *DNM1* allele in Labrador retriever subpopulations and other breeds, to estimate the penetrance of the homozygous mutant (EE) genotype and to evaluate its significance for the overall health and well-being of these populations.

Materials and methods

Labrador retriever samples solicited by the investigators

Several Labrador retriever populations were sampled to estimate the *DNM1* allele and genotype frequencies (Table 1). Samples from Labrador retrievers were collected at American Kennel Club (AKC) field trial competitions during the summer of 2007 ($n = 396$), Canadian Kennel Club (CKC) field trial and hunt test competitions held during the summer of 2007 ($n = 63$) and the Canadian National Retriever Championship held in September 2007 ($n = 23$). Dogs from throughout the US and Canada participated and sample collection was performed during the Open All Age competitions to maximize participation of competitive dogs being handled by professional retriever trainers. All Labradors retrievers present were eligible for inclusion and participation levels were high (>80%) at all locations.

Samples from conformation (dog show) lines of Labrador retrievers were collected using kits distributed at the USA Labrador Retriever Club 2007 National Specialty ($n = 198$). DNA samples from Labrador retrievers used as service dogs were obtained from a DNA repository maintained by the University of Missouri – Columbia College of Veterinary Medicine ($n = 112$). Additional samples from pet Labrador retrievers were obtained from a random sample of all dogs presenting to the University of California – Davis Veterinary Clinic ($n = 68$).

Labrador retriever samples submitted by veterinarians, breeders and owners

A large data set containing Labrador retriever samples submitted by the public was also available (Table 2). Samples from Labrador retrievers participating in the original genetic study ($n = 391$) (Patterson et al., 2008) and samples submitted to the University of Minnesota – Veterinary Diagnostic Laboratory[1] since the public offering of the genetic test for d-EIC in July 2008 ($n = 9125$), were included in the calculations of the prevalence of collapse or exercise intolerance for each genotype. Submitters were asked to classify the dog's lineage as field trial, hunt test, service, pet, conformation or a combination of these. More than 95% of these samples were from the USA or Canada. European dogs ($n = 2759$) submitted for d-EIC genotyping at the Laboklin Diagnostic Laboratory, Bad Kissingen, Germany,[2] were also evaluated to estimate the geographic distribution of EE-affected dogs in Europe. These dogs had limited phenotypic information available and their data was not used for calculations of the prevalence of collapse.

Assessment of Labrador retriever phenotype

Whenever possible, owners of tested Labrador retrievers were given questionnaires inquiring about their dog's collapse status (history of collapse or no observed collapse). In dogs with a history of collapse, additional questions included the frequency of collapse, age of onset of collapse episodes and descriptions of the collapse episodes. Questionnaires were also periodically solicited from owners of all dogs that were found to have the homozygous mutant genotype, but no history of collapse, in order to ascertain the onset of the collapse phenotype.

Samples submitted from other breeds

Samples from 22 other breeds were obtained from submissions to the University of Minnesota Veterinary Diagnostic Laboratory or the University of Minnesota Canine Neuromuscular Disease Genetics Laboratory. These breeds were selected based either on a documented or presumed close relationship to Labrador retrievers on the basis of natural histories and/or genetic marker clustering data (Parker et al., 2007), a suspected breed-associated increased prevalence of weakness or collapse with exercise or the availability of DNA samples (Tables 3 and 4). Samples were also collected from mixed breed dogs, primarily from purpose-bred Labrador retriever mixes.

Samples from non-Labrador retrievers with signs of collapse

[1] See: www.vdl.umn.edu/ourservices/canineneuromuscular/eic/home.html.

[2] See: www.laboklin.de/index.php?link = labogen/pages/html/en/geneticdiseases/dog/dog_EIC.htm.

Table 1
Dynamin 1 (*DNM1*) genotype numbers and frequencies in investigator-solicited Labrador retriever populations.

Population	Total	Genotype[a]			HWE[b]
		NN	EN	EE	*P* value
Conformation	198	96 (48.5%)	75 (37.9%)	27 (13.6%)	ns
Field trial/hunt test	482	276 (57.2%)	183 (38.0%)	23 (4.8%)	ns
Pet	68	47 (69.1%)	19 (27.9%)	2 (2.9%)	ns
Service	112	90 (80.4%)	20 (17.9%)	2 (1.8%)	ns
All	860	509 (59.2%)	297 (34.5%)	54 (6.3%)	ns

[a] EE, homozygous for the mutant allele; NN, homozygous for the normal allele; EN, heterozygous/carrier (one copy of mutant allele and one copy of normal allele).
[b] Test for fit of the observed genotype frequencies to Hardy–Weinberg equilibrium (HWE); ns, not significant ($P > 0.05$).

Table 2
DNM1 genotype numbers and frequencies of Labrador retrievers submitted to the University of Minnesota Veterinary Diagnostic Laboratory by owners, breeders and veterinarians.

Population	Total	Genotype[a]			HWE[b]
		NN	EN	EE	*P* value
Conformation	943	463 (49.1%)	356 (37.8%)	124 (13.1%)	5×10^{-5}
Field trial and/or hunt test and conformation	496	255 (51.4%)	186 (37.5%)	55 (11.1%)	2×10^{-2}
Field trial and/or hunt test	6500	3550 (54.6%)	2476 (38.1%)	474 (7.3%)	ns
Pet	743	331 44.5%	212 (28.5%)	200 (26.9%)	4×10^{-29}
Service	443	227 (51.2%)	162 (36.6%)	54 (12.2%)	4×10^{-3}
All	9125	4826 (52.9%)	3392 (37.2%)	907 (9.9%)	3×10^{-17}

[a] EE, homozygous for the mutant allele; NN, homozygous for the normal allele; EN, heterozygous/carrier (one copy of mutant allele and one copy of normal allele).
[b] Test for fit of the observed genotype frequencies to Hardy–Weinberg equilibrium (HWE); ns, not significant ($P > 0.05$).

Table 3
DNM1 genotype numbers and frequencies in non-Labrador retriever breeds.

Breed	Total	Genotype[a]			HWE[b]
		NN	EN	EE	*P* value
Chesapeake Bay retriever	320	256 (80.0%)	56 (17.5%)	8 (2.5%)	3×10^{-2}
Curly-coated retriever	251	118 (47.0%)	84 (33.5%)	49 (19.5%)	1×10^{-5}
Flat-coated retriever	99	99 (100.0%)	0 (0.0%)	0 (0.0%)	–
Golden retriever[c]	270	269 (100.0%)	0 (0.0%)	0 (0.0%)	–
Nova Scotia duck tolling retriever	78	78 (100.0%)	0 (0.0%)	0 (0.0%)	–

[a] EE, homozygous for the mutant allele; NN, homozygous for the normal allele; EN, heterozygous/carrier (one copy of mutant allele and one copy of normal allele).
[b] Test for fit of the observed genotype frequencies to Hardy–Weinberg equilibrium (HWE).
[c] One dog reported as a Golden retriever was genotyped as EN but its parentage and breed registration could not be confirmed.

Table 4
DNM1 genotype numbers and frequencies in non-retriever breeds in which the mutation was found.

Breed	Total	Genotype[a]			HWE[b]
		NN	EN	EE	*P* value
Boykin spaniel	125	70 (56.0%)	45 (36.0%)	10 (8.0%)	ns
Mixed breed	16	15 (93.8%)	1 (6.2%)	0 (0.0%)	–
Mixed breed (Labrador cross)	107	77 (72.0%)	24 (22.4%)	6 (5.6%)	–
Pembroke Welsh corgi	80	69 (86.3%)	10 (12.5%)	1 (1.2%)	ns

[a] EE, homozygous for the mutant allele; NN, homozygous for the normal allele; EN, heterozygous/carrier (one copy of mutant allele and one copy of normal allele).
[b] Test for fit of the observed genotype frequencies to Hardy–Weinberg equilibrium (HWE); ns, not significant ($P > 0.05$).

Samples from dogs with a history of exercise intolerance or collapse were submitted by owners or veterinarians to the University of Minnesota Veterinary Diagnostic Laboratory. The retriever breeds included Chesapeake Bay retrievers ($n = 13$), Curly-coated retrievers ($n = 5$), Flat-coated retrievers ($n = 5$), Golden retrievers ($n = 13$) and Nova Scotia duck tolling retrievers ($n = 2$). The non-retriever breeds included Australian kelpie ($n = 1$), Australian shepherd dog ($n = 2$), Beagle ($n = 2$), Bearded collie ($n = 2$), Belgian malinois ($n = 3$), Belgian shepherd dog ($n = 1$), Border collie ($n = 23$), Boykin spaniel ($n = 10$), Chihuahua ($n = 3$), Dutch shepherd dog ($n = 1$), English cocker spaniel ($n = 1$), English setter ($n = 2$), English springer spaniel ($n = 8$), French Brittany spaniel ($n = 1$), German shepherd dog ($n = 4$), German shorthaired pointer ($n = 4$), German wire-haired pointer ($n = 1$), Great Dane ($n = 1$), Greater Munsterlander ($n = 2$), Icelandic sheepdog ($n = 1$), Koolie ($n = 1$), Macnab ($n = 1$), Pembroke Welsh corgi ($n = 1$), Poodle ($n = 1$), Hungarian Vizsla ($n = 1$), Whippet ($n = 2$) and mixed breed dogs ($n = 36$).

Ethical use of animals

University of Minnesota Institutional Animal Care and Use Committee (IACUC) approval and client consent was obtained for all prospective investigator-solicited DNA samples (University of Minnesota IACUC approval number 0711A21742). All respective University regulations relating to confidentiality and/or research in animals were followed for all retrospective banked samples at the Universities of Minnesota, Missouri–Columbia and California–Davis. Furthermore, all University of Minnesota Veterinary Diagnostic Laboratory and University of Minnesota regulations relating to confidentiality and/or research in animals were followed for all samples submitted to the University of Minnesota Veterinary Diagnostic Laboratory for d-EIC genetic testing.

Genotyping the DNM1 G767T mutation

DNA isolation from buccal swabs or whole blood, as well as *DNM1* genotyping, were performed as described by Patterson et al. (2008). The *DNM1* genotypes were identified as two copies of the mutant allele (homozygous E, EE), two copies of the normal allele (homozygous N, NN) or a copy of each allele (heterozygous/carrier, EN). Tests of the fit of the observed *DNM1* genotype frequencies to those predicted by Hardy–Weinberg equilibrium were performed with an online calculator.[3]

Results

DNM1 genotype frequencies in investigator-solicited Labrador retriever samples

The homozygous mutant (EE) genotype that confers susceptibility to d-EIC was found in all Labrador retriever sub-populations (Table 1) and ranged from 1.8% in service dogs to 13.6% in conformation show dogs. The heterozygote/carrier (EN) genotype frequency ranged from 17.9% in service dogs to 38.0% in field trial/hunt test and conformation show dogs. While the frequency of the homozygous mutant was apparently lower in the field trial/hunt test group compared to the conformation group (4.8% vs. 13.6%, respectively), the frequency of the heterozygous genotype was the approximately the same between these two groups (37.9% vs. 38.0%).

DNM1 genotype frequencies in Labrador retriever samples submitted by veterinarians, breeders and owners

The EE and EN genotypes were again found in all Labrador subpopulations (Table 2). The EE genotype frequency ranged from 7.3% in field trial/hunt test dogs to 26.9% in pet dogs. The EE genotype frequency was higher in this group of samples (Table 2) than in the investigator-solicited samples (Table 1) for all groups except conformation show dogs. The EN genotype frequency ranged from 28.5% in pet dogs to 38.1% in field trial/hunt test dogs (Table 2).

Two or more EE dogs were documented in the following countries outside of North America; Australia ($n = 2$), Austria ($n = 2$), Belgium ($n = 5$), Czech Republic ($n = 13$), Denmark ($n = 40$), Germany ($n = 146$), Finland ($n = 89$), France ($n = 3$), Great Britain ($n = 29$), Italy ($n = 3$), Norway ($n = 2$), The Netherlands ($n = 25$), Poland ($n = 3$), Slovakia ($n = 9$), Sweden ($n = 11$) and Switzerland ($n = 2$). The *DNM1* genotype frequency of this combined non-North American population was 52.6% NN, 35.8% EN and 11.6% EE.

Association between DNM1 genotype and collapse phenotype in Labrador retrievers

Basic questionnaires were available from the owners of 10,377 genotyped Labrador retrievers. Age of onset information was supplied in detailed questionnaires from 307 EE dogs identified with a collapse phenotype. These dogs were first observed to have collapsed between 3 months and 10 years of age (Fig. 1). The mean age and standard deviation at the time of the first collapse event was 17 ± 15 months and 95.9% of all EE dogs reported to collapse did so at or before 48 months of age.

The prevalence of a collapse phenotype in Labrador retriever subgroups was analyzed according to genotype. For this data, dogs collapsing before 4 years of age and dogs that had attained 4 years without evidence of collapse were included, but dogs younger than 4 years of age with no history of collapse were not included because they were less likely to have yet expressed the phenotype. Overall, at least one episode of exercise intolerance or collapse was recognized in 961/4359 (22.0%) Labrador retrievers eligible for inclusion and 649/961 (67.5%) of these collapsing dogs were EE. Table 5 shows that 649/776 (83.6%) EE Labrador retrievers, 210/2271 (9.2%) NN Labrador retrievers and 102/1312 (7.8%) EN Labrador retrievers had reports of collapse. EE dogs from the field trial and hunt test categories appeared to be more likely to have a reported collapse than EE dogs from conformation show lines (85.7% vs. 60%, respectively).

DNM1 genotype frequencies and collapse phenotype in non-Labrador retriever breeds

The *DNM1* mutation was detected in Chesapeake Bay and Curly-coated retrievers, with an EE genotype frequency of 2.5% and 19.5%, respectively, and an EN genotype frequency of 17.5% and 33.5%, respectively (Table 3); 3/3 EE Chesapeake Bay retrievers and 4/20 EE Curly-coated retrievers were reported to have experienced collapse by 48 months of age. The mutation was not observed in the other three retrieving breeds for dogs with verified purebred registration (Table 3).

DNM1 genotype frequencies and collapse phenotypes in non-retriever breeds

The EE and EN *DNM1* genotypes were detected in Boykin spaniels, Pembroke Welsh corgis, a group of mixed breed dogs thought

[3] See: www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls.

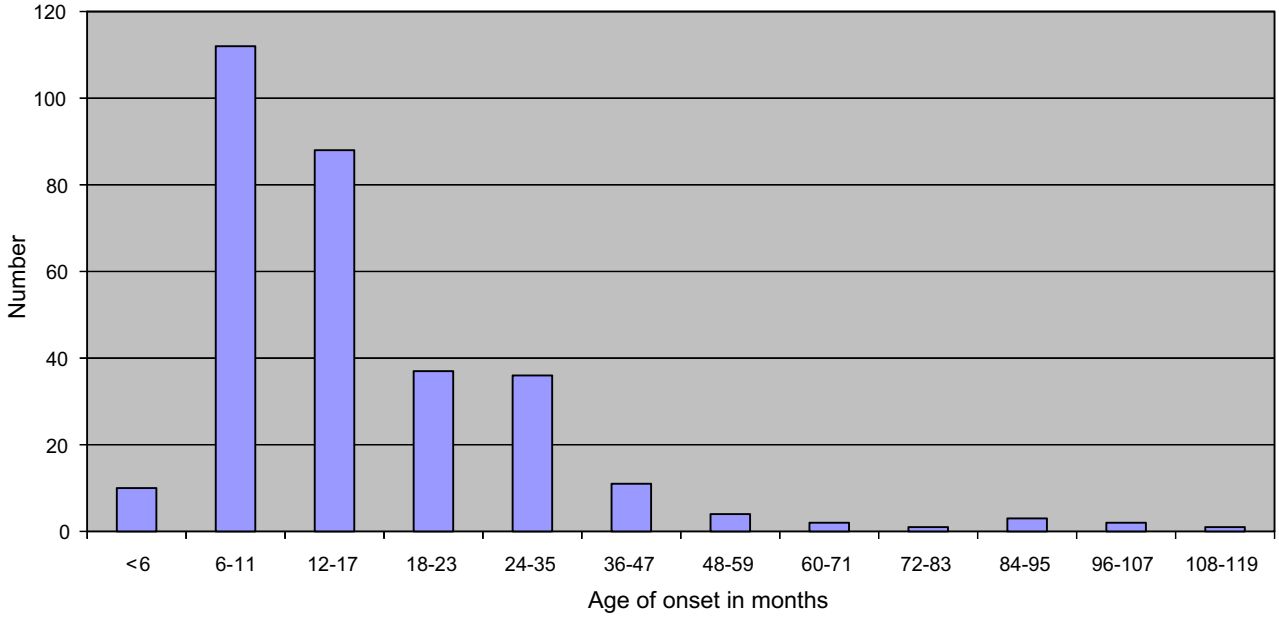

Fig. 1. Age (in months) of first observed collapse in 307 EE (homozygous for the *DNM1* mutation) Labrador retrievers. The mean age and standard deviation of first collapse was 17 ± 15 months, and 95.9% of all EE dogs that were reported to collapse did so at or before 48 months of age.

Table 5
Prevalence of a collapse phenotype in Labrador retrievers with different *DNM1* genotypes.

Population	Genotype and collapse phenotype[a]		
	NN with collapse	EN with collapse	EE with collapse
Conformation	8/193 (4.1%)	11/181 (6.1%)	63/105 (60.0%)
Field trial and/or hunt test	76/1730 (4.4%)	38/942 (4.0%)	394/460 (85.7%)
Field trial and/or hunt test and conformation	5/92 (5.4%)	1/65 (1.5%)	13/25 (52.0%)
Pet	112/158 (70.9%)	49/77 (63.6%)	163/168 (97.0%)
Service	9/98 (9.2%)	3/47 (6.4%)	16/18 (88.9%)
Total	210/2271 (9.2%)	102/1312 (7.8%)	649/776 (83.6%)

[a] *DNM1* genotype and collapse phenotype are tabulated in Labrador retriever samples that were investigator-solicited or submitted to either the original genetic study or to the University of Minnesota Veterinary Diagnostic Laboratory for *DNM1* genotyping by owners and veterinarians. Collapse phenotype was determined by questionnaire. The number of dogs that collapsed relative to the total number of dogs in that category is provided, along with the percentage affected. Non-collapsing dogs must have attained at least 48 months to be considered non-collapsing and to be included in these calculations.

to be Labrador retriever crosses and one mixed breed dog of unknown parentage, which was EN (Table 4); 8/10 EE Boykin spaniels and 1/1 EE Pembroke Welsh corgis were reported to have experienced collapse episodes by 48 months of age. None of the other 69 collapsing dogs from 24 other breeds tested contained the mutant *DNM1* allele.

The mutation was not observed in any of the other 15 non-retriever breeds examined for which breed registration could be confirmed. This included American water spaniels ($n = 114$), Australian shepherd dogs ($n = 40$), Beagles ($n = 42$), Border collies ($n = 151$), Boxers ($n = 32$), Dalmatians ($n = 71$), German shorthaired pointers ($n = 38$), Jack Russell terriers ($n = 99$), Leonbergers ($n = 38$), Newfoundlands ($n = 196$), Portuguese water dogs ($n = 49$), Soft-coated wheaten terriers ($n = 72$), Staffordshire bull terriers ($n = 66$), Standard schnauzers ($n = 84$) and Hungarian Vizslas ($n = 78$). One dog reported as a Border collie and one reported as a Staffordshire bull terrier were genotyped as EN, but their parentage and breed registration could not be confirmed.

Discussion

The discovery of a *DNM1* mutation very strongly associated with an exercise-inducible collapse in Labrador retrievers (Patterson et al., 2008) has provided a genetic test for the condition now known as d-EIC. The conservation of amino acid sequences at the site of the mutation and the role of dynamin 1 in synaptic vesicle recycling and in sustaining neurotransmission support a causal role for this mutation in d-EIC. Testing for the mutation has allowed veterinarians to specifically diagnose the condition, breeders to manage matings to reduce its incidence and researchers to further define its role in the larger group of collapse phenotypes that occur with exercise in dogs.

This study documents that *DNM1* EE and EN genotypes are present at a high and varied frequency in Labrador retrievers throughout the world and in all Labrador retriever breed subgroups in the USA (Table 1). Based on investigator-solicited samples, the EE genotype appears to be highest in populations of Labrador retrievers used for conformation shows (13.6%), lowest in dogs bred for service work (1.8%) and intermediate in dogs from field trial/ hunt test lines (4.8%). The EN genotype frequency ranged from 17.9 to 38.0% and was highest in conformation and field trial/hunt test dogs. The mean age at which EE Labrador retrievers with a collapse phenotype experienced their first episode was 17 months and >95.9% of all collapsing EE dogs did so before 4 years of age. This finding is similar to observations made prior to the discovery of the *DNM1* mutation (Taylor et al., 2008).

In our study, 83.6% of EE Labrador retrievers were reported to have experienced a collapse event by 4 years of age (Table 5), indicating less than 100% penetrance of the EE genotype. There was also an apparent difference in penetrance between subpopulations, with signs of collapse being experienced by 85.7% of EE dogs par-

ticipating in field trials and hunt tests (the most physically demanding and intense tests of performance) and 60% of EE dogs participating solely in conformation shows. Thus, continuous, intense exercise, particularly when accompanied by a high level of excitement, anxiety or stress, is most likely to cause collapse in dogs with d-EIC (Taylor, 2007). We hypothesize that many non-collapsing EE dogs may never have been exposed to sufficient conditions to initiate collapse. It is also possible that certain EE dogs have modifying genes that increase or decrease their susceptibility to d-EIC-related collapse. The high rate of a collapse phenotype in NN Labradors specified as pets most likely reflects bias from the widespread use of the genetic test by veterinarians as an early step in the diagnostic approach to exercise intolerance in this group.

The EE genotype was present in 649/961 (67.5%) of collapsing Labrador retrievers, confirming that d-EIC is likely to be the major cause of recurrent episodes of collapse with exercise in this breed. There was no evidence for increased susceptibility to collapse in EN Labradors (7.8%) vs. NN Labradors (9.2%) (Table 5). These results are consistent with d-EIC being an incompletely penetrant autosomal recessive disease and suggest that individuals in all groups had exercise intolerance or collapse from condition(s) other than d-EIC. Metabolic, orthopedic, cardiac, respiratory, muscular and neurologic causes of weakness and collapse occur in dogs; even in EE dogs it is important to establish whether collapse is due to their *DNM1* genotype or other causes (Cosford and Taylor, 2010).

This study confirms our previous report of a relatively high frequency of EE and EN genotypes in Chesapeake Bay retrievers and Curly-coated retrievers (Patterson et al., 2008). However, the *DNM1* mutation was not identified in any of the other retriever breeds tested, including individuals with a history of exercise intolerance or collapse. Although 19.5% of Curly-coated retrievers had the EE genotype, only 4/20 dogs were reported to have experienced a collapse event by 4 years of age, so it appears that the EE genotype is not as likely to result in a collapse phenotype in this breed compared to Labrador retrievers. Again, the level of activity, excitement and drive, as well as other potential genetic modifiers, could be responsible for the apparently lower penetrance of the EE genotype in Curly-coated retrievers.

A common founder in the Labrador retriever breed many generations ago and recent high usage of popular sires for breeding within each subpopulation could explain the widespread distribution of the *DNM1* mutation in Labrador retrievers and related retrieving breeds, but the presence of the *DNM1* E allele in Pembroke Welsh corgis suggests a far older mutation.

There are a number of limitations to this study. Firstly, random sampling of the breeds and populations was not possible. Thus, a statistical comparison of genotype frequencies between Labrador retriever populations was not performed and only trends were noted. Sampling retrievers at field competition events may preferentially select NN and EN dogs, since collapsing EE dogs are often unable to train and compete at a high level. Evaluating samples submitted for pre-breeding evaluation will also underestimate E allele frequency, because samples from collapsing dogs would be unlikely to be submitted. Conversely, evaluating samples submitted by owners and veterinarians for diagnosis of collapsing dogs will overestimate E allele frequency. These biases are likely to be reflected in some of our data. For example, genotypic frequencies in samples submitted to the diagnostic laboratory indicated a relatively high EE frequency (26.9%) in pet dogs, a population less likely to receive pre-breeding screening and thus likely to be more representative of collapsing dogs (Table 2).

In contrast, in investigator-solicited samples from mostly non-collapsing dogs, only 2.9% of pet dogs had the EE genotype (Table 1). It is of interest that the investigator-initiated Labrador retriever populations in Table 1 are in Hardy–Weinberg equilibrium (HWE), possibly reflecting a relatively inclusive and random sampling of these populations, while many of the public-provided samples both from Labrador retrievers (Table 2) and other retriever breeds (Table 3) are not in HWE. Another concern is that there were insufficient samples examined from many breeds to be confident that the *DNM1* E allele would be detected, even if it was present. In a breed with a true *DNM1* E allele frequency of 5%, there is >99% chance of detecting E if 100 dogs are tested, but only 90% chance of detection if 45 dogs are tested (Gregorius, 1980). Thus, failure to detect the E allele in breeds where fewer dogs were sampled should not be interpreted to mean that there is no risk of d-EIC in these breeds.

Conclusions

While d-EIC is only one of many possible causes of exercise intolerance with collapse in dogs, it is a major contributor to the collapse phenotype within Labrador retrievers in North America and Europe and is also a cause for concern in Chesapeake Bay retrievers, Curly-coated retrievers, Boykin spaniels and Pembroke Welsh corgis. Homozygosity for the mutant allele of the *DNM1* gene makes it likely that a dog will collapse under conditions of intense exercise and/or excitement, but it does not guarantee that the dog will suffer from d-EIC episodes. A comprehensive breeding program to gradually eliminate the mutant allele in Labrador, Chesapeake Bay and Curly-coated retrievers, as well as Boykin spaniels and Pembroke Welsh corgis, could be implemented with testing, while still taking care to preserve genetic diversity and reduce the prevalence of other common inherited diseases.

Conflict of interest statement

The d-EIC test was developed through financial support from the AKC Canine Health Foundation. A portion of the proceeds from the test are returned to the AKC Canine Health Foundation to further its mission to improve the health of all dogs. Drs Mickelson, Patterson and Taylor and Ms Minor have a pending patent in the USA, Canada and the Bahamas for the d-EIC test and they receive a portion of the testing royalties. These results have been presented in part at the 27th Annual Forum of the American College of Veterinary Internal Medicine, Montreal, Canada, 3–6 June 2009. The contents of this publication are solely the responsibility of the authors and do not necessarily reflect the views of the Canine Health Foundation.

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