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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.