Title
Association of Dilated Cardiomyopathy with the Striatin Mutation Genotype in Boxer Dogs.

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Three forms of Boxer cardiomyopathy were originally described by Harpster in the early 1980s. Two were characterized by ventricular tachyarrhythmias either with or without clinical manifestations and a third, less common form, included dogs that presented with cardiac arrhythmias and congestive heart failure. The third is now considered to be a form of dilated cardiomyopathy (DCM), characterized primarily by left and sometimes right ventricular myocardial systolic dysfunction and chamber dilatation. Ventricular tachyarrhythmias and sometimes supraventricular tachyarrhythmias are also commonly present in this form of the disease.

The arrhythmic form of Boxer cardiomyopathy (Boxer cardiomyopathy forms 1 and 2 as described by Harpster) is now often referred to as arrhythmogenic right ventricular cardiomyopathy (ARVC). Arrhythmogenic right ventricular cardiomyopathy in Boxer dogs is an inherited disease that has been shown to be associated, at least in some families, with a deletion in the 3′ untranslated region of the striatin gene.
the striatin mutation and might be associated with the homozygous genotype.

Materials and Methods

This study was conducted in accordance with the guidelines of the Cummings School of Veterinary Medicine at Tufts University, Ohio State University, Oregon State University, North Carolina State University, and Washington State University Animal Care and Use committees.

Dogs were recruited for the study with the assistance of several veterinary cardiologists who supplied clinical information and DNA samples from adult Boxer dogs with a DCM phenotype. In addition, DNA samples were selected from an archival database of DNA samples from Boxers that had participated in a prospective study of Boxer ARVC. The database was searched for dogs that met the criteria for DCM. The DCM phenotype was defined as left ventricular fractional shortening (FS%) <21%, left ventricular internal diastolic dimension (LVIDD) >4.8 centimeters (cm), and left ventricular systolic internal dimension (LVIDS) >3.3 cm. These values were chosen based on being outside of (either above for LVIDD and LVIDS, or below for FS%) the reference range as defined for normal adult Boxer dogs.10

M-mode echocardiographic measurements were recorded from 2-dimensional guided right parasternal short axis views obtained with either a 3.5 or 5-MHz transducer with the dog positioned in right lateral recumbency. Measurements were made in accordance with the American Society of Echocardiography standards by board-certified veterinary cardiologists or their residents under the supervision of the veterinary cardiologist.11 Dogs with physical or biochemical evidence of serious systemic disease or a history of hypothyroidism (or treatment for hypothyroidism) were excluded from the study, but current thyroid hormone measurements were not required for inclusion in this investigation.

Two control groups of Boxer dogs were selected to evaluate the relationship of the striatin deletion to the DCM phenotype. Both groups were selected from a population of Boxer dogs that had been evaluated in a study of clinical and genetic aspects of ARVC. The 1st group was selected from adult Boxer dogs that were not currently affected with ARVC. They served as controls to evaluate the association of the striatin mutation with the DCM phenotype as compared to a normal Boxer population. They also served as controls to evaluate the association of the homozygous genotype to the DCM and ARVC phenotypes. These dogs were selected for inclusion if they were at least 7 years of age and had been evaluated at least 3 years consecutively by physical examination, echocardiography, 24-hour ambulatory electrocardiogram (AECG), and DNA control. Control dogs could have no more than 90 VPCs/24 hours during any examination.12 History of syncope, signs consistent with congestive heart failure, or both were exclusionary criteria. The 2nd group was selected from adult Boxer dogs with ARVC (Boxer dogs with frequent ventricular tachyarrhythmias and normal LV systolic function) that were positive for the striatin mutation. They served as a comparison group to evaluate the association of the homozygous genotype to the DCM or ARVC phenotypes. They were selected for inclusion if they had been evaluated by physical examination, echocardiography, and 24-hour AECG, had at least 90 VPCs/24 h, and were positive for the striatin gene deletion.

Age, sex, and echocardiographic data including LVIDD, LVIDS, and FS% were collected for each dog (DCM, ARVC, control groups). A list of medications being administered at the time of DCM diagnosis also was obtained.

DNA samples were obtained either from a blood sample collected in an EDTA tube or a buccal swab. DNA was extracted from the sample and evaluated by amplified fragment length polymorphism (AFLP)-polymerase chain reaction. Polymerase chain reaction (PCR) was used to amplify an approximately 350 base pair (bp) region of the striatin gene that contains the mutation. Standard PCR amplifications were carried out with NH₄SO₄ amplification buffer, Taq DNA polymerase (0.1 units/µL of reaction volume), 2.5 mM MgCl₂, 12.5 µM of each dNTP, and 2.5 mM of each primer (the 5′ end of the forward primer labeled fluorescently with 5′-FAM). Amplicons then were evaluated by AFLP on a 3730 DNA Analyzer.5 Alleles were scored by Peak Scanner Software version 1.0 according to fragment size (352 bp for wildtype, 343 bp and 352 bp for positive heterozygote fragments, and 343 bp for positive homozygous).

Association of the striatin deletion with the DCM phenotype was tested using a case-control design with a Fisher’s exact test with genotypic encoding (such that heterozygotes and homozygotes were treated as separate groups to avoid any genetic model assumptions). Association of the homozygous genotype with DCM phenotype was evaluated by a Fisher’s exact test performed with the DCM phenotypes and the control population. For comparison, the association of the homozygous genotype with the ARVC phenotype also was evaluated by a Fisher’s exact test with the ARVC phenotypes and the control population. T-tests were used to evaluate potential differences between the positive heterozygous and positive homozygous groups with DCM with regard to age, LVIDD, LVIDS, and FS%. To control for concerns about multiple comparisons, a Bonferroni corrected alpha was used to control the family-wise error rate. Based on the number of tests performed, an alpha of $P < .05$ was considered statistically significant. Confidence intervals were constructed to determine the lower and upper limits of the 95% confidence interval for the proportion of dogs with DCM that were likely to have the mutation.

Statistical analysis was performed by Stata v10 software (www.stata.com).

Results

Thirty-three Boxer dogs (18 male [9 intact, 9 castrated], 15 female [4 intact, 11 spayed]) with DCM were evaluated from 6 clinics: North Carolina State University (14), Oregon State University (6), University of California Davis (4), The Ohio State University (4), Cummings School of Veterinary Medicine at Tufts (3), and Chicago Veterinary and Emergency Center (2). Median age was 7 years (range 1–11 years). A medication history was available for 27 of the dogs in the DCM group at the time of diagnosis. Twelve of the 27 were not on any medication, 2 were only on an antibiotic (eg, amoxicillin, ciprofloxacin) and 13 were on a cardiac medication or nutritional supplement. Cardiac medications or supplements included atenolol (3), digoxin (3), enalapril (8), furosemide (5), mexiletine (3) pimobendan (4), sotalol (5), spironolactone (3), taurine (1), and L-carnitine (4).

The control group included 16 Boxer dogs (5 positive heterozygous, 11 negative). There were 11 female (4 intact, 7 spayed) and 5 males (1 intact, 4 castrated). Median age was 9 years (range, 5–10).
The ARVC group included 29 Boxer dogs (23 positive heterozygous, 6 positive homozygous). There were 19 female (4 intact, 15 spayed) and 11 male (5 intact, 6 castrated). Median age was 7 years (range, 2–10 years; Table 1). Thirty of the 33 dogs with DCM (95% confidence intervals, 78–97%) were positive for the striatin mutation (15 positive heterozygous, 15 positive homozygous). Three dogs with DCM were negative for the striatin mutation. (Table 1) The mutation was strongly associated with the DCM phenotype ($P < .001$).

The homozygous genotype was strongly associated with the DCM phenotype ($P = .005$), but not the ARVC phenotype ($P = .07$). There was no statistical difference between the Boxer dogs with DCM that were homozygous and those that were heterozygous for the striatin mutation with regard to age, sex, LVIDD, LVIDS, and FS%.

Comparison of age, sex, LVIDD, LVIDS, and FS% for the dogs with DCM and the striatin mutation to the dogs with DCM that did not have the striatin mutation was not performed because of the small size (N = 3) of the group that did not have the mutation.

### Discussion

The results of this study indicate a positive association between the development of DCM in the Boxer dog and the presence of the striatin mutation in comparison with the control groups and suggest that DCM in many Boxer dogs is another manifestation of ARVC. The homozygous genotype also was strongly associated with the DCM phenotype suggesting that homozygous dogs may be at an increased risk of developing the DCM phenotype.

Striatin is a desmosomal protein that colocalizes to plakophilin, a desmosomal protein associated with the development of ARVC in human beings. In the original report of the discovery of the striatin mutation in Boxer dogs with ARVC, dogs that were homozygous for the striatin mutation had decreased striatin RNA and protein in comparison with normal controls. Decreased amounts of this desmosomal protein could lead to a reduction in myocardial desmosomal integrity in Boxer dogs with ARVC. A central hypothesis of ARVC in human beings is that this disease results in loss of desmosomal integrity, impaired cell-to-cell adhesion, myocyte detachment, and cell death. Subsequently, these changes may lead to decreased resilience to mechanical stress and pressure. This is also a plausible explanation of the eventual development of DCM.

Dilated cardiomyopathy, affecting left ventricular or biventricular function, has been described as a form of ARVC in human beings. Although ARVC originally was thought of as only a right ventricular disease, involvement of the left ventricle is more common than originally thought and the term arrhythmogenic cardiomyopathy may be more appropriate. The various factors that lead to the development of the left and biventricular forms of this disease are not well understood. At least in some cases in human beings, the same genetic mutation can be associated with the development of both the arrhythmogenic form of ARVC and the DCM form within the same family.

Clearly, the contribution of other factors impacts the development of the penetrant form of this disease because not all dogs that are positive heterozygotes or homozygotes for the mutation develop either DCM or ARVC. We have previously estimated that penetrance in Boxer dogs with the striatin mutation, as evidenced by the development of ventricular arrhythmias, is approximately 82% in positive heterozygotes (82% of dogs heterozygous for the mutation will show evidence of the disease) and nearly 100% in positive homozygotes. This variation in penetrance may be an explanation of the 5 dogs in the control group that were positive heterozygous for the mutation, but did not demonstrate the ARVC phenotype when evaluated. In human beings, the penetrance of the disease has been shown to vary widely and may be as low as 20% (only 20% of people with the causative mutation will show the disease) and as high as 75% depending on the mutation. Environmental factors and modifying genes also are believed to have an impact on the development and variability in the final phenotype.

This study demonstrated a strong positive association between the presence of the striatin mutation and DCM in the Boxer dog. However, other causes of DCM must exist in this breed because 3 dogs with DCM were
negative for the mutation. These dogs may have a different form of Boxer DCM related to a 2nd genetic mutation or may have a nutritional (eg, carnitine deficiency) deficiency or even a viral etiology. In human beings, several viruses, including adenovirus, coxsackievirus, enterovirus, and parvovirus, among others, have been identified in the myocardium of some patients with ARVC, although causality has not been demonstrated.

Ours was a prospective multicentered study evaluating a fairly uncommon form of heart disease in the Boxer dog. As is the case for many clinical studies, it has several limitations. First, the clinical evaluations were performed by several different veterinary cardiologists. Although the clinical information was obtained by standard techniques, there may have been some variation in measurement techniques among the clinicians. In addition, many of the dogs were on some type of cardiac medication, at the time of diagnosis. Some of these medications, including furosemide, enalapril, and pimobendan, may have influenced blood volume, cardiac size, or both. Both of these issues could have led to an inability to detect differences in cardiac size or fractional shortening that might have existed between the positive heterozygous and positive homozygous groups. Finally, there were limitations to both the control and ARVC comparison groups. The control population of adult Boxer dogs was selected based on an age of at least 7 years. However, the age of the onset of cardiac disease in the Boxer dog is variable and some of the dogs chosen as controls might have gone on to develop cardiac disease. Finally, the selection of 90 as the number of VPCs/24 h used to separate the control group from the ARVC group was selected from a previous study that demonstrated that most normal adult Boxers <90 VPCs/24 h. However, ARVC is a complex disease and the use of a single diagnostic 24-hour AECG to diagnose ARVC is likely to lead to occasional misdiagnosis.

In conclusion, this study demonstrates an association between DCM in the Boxer dog and the striatin mutation, particularly with the homozygous genotype. The mating of 2 positive heterozygous dogs should be discouraged when possible to decrease the risk of producing dogs with the positive homozygous genotype. The observation that 3/33 dogs developed DCM but lacked the striatin mutation suggests that there is at least 1 other cause of DCM in the Boxer dog.

Acknowledgments

Conflict of Interest Declaration: Dr Meurs is listed as the investigator of the striatin mutation held by Washington State University.

Footnote

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References