Arrhythmogenic Right Ventricular Cardiomyopathy Causing Sudden Cardiac Death in Boxer Dogs
A New Animal Model of Human Disease

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Background—Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a primary familial cardiomyopathy associated with substantial cardiovascular morbidity and risk of sudden death. Efforts to discern relevant pathophysiological mechanisms have been impaired by lack of a suitable animal model.

Methods and Results—ARVC was diagnosed in 23 boxer dogs (12 male; 9.1 ± 2.3 years old). Clinical events alone or in combination included sudden death (n = 9; 39%), ventricular arrhythmias of suspected right ventricular (RV) origin (n = 19; 83%), syncope (n = 12, 52%), and heart failure (n = 3; 13%). Right ventricular enlargement or aneurysms occurred in 10 (43%). Striking histopathological abnormalities were present in each boxer dog but not in controls, including severe RV myocyte loss with replacement by fatty (n = 15, 65%) or fibrofatty (n = 8, 35%) tissue. Focal fibrofatty lesions were also present in both atria (n = 8) and the left ventricle (LV) (n = 11). Fatty replacement occupied substantially greater RV wall area in ARVC dogs than controls (40.4 ± 18.8% versus 13.8 ± 3.4%, respectively) (P < 0.001); residual myocardium was correspondingly reduced (56.6 ± 19.2% versus 84.8 ± 3.8% in controls) (P < 0.001). MRI demonstrated bright anterolateral and/or infundibular RV myocardial signals, confirmed as fat by histopathology. Myocarditis appeared in the RV (n = 14, 61%) and LV (n = 16, 70%) and in each dog with sudden death, but not in controls. Familial transmission was evident in 10 of the 23.

Conclusions—We describe a novel, spontaneous, and genetically transmitted animal model of ARVC associated with sudden death in the boxer dog, closely resembling the human disease. This model may aid in understanding the pathogenic mechanisms of ARVC. (Circulation. 2004;109:1180-1185.)

Key Words: models, animal ■ cardiomyopathy ■ pathology ■ death, sudden

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a primary familial cardiomyopathy associated with substantial cardiovascular morbidity and sudden death in the young.1-3 ARVC is transmitted as an autosomal dominant trait, and 2 mutations have been identified at the cardiac ryanodine receptor 2 gene (ARVD2) and the desmoplakin gene (ARVD8).4,5

It has been noted for many years that the boxer canine breed is predisposed to ventricular arrhythmias and sudden death,6,7 but the underlying disease responsible for these clinical features has been incompletely defined. In light of advances in genomic mapping of the domestic dog,8 a spontaneous canine model of ARVC and sudden death would provide a unique opportunity to study this disease genome and contribute valuable insights into its pathogenesis. Therefore, the purpose of the present study was to define the clinical and pathological features of a naturally occurring myocardial disease in boxer dogs and assess its suitability as an animal model of ARVC.

Methods

Selection of Animal Subjects
As part of an ongoing study to evaluate the heritability of ventricular arrhythmias in boxer dogs, 239 such animals, including 6 large families, were prospectively recruited for Holter ECG between 1997 and 1999 at the Ohio State University College of Veterinary Medicine. Of those with substantial ectopy or syncope that died or were euthanized, 23 boxer dogs (12 male, 11 female) had autopsy examination and constitute the study group. Animal handling was in accordance with the Ohio State University Institutional Guidelines for Use and Care of Animals, which approved experimental procedures. The boxer dogs were 4.5 to 13.7 years old (mean, 9.1 ± 2.3 years) and weighed 22.7 to 44.0 kg (mean, 30 ± 5.4 kg). Seven healthy mongrel dogs (3 male, 4 female; 5.4 to 9.8 years old [mean, 7 ± 4 years], weighing 20.4 to 33 kg [mean, 29 ± 5 kg]) that died or were euthanized for noncardiac causes were selected as controls.
Boxer dogs and controls did not differ with respect to age, sex, or body weight ($P>0.05$).

**Electrocardiography**
Standard, 9-lead ECGs were recorded. In addition, 3-channel, 24-hour ambulatory ECGs (Delmar Accuplus 363 Holter Analysis System) were obtained by a previously described technique. Morphology of ventricular tachycardia was evaluated on the basis of criteria derived from experimentally paced dogs.

**Magnetic Resonance Imaging**
Formalin-fixed hearts were viewed along their long and short axes in a water-filled box using spin-echo T-weighted multislice MRI scanning (Philips Gyroscan T5, 0.5 T). This technique allows fat (high intensity, bright signal) and fibrous tissue (low intensity, dark signal) to be differentiated from each other and from normal myocardium. MRI studies were interpreted with the researcher blinded to pathological results and animal identity.

**Gross Pathology**
Hearts were fixed in 10% phosphate-buffered formalin and weighed, and longitudinal and transverse diameters were measured. Wall thickness and chamber sizes were routinely assessed. Transverse, 5-mm sections were obtained perpendicular to the longitudinal axis of the RV and left ventricular (LV) chambers, two thirds of the distance distal to the atrioventricular valves toward the apex. Thicknesses of the RV and LV walls and ventricular septum were measured (aided by light microscopy and micrometer) from endocardium to epicardium, excluding epicardial fat and trabeculae.

**Histopathology**
Full-thickness tissue blocks were removed from the anterior, lateral, and posterior RV; anterior, lateral, and posterior LV; anterior, medial, and posterior ventricular septum; and left and right atrial walls. Longitudinal samples were also taken at the RV apex and RV outflow tract. Tissue sections 5 μm thick were cut and stained with hematoxylin and eosin and Heidenhain (azan) trichrome stains. Quantitative computerized morphometry was performed on panoramic, full-thickness RV wall samples stained by Heidenhain trichrome using an image analyzer system and commercially available software (Image-Pro Plus 4.0, Media Cybernetics).

Amounts of fatty tissue and residual myocardium were quantified as the sum of all adipose tissue or myocardium divided by the total area of tissue analyzed.

**Immunohistochemistry**
Myocardial inflammatory infiltrates were evaluated using antibodies against endothelial cells (von Willebrand factor, polyclonal), leukocytes (CD45), T lymphocytes (CD43 and CD4R5O), macrophages (CD68), and B lymphocytes (C20) (all monoclonal antibodies from Dako, except CD43 from Novocastra) according to the avidin-biotin complex method (Vector) and by quantitative immunohistochemistry criteria reported for myocarditis. Apoptosis was identified by the terminal deoxynucleotidyl transferase –dUTP-biotin nick end-labeling method (TUNEL). Sections were processed in accordance with the method of Gavrieli et al. An index was calculated as the number of apoptosis-positive myocytes divided by the total number of myocytes, multiplied by 100.

**Statistical Methods**
Data are reported as mean±SD. Continuous variables were compared using Student’s $t$ test or Mann-Whitney rank-sum test. One-way ANOVA was used for multiple comparisons, and Bonferroni’s procedure was used to control type I error. Categorical variables were assessed using Fisher’s exact test. A value of $P<0.05$ was considered statistically significant.

Clinical Course and Outcomes
Nine of the 23 boxer dogs (39%) died suddenly and unexpectedly, during vigorous exercise in 3, leisurely walking in 4, or while sleeping in 2. Three others were euthanized for severe, drug-refractory, and predominantly right-sided congestive heart failure associated with tachypnea, pleural effusion, jugular venous distention, and/or hepatosplenomegaly. Syncope occurred in 12 of the 23 boxer dogs (52%), including 6 of the 9 that subsequently died suddenly. Syncope was associated with exercise in 5 of these 9 that experienced sudden death. The remaining 11 animals died or were euthanized because of noncardiac conditions, including cancer (n=8), degenerative myelopathy (n=1), stroke of undetermined pathogenesis (n=1), and arthritis (n=1).

**Arrhythmias**
Ventricular premature complexes (PVCs) with left bundle-branch block morphology were documented by 24-hour Holter ECG in 19 (83%) of the 23 boxer dogs. In addition, PVCs of right bundle-branch block morphology were also detected in 4 of these 19 animals. The frequency of PVCs in a 24-hour period was ≥28 000 in 2 dogs; 7000 to 9000 in 4; 1000 to 5000 in 5, and zero to 999 in 8 dogs. Ventricular tachycardia (VT) with left bundle-branch block was detected in 11 boxer dogs (48%) (Figure 1). Of the 9 dogs that died suddenly, 1 had sustained VT, 2 had nonsustained VT with PVCs, and 3 had PVCs alone.

**Familial Occurrence**
Ten of the 23 boxer dogs with ARVC showed familial occurrence of the disease. One mother and male offspring both died suddenly of ARVC (both had documented ventricular arrhythmias, and the mother had sustained ventricular tachycardia). Also, 2 sibling pairs, another mother and male...
TABLE 1. Gross and Histopathological Characteristics of Boxer Dogs With ARVC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ARVC (n=23)</th>
<th>Controls (n=7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, g</td>
<td>252±35</td>
<td>245±63</td>
<td>0.4</td>
</tr>
<tr>
<td>RV thickness, mm</td>
<td>4.3±1.2</td>
<td>3.7±0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>LV thickness, mm</td>
<td>11.4±1.9</td>
<td>11.7±1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Myxomatous degeneration, mitral valve</td>
<td>7 (32)</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>Myocardial injury/repair, No. of dogs (%)</td>
<td>RV</td>
<td>23 (100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fibrofatty</td>
<td>8 (35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatty</td>
<td>15 (65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LV free wall*</td>
<td>11 (48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ventricular septum*</td>
<td>7 (30)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA or RA†</td>
<td>8 (35)</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Myocarditis,‡ No. of dogs (%)</td>
<td>RV</td>
<td>14 (61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>LV free wall</td>
<td>16 (70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>LA or RA</td>
<td>4 (17)</td>
<td>0.4</td>
</tr>
<tr>
<td>Apoptosis,§ No. dogs (%)</td>
<td>9 (39)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

LA indicates left atrium; RA, right atrium.
*Focal, fibrous tissue replacement with mild adipose replacement.
†Fatty (n=4) or fibrofatty (n=4) replacement.
‡Significant inflammatory infiltrates by immunohistochemistry quantitative criteria.13
§Mean apoptotic index in positive dogs was 4.0±3 in RV and 2.0±0.7 in LV.

Pathological Findings

Gross Pathology

Moderate RV chamber dilatation was judged on inspection to be present in 8 ARVC dogs (35%). Infundibular aneurysms were evident in 1 dog with RV chamber dilatation and also in 2 others. ARVC boxer dogs and controls did not differ significantly with regard to heart weight (252±35 versus 245±63 g), RV wall thickness (4.3±1.2 versus 3.7±0.9 mm), or LV thickness (11.4±1.9 versus 11.7±1.2 mm) (Table 1).

Histopathology

All 23 boxer dogs displayed histopathological lesions that closely resembled those characteristic of human patients with ARVC11,15 (Table 1). Most distinguishing was the substantial replacement of RV cardiac myocytes by adipose or fibrous tissue. This occurred in 2 patterns: a fatty form (n=15; 65%) (Figure 2) or a fibrofatty form (n=8; 35%) (Figure 3).

The fatty form was characterized by diffusely distributed, multifocal regions of adipose cell replacement within the RV wall and trabeculae, extending from epicardium toward endocardium, often in association with mild interstitial fibrosis (Figure 2, C and E). The fibrofatty form consisted of focal or diffuse regions of adipose cell replacement associated with areas of replacement fibrosis (Figure 3, A and B). Both the fatty and fibrofatty forms were characterized by residual surviving myocytes embedded within regions of fat, and fatty and fibrous tissue, respectively (Figures 2E and 3B).

Fatty tissue replacement was more extensive in the RV of ARVC boxer dogs than in controls (mean % area occupied by fatty tissue, 40.4±18.8% versus 13.8±3.4%, respectively; P<0.001). In ARVC dogs, mean % area of RV fat did not differ significantly between anterolateral (46.7±19.7%) and infundibular (45.2±12.2%) sites but was lower in the posterior wall (29.2±18.9%) (P=0.008) (Figure 4). Replacement of RV myocardium by fat was diffuse (involving ≥2 regions) in 16 ARVC dogs (70%) and segmental in the remaining 7 (30%). Residual RV area occupied by myocytes was correspondingly reduced in ARVC dogs (56.6±19.2%) compared with controls (84.8±3.8%) (P<0.001).

Left ventricular lesions were present in 11 of 23 ARVC hearts (48%) and consisted largely of focal, fibrous tissue replacement with some mild fatty tissue replacement; these 11 hearts included 6 with the fatty and 5 with the fibrofatty pattern. In addition, left or right atrial walls in 8 of the 23 dogs (35%) displayed myocyte loss with fatty or fibrofatty replacement. Myxomatous degeneration of the mitral valve leaflets was identified in 7 ARVC hearts but not in controls.
MRI scans were acquired in 19 of 23 ARVC boxer hearts and in 7 controls. ARVC hearts displayed high transmural signal intensity in the anterolateral and/or infundibular regions of the RV in all 14 hearts examined with fatty replacement, corresponding anatomically to those areas of RV fat identified by histopathology (Figure 2).

**Myocarditis and Apoptosis**

Myocarditis characterized by focal or multifocal lymphocytic infiltrates (CD45-, CD45RO-, and CD43-positive) and associated with myocyte death was identified in the RV of 14 of 23 ARVC boxer dogs (61%; fatty form in 7 and fibrofatty in 7) (Figure 3C). Myocarditis was also present in the LV free wall of 16 ARVC dogs (70%) and in the atrium of 4 (17%) but not in the controls. Myocyte apoptosis was identified in 9 ARVC hearts (39%) (Figure 3D) and not in controls. Mean apoptotic index was 4 ± 3 in the RV and 2 ± 0.7 in the LV.

**Clinicopathological Relationships**

Myocarditis and fibrofatty myocardial injury and repair were characteristic of boxer dogs with ARVC that died suddenly. Myocarditis was detected in the RV and/or LV in each of the 9 boxer dogs with sudden death (Table 2). There were no significant differences in demographics, clinical features, or extent of RV fat replacement between boxer dogs that died of ARVC and those that died of other causes. The percentage of RV wall fatty tissue did not correlate significantly with age, body weight, or heart weight.

**Discussion**

We have documented a novel, spontaneous animal model of ARVC and sudden death in the boxer dog that is strikingly similar to clinical and pathological features of the human condition.²,¹¹,¹⁶ The present study characterizes clinical, ECG, gross morphological, histopathological, morphometric, and MRI aspects of ARVC in this species. The combined clinical profile (sudden death, ventricular arrhythmias of suspected RV origin, and syncope) and pathological abnormalities (RV chamber enlargement and aneurysm, RV myocyte loss and fatty replacement, myocarditis, and apoptosis) provide compelling evidence for this canine model. Furthermore, this represents another example of spontaneous and previously unrecognized heart disease in animals closely resembling the human condition.¹²,¹⁷,¹⁸

Similar to the findings of the present study in canine ARVC, other naturally occurring animal models of cardiovascular disease have as a consequence sudden death. These include feline hypertrophic cardiomyopathy,¹⁷ ventricular arrhythmias associated with autonomic dysfunction in German shepherd dogs,¹⁹ and left ventricular dysfunction in Doberman pinscher dogs.²⁰ In contrast, however, sudden...
consider fatty and fibrofatty patterns to be consecutive stages of disease, mediated by myocarditis, in which the fatty form is an early feature and fibrofatty repair results from myocarditis-induced injury.\textsuperscript{21,22} Consistent with this hypothesis, RV inflammatory infiltrates were pronounced in boxer dogs with the fibrofatty form, whereas areas of myocarditis were small and uncommon when associated with purely fatty replacement. It is also noteworthy that in the feline model of ARVC, RV myocarditis was also a characteristic feature in the fibrofatty pattern.\textsuperscript{12} Furthermore, our finding of apoptotic myocytes in boxer dogs with ARVC is consistent with the hypothesis that loss of RV myocardial cells and replacement by fat and fibrosis is mediated by programmed cell death.\textsuperscript{15,23}

In this canine model of ARVC, striking histopathological lesions were associated with clinical findings and events because of electrical instability, including ventricular tachycardia and sudden death. For example, all boxer dogs (including those that died suddenly) had fatty or fibrofatty replacement that surrounded and embedded strands of surviving myocytes. Moreover, myocarditis was conspicuously present in all ARVC dogs with sudden death, suggesting that myocardial inflammation may also play a role in arrhythmogenesis. As with young ARVC patients who die suddenly,\textsuperscript{24–26} the presence of diffuse RV myocyte loss, fatty replacement, and residual myocytes embedded within fat, as well as myocarditis, in our boxer dogs suggests a substrate predisposed to sudden death.

In the present study, ARVC occurred in siblings, parents, and offspring, indicating familial transmission. We did not, however, systematically study those pedigrees, and therefore, the precise inheritance pattern remains unresolved. Nevertheless, examples of transmission from one generation to another suggest the likelihood of dominant inheritance which, in turn, would be consistent with that of the human disease.\textsuperscript{4,27} Of note, Meurs et al\textsuperscript{7} reported autosomal dominant inheritance in boxer dogs with ventricular arrhythmias. Although undocumented, it is possible that the familial occurrence of ventricular arrhythmias in such dogs was a marker for ARVC. Endeavors to identify a genetic basis for ARVC in the dog should accelerate as the canine genome and the genetic mutations responsible for ARVC in humans become clarified.

In conclusion, we report a novel, spontaneous animal model of ARVC and sudden death in boxer dogs that closely resembles the clinical and pathological features of the human disease. In addition to sudden death, this canine model is characterized by ventricular tachycardia of suspected RV origin and structural RV abnormalities distinguished by RV enlargement, myocyte loss with fatty or fibrofatty replacement, myocarditis, and apoptosis. Several of these animals were related, suggesting that canine ARVC is inherited. This animal model of human ARVC constitutes a new and potentially useful investigative tool to understand the complex clinical and pathogenic mechanisms responsible for sudden death and disease progression.

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**TABLE 2. Comparison of Clinical and Pathological Findings of Boxer Dogs With ARVC That Died Suddenly or of Other Causes**

<table>
<thead>
<tr>
<th></th>
<th>ARVC With Sudden Death (n=9)</th>
<th>ARVC Without Sudden Death (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>8.9±1.7</td>
<td>9.3±2.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Male</td>
<td>3 (33)</td>
<td>9 (64)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6 (67)</td>
<td>5 (36)</td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>27.6±3.5</td>
<td>32.5±6.1</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syncpe</td>
<td>6 (67)</td>
<td>6 (43)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ventricular arrhythmias (LBBB pattern)</td>
<td>6 (67)</td>
<td>13 (93)</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Pathological characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial injury/repair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphological pattern of ARVC</td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Fatty form</td>
<td>3 (33)</td>
<td>12 (86)</td>
<td></td>
</tr>
<tr>
<td>Fibrofatty form</td>
<td>6 (67)</td>
<td>2 (14)</td>
<td></td>
</tr>
<tr>
<td>Myocarditis</td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>RV and LV myocarditis</td>
<td>8 (89)</td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>LV myocarditis</td>
<td>1 (11)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>3 (33)</td>
<td>6 (43)</td>
<td>1.0</td>
</tr>
<tr>
<td>Myxomatous valve degeneration</td>
<td>1 (11)</td>
<td>6 (43)</td>
<td>0.2</td>
</tr>
<tr>
<td>Fatty tissue, mean % area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterolateral RV</td>
<td>51.2±21.9</td>
<td>43.3±17.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Posterior RV</td>
<td>35.7±21.7</td>
<td>25.1±16.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Infundibular RV</td>
<td>42.4±9.3</td>
<td>47±13.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%). LBBB indicates left bundle-branch block.

death is not a feature of the spontaneous feline model of ARVC.\textsuperscript{12}

Loss of RV myocytes with replacement by fat or fibrofatty tissue is the pathological hallmark of human ARVC.\textsuperscript{2,16,21} Postmortem MRI accurately identified intramyocardial fat in the RV locations confirmed by histopathology, suggesting that clinical recognition and assessment of disease progression is readily possible in this canine ARVC model.

Purely fatty replacement of RV represented the predominant morphological variant in two thirds of our boxer dogs, most substantially in the infundibular and anterolateral regions. In these dogs, the RV wall thickness remained normal, and RV aneurysms were uncommon. Although the fibrofatty pattern of ARVC was present in one third of dogs in the present study, in contrast, it constitutes the predominant morphological pattern in feline ARVC and is associated with extensive RV myocardial loss, replacement fibrosis, and correspondingly, frequent wall thinning and RV aneurysms.\textsuperscript{12} This latter association between fibrofatty replacement and RV wall thinning and aneurysmal formation is most similar to that observed in patients with ARVC.\textsuperscript{11}

Although the precise role of myocarditis and apoptosis in the pathogenesis of ARVC is unresolved, the findings of the present study support the view that these processes modulate disease morphology and progression. Some investigators...
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References
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