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

Cristina Basso, MD, PhD; Philip R. Fox, DVM; Kathryn M. Meurs, DVM, PhD; Jeffrey A. Towbin, MD; Alan W. Spier, DVM, PhD; Fiorella Calabrese, MD; Barry J. Maron, MD; Gaetano Thiene, MD

Background—Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a primary familial heart muscle disease 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.

Received July 24, 2003; revision received October 23, 2003; accepted October 28, 2003.
From the Institute of Pathology, University of Padua Medical School, Padua, Italy (C.B., F.C., G.T.); Caspary Research Institute of the Animal Medical Center, New York, NY (P.R.F.); the Ohio State University College of Veterinary Medicine, Columbus (K.M.M., A.W.S.); the Department of Pediatrics, Baylor College of Medicine, Houston, Tex (J.A.T.); and the Minneapolis Heart Institute Foundation, Minneapolis, Minn (B.J.M.).
Guest Editor for this article was Hein J.J. Wellens, MD, Cardiovascular Research Institute, Maastricht, the Netherlands.
© 2004 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000118494.07530.65

1180
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 Accumulus 363 Holter Analysis System) were obtained by a previously described technique.\(^9\) Morphology of ventricular tachycardia was evaluated on the basis of criteria derived from experimentally paced dogs.\(^{10}\)

**Magnetic Resonance Imaging**

Formalin-fixed hearts were viewed along their long and short axes in a water-filled box using spin-echo \( T_1 \)-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.\(^ {11}\) 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.\(^ {12}\) 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 from 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 \( \mu \)m 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-peroxidase complex method (Vector) and by quantitative immunohistochemistry criteria reported for myocarditis.\(^ {13}\) Apoptosis was identified by the terminal deoxynucleotidyl transferase–mediated dt/TP-biotin nick end-labeling method (TUNEL). Sections were processed in accordance with the method of Gavrieli et al.\(^ {14}\) An index was calculated as the number of apoptosis-positive myocytes divided by the total number of myocytes, multiplied by 100.\(^ {15}\)

**Statistical Methods**

Data are reported as mean \( \pm \) 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.
All 23 boxer dogs displayed histopathological lesions that closely resembled those characteristic of human patients with ARVC (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%) (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. 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.

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, ventricular arrhythmias associated with autonomic dysfunction in German shepherd dogs, and left ventricular dysfunction in Doberman pinscher dogs. In contrast, however, sudden...
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 a potentially useful investigative tool to understand the complex clinical and pathogenic mechanisms responsible for sudden death and disease progression.

Acknowledgments

This study was supported by the Ministry of Health and Telethon, Rome, Italy; the ARVC/D Project, QLG1-CT-2000-01091 5th Framework Programme, European Commission, Brussels, Belgium; and the Telethon, Rome, Italy.
the AKC Canine Health Foundation; ABT; NIH grant U01HL65652, Multidisciplinary Study of Right Ventricular Dysplasia; and the Caspari Research Institute. We are indebted to Drs Sydney Moise and Rebecca Stepien, who contributed normal canine hearts, and Mila Della Barbera, BSc, for histomorphometric analysis. We thank Alessandra Dubrovich and Mauro Pagetta for technical assistance.

References

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

Cristina Basso, Philip R. Fox, Kathryn M. Meurs, Jeffrey A. Towbin, Alan W. Spier, Fiorella Calabrese, Barry J. Maron and Gaetano Thiene

_Circulation_. 2004;109:1180-1185; originally published online March 1, 2004; doi: 10.1161/01.CIR.0000118494.07530.65

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/9/1180

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/