JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2015 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.

VOL. 65, NO. 14, 2015
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2015.01.045

Exercise Triggers ARVC Phenotype in Mice Expressing a Disease-Causing Mutated Version of Human Plakophilin-2

Francisco M. Cruz, PhD,* David Sanz-Rosa, PhD,† Marta Roche-Molina, PhD,* Jaime García-Prieto, BSc,† José M. García-Ruiz, MD,† Gonzalo Pizarro, MD,† Luis J. Jiménez-Borreguero, MD,† Miguel Torres, PhD,* Antonio Bernad, PhD,* Jesús Ruíz-Cabello, PhD,‡ Valentín Fuster, MD, PhD,†§ Borja Ibáñez, MD, PhD,†‖ Juan A. Bernal, PhD*

ABSTRACT

BACKGROUND Exercise has been proposed as a trigger for arrhythmogenic right ventricular cardiomyopathy (ARVC) phenotype manifestation; however, research is hampered by the limited availability of animal models in which disease-associated mutations can be tested.

OBJECTIVES This study evaluated the impact of exercise on ARVC cardiac manifestations in mice after adeno-associated virus (AAV)–mediated gene delivery of mutant human *PKP2*, which encodes the desmosomal protein plakophilin-2.

METHODS We developed a new model of cardiac tissue-specific transgenic-like mice on the basis of AAV gene transfer to test the potential of a combination of a human *PKP2* mutation and endurance training to trigger an ARVC-like phenotype.

RESULTS Stable cardiac expression of mutant *PKP2* (c.2203C>T), encoding the R735X mutant protein, was achieved 4 weeks after a single AAV9-R735X intravenous injection. High-field cardiac magnetic resonance over a 10-month postinfection follow-up did not detect an overt right ventricular (RV) phenotype in nonexercised (sedentary) mice. In contrast, endurance exercise training (initiated 2 weeks after AAV9-R735X injection) resulted in clear RV dysfunction that resembled the ARVC phenotype (impaired global RV systolic function and RV regional wall motion abnormalities on cardiac magnetic resonance). At the histological level, RV samples from endurance-trained R735X-infected mice displayed connexin 43 delocalization at intercardiomyocyte gap junctions, a change not observed in sedentary mice.

CONCLUSIONS The introduction of the *PKP2* R735X mutation into mice resulted in an exercise-dependent ARVC phenotype. The R735X mutation appears to function as a dominant-negative variant. This novel system for AAV-mediated introduction of a mutation into wild-type mice has broad potential for study of the implication of diverse mutations in complex cardiomyopathies. (J Am Coll Cardiol 2015;65:1438–50) © 2015 by the American College of Cardiology Foundation.

From the *Cardiovascular Development and Repair Department, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain; †Epidemiology, Atherothrombosis and Imaging Department, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain; ‡Advanced Imaging Unit, Epidemiology, Atherothrombosis and Imaging Department, Ciber de Enfermedades Respiratorias and Universidad Complutense, Madrid, Spain; §The Zena and Michael A. Wiener Cardiovascular Institute, Mount Sinai School of Medicine, New York, New York; and the ‖Cardiovascular Institute, Hospital Clínico San Carlos, Madrid, Spain. This study was supported by grants from the Spanish Ministry of Economy and Competitiveness and the "Fondo de Investigacion Sanitaria" (BFU2012-35258 grant to Dr. Bernal, Ramón y Cajal Program grant RYC-2009-04341 to Dr. Bernal, and FIS10/02268 to Dr. Ibáñez), and fellowship FPU-AP2010-5951 to Dr. Cruz. The Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) is supported by the Ministerio de Economia y Competitividad (MINECO) and the Pro-CNIC Foundation. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Hugh Calkins, MD, served as Guest Editor for this article.
Listen to this manuscript's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.
You can also listen to this issue's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.

Manuscript received October 1, 2014; revised manuscript received January 27, 2015, accepted January 29, 2015.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heart muscle disease, clinically characterized by right ventricular (RV) anatomic abnormalities and an above-normal incidence of ventricular arrhythmia that can lead to sudden cardiac death, especially in young people. Many cases involve a familial association, and several mutations have been identified (1,2).

SEE PAGE 1451

ARVC onset is unpredictable; a gene-environment interaction has been suggested to trigger the disease's anatomic/electrical development, but this proposal is still deliberated. Ventricular arrhythmias and sudden death have been linked to exercise, which led to the recommendation that patients carrying an ARVC-causing mutation withdraw from endurance exercise (3); however, the effect of exercise on the onset and development of the anatomic ARVC phenotype is strongly debated.

In most cases, ARVC first manifests, after an initial concealed phase, as areas of RV dyskinesis that develop into isolated right-sided heart dysfunction and finally biventricular failure and fibrofatty replacement of heart muscle (4). Beyond reducing the incidence of arrhythmias with antiarrhythmic drugs or an implantable cardioverter-defibrillator, no treatment can effectively prevent disease progression.

ARVC is a paradigm of a complex cardiomyopathy caused by an autosomal dominant trait (5,6). Of the 8 genes linked to ARVC (7), 5 encode desmosomal proteins and account for ~50% of ARVC probands (5). The more than 380 mutations identified in ARVC patients include 161 pathogenic variants of *PKP2*, which encodes the desmosomal protein plakophilin-2. In several series, the prevalence of *PKP2* mutations in ARVC patients was >40% (8,9). Despite the strong implication of *PKP2* mutations in ARVC, there are no available transgenic disease models that express *PKP2* mutant alleles (10).

To study the effect of exercise on hearts of mice carrying the most prevalent ARVC-associated mutation (*PKP2*), we used adeno-associated virus (AAV)-mediated *PKP2* mutant gene transfer to express an ARVC-causing mutation in the cardiomyocytes of wild-type mice. After stable expression of mutant *PKP2*, we studied RV function by high-field cardiac magnetic resonance (CMR) in sedentary and endurance-trained mice.

METHODS

Four- to 6-week-old wild-type C57BL6/J mice were injected with 3.5×10^{10} viral genomes encoding luciferase (*Luc*), wild-type human *PKP2*, or the C-terminal deletion mutant version, R735X. Animals were divided into group A (trained) and group B (sedentary). Training in group A started 2 weeks after injection and continued for 8 consecutive weeks. At the end of that period, *Luc*, *PKP2*, and R735X mice were imaged by CMR and euthanized for heart sampling. Animals in group B were analyzed by CMR 6 and 10 months after infection with AAV particles.

All CMR images were analyzed with dedicated software (QMass MR version 7.5, Medis, Leiden, the Netherlands) by 2 experienced observers blinded to the study allocation. All CMR images were of good quality and could be analyzed. The short-axis dataset was analyzed quantitatively by manual detection of endocardial borders in end diastole and end systole with exclusion of papillary muscles and trabeculae to obtain both left and right end-diastolic volume, end-systolic volume, and ejection fraction (EF). Wall motion was classified as abnormal in the presence of akinesia, dyskinesia (in ventricular systole), or bulging (in ventricular diastole).

Experiments used the minimum number of mice needed to give sufficient statistical power, and no animals were excluded from the analyses. Data were analyzed by 1-way analysis of variance, 2-way analysis of variance, and Student *t* test. Relative risk analysis was assessed by 2-tailed Fisher exact test. CMR measure reliability was assessed by interobserver intraclass correlation coefficient (absolute agreement) and mean bias.

Additional materials and methods are available in the Online Appendix.

RESULTS

We generated enhanced green fluorescence protein (EGFP)-reporter AAV vectors driven from the cardiomyocyte-specific cardiac troponin T proximal promoter and encoding Luc, wild-type human PKP2a (the major splice variant in the heart), or a C-terminal deletion PKP2a mutant (R735X) (**Figures 1A and 1B**). We chose the R735X mutation because exon 11 is a hot spot for mutations that give rise to truncated PKP2 products found in ARVC patients (11–13) (**Figure 1C**). To further test whether genetic haploinsufficiency operates after expression in trans of R735X mutant, we measured the endogenous mouse *Pkp2* transcript level. In AAV-R735X transduced mice, endogenous *Pkp2* messenger ribonucleic acid (mRNA) levels remained stable (**Figure 1D**), which demonstrates that

ABBREVIATIONS AND ACRONYMS

AAV = adeno-associated virus

ARVC = arrhythmogenic right ventricular cardiomyopathy

CMR = cardiac magnetic resonance

Cx43 = connexin 43

EF = ejection fraction

EGFP = enhanced green fluorescence protein

Luc = luciferase

LV = left ventricle

mRNA = messenger ribonucleic acid

PKP2 = plakophilin-2

R735X = C-terminal deletion PKP2a mutant

RV = right ventricle