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

VOL. 65, NO. 6, 2015
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.10.069

Irreversible Triggers for Hypertrophic Cardiomyopathy Are Established in the Early Postnatal Period

Leah Cannon, PhD,* Ze-Yan Yu, MBBS, PhD,† Tadeusz Marciniec, PhD,* Ashley J. Waardenberg, PhD,‡ Siiri E. Iismaa, PhD,*§ Vesna Nikolova-Krstevski, PhD,*§ Elysia Neist, B Biotech (Hons),† Monique Ohanian, BMedSci (Hons),* Min Ru Qiu, MBBS, PhD,‖ Stephen Rainer, MBBS,‖ Richard P. Harvey, PhD,‡§¶ Michael P. Feneley, MD,†§# Robert M. Graham, MD,*§¶# Diane Fatkin, MD*§#

ABSTRACT

BACKGROUND Hypertrophic cardiomyopathy (HCM) is caused by mutations in sarcomere protein genes, and left ventricular hypertrophy (LVH) develops as an adaptive response to sarcomere dysfunction. It remains unclear whether persistent expression of the mutant gene is required for LVH or whether early gene expression acts as an immutable inductive trigger.

OBJECTIVES The aim of this study was to use a regulatable murine model of HCM to study the reversibility of pathological LVH.

METHODS The authors generated a double-transgenic mouse model, tTA x αMHC^{R403Q}, in which expression of the HCM-causing Arg403Gln mutation in the α-myosin heavy chain (MHC) gene is inhibited by doxycycline administration. Cardiac structure and function were evaluated in groups of mice that received doxycycline for varying periods from 0 to 40 weeks of age.

RESULTS Untreated tTA x αMHC^{R403Q} mice showed increased left ventricular (LV) mass, contractile dysfunction, myofibrillar disarray, and fibrosis. In contrast, mice treated with doxycycline from conception to 6 weeks had markedly less LVH and fibrosis at 40 weeks. Transgene inhibition from 6 weeks reduced fibrosis but did not prevent LVH or functional changes. There were no differences in LV parameters at 40 weeks between mice with transgene inhibition from 20 weeks and mice with continuous transgene expression.

CONCLUSIONS These findings highlight the critical role of the early postnatal period in HCM pathogenesis and suggest that mutant sarcomeres manifest irreversible cardiomyocyte defects that induce LVH. In HCM, mutation-silencing therapies are likely to be ineffective for hypertrophy regression and would have to be administered very early in life to prevent hypertrophy development. (J Am Coll Cardiol 2015;65:560–9) © 2015 by the American College of Cardiology Foundation.

From the *Molecular Cardiology and Biophysics Division, Victor Chang Cardiac Research Institute, Darlinghurst, Australia; †Cardiac Physiology and Transplantation Division, Victor Chang Cardiac Research Institute, Darlinghurst, Australia; ‡Cardiac Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, Australia; §Faculty of Medicine, University of New South Wales, Kensington, Australia; ¶School of Biotechnology and Biomolecular Sciences, University of New South Wales, Kensington, Australia; ‖Anatomical Pathology Department, St. Vincent's Hospital, Darlinghurst, Australia; and the #Cardiology Department, St. Vincent's Hospital, Darlinghurst, Australia. This work was supported by the National Health and Medical Research Council, Canberra, Australian Capital Territory (grant numbers 142009, 354400, 404808, 573731, and 573732). Dr. Cannon was the recipient of an Australian Postgraduate Award (University of New South Wales, Kensington, Australia). All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Graham and Fatkin are joint senior authors.

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 August 5, 2014; revised manuscript received October 9, 2014, accepted October 28, 2014.

Hypertrophic cardiomyopathy (HCM) is a heritable myocardial disorder associated with an increased risk of congestive heart failure, myocardial ischemia, cardiac arrhythmias, and sudden death, particularly in young individuals (1,2). Given the substantial morbidity and mortality of HCM, discovering strategies that induce regression or prevent left ventricular hypertrophy (LVH) would have substantial clinical impact.

SEE PAGE 570

The contemporary mechanistic paradigm for HCM is that primary perturbations of sarcomere function resulting from disease gene mutations trigger a cascade of cardiomyocyte responses including changes in calcium homeostasis, energy use, and gene expression that culminate in the hallmark pathological features of myocardial hypertrophy, disarray, and fibrosis (1,2). Although numerous interventions that modify downstream hypertrophic pathways have been evaluated, the effects of therapies that primarily inactivate or interfere with expression of mutant HCM genes have not been fully explored.

The first mutation discovered to have an association with HCM was an Arg403Gln substitution in the β-myosin heavy chain (MHC) gene (3), and this mutation has been studied in murine and rabbit models (4–10). The 2 isoforms of cardiac MHC, α and β, vary in expression among species and developmental stages (11). In humans and rabbits, β is the major isoform in the normal adult ventricle. In mice, there is a postnatal isoform switch from β to α, and thus human β mutations are typically modeled in the murine α gene. The first Arg403Gln α-MHC model was generated using a "hit-and-run" approach (4). Heterozygous mice developed HCM during adult life, whereas their homozygous littermates showed fulminant dilated cardiomyopathy (DCM) and died during the first postnatal week (4–6). In a seminal study, Jiang et al. (12) reported that ribonucleic acid interference (RNAi)-induced silencing of the mutant allele in heterozygous mice suppressed the development of HCM only when silencing was induced in mice at 1 day of age, with no effect on mice with overt HCM.

Here we report a novel regulatable mouse model of HCM in which mice carrying an Arg403Gln α-MHC transgene under the control of a tetracycline operon were crossed to mice that carried a tetracycline transactivator (tTA) transgene under the control of the α-MHC promoter (13). In the double-transgenic mice, tTA x α-MHC^{R403Q}, Arg403Gln is constitutively expressed but can be inhibited by adding the tetracycline analogue, doxycycline, to drinking water. We used this model to determine whether transgene inhibition at varying times in adult mice would enable HCM to be reversed or prevented. Our data support the use of mutation-silencing strategies for primary prevention of HCM but suggest that there is a limited time window in which such therapies are likely to be effective.

ABBREVIATIONS AND ACRONYMS

CVF = collagen volume fraction

DCM = dilated cardiomyopathy

HCM = hypertrophic cardiomyopathy

LV = left ventricular

LVDD = left ventricular end-diastolic diameter

LVFS = left ventricular fractional shortening

LVH = left ventricular hypertrophy

MHC = myosin heavy chain

RNAi = ribonucleic acid interference

tTA = tetracycline transactivator

WT = wild-type

METHODS

ANIMAL MODEL. A construct containing murine *Myh6* complementary deoxyribonucleic acid (cDNA) with the Arg403Gln mutation and a Kozak consensus sequence (accAUGg) was cloned into a pTet splice plasmid vector downstream of 7 copies of the tetracycline operator and a single copy of the human cytomegalovirus (CMV) minimal promoter (**Figure 1A**). DNA was injected into pronuclei of zygotes from FVB/N mice, and transgenic mice were generated and maintained on an FVB/N background. Heterozygous carriers of the Arg403Gln α-MHC (αMHC^{R403Q}) transgene were then crossed to mice carrying a single tTA transgene under the control of the α-MHC promoter on an FVB/N background (Jackson Laboratory, Bar Harbor, Maine) (13) to produce double-transgenic offspring (referred to as tTA x αMHC^{R403Q}). Expression of the αMHC^{R403Q} transgene was turned off by administering 1 mg/ml doxycycline mixed with 25 mg/ml sucrose in mouse drinking water. Wild-type (WT) controls were littermates of tTA x αMHC^{R403Q} mice that did not carry either the αMHC^{R403Q} or tTA transgenes. Mice were genotyped by polymerase chain reaction (PCR) amplification of tail genomic DNA and were maintained and evaluated according to protocols approved by the Garvan-St Vincent's Hospital Animal Ethics Committee (Darlinghurst, Australia).

ECHOCARDIOGRAPHY. Mice were sedated with 2.5% tribromoethanol (Avertin), 0.005 to 0.01 ml/g) by intraperitoneal injection, and transthoracic echocardiography was performed using a Sonos 5500 ultrasonograph (Philips Medical Systems, Andover, Massachusetts) and a 12- to 15-mHz probe. Heart rate was monitored by continuous electrocardiography.

MYOCARDIAL HISTOPATHOLOGY. Hearts were dissected, washed in 0.9% sodium chloride, fixed in 4% paraformaldehyde overnight, dehydrated in 70% ethanol, and embedded in paraffin. Tissue blocks were cut into 3-μm sections, placed on glass slides, and stained with hematoxylin and eosin or picrosirius red. Sections were reviewed by an independent