JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 72, NO. 1, 2018

PUBLISHED BY ELSEVIER

Genome Editing of Induced Pluripotent Stem Cells to Decipher Cardiac Channelopathy Variant

Priyanka Garg, PhD,[a] Angelos Oikonomopoulos, PhD,[a] Haodong Chen, PhD,[a] Yingxin Li, PhD,[a] Chi Keung Lam, PhD,[a] Karim Sallam, MD,[a] Marco Perez, MD,[a,b] Robert L. Lux, PhD,[c] Michael C. Sanguinetti, PhD,[c,d] Joseph C. Wu, MD, PhD[a]

ABSTRACT

BACKGROUND The long QT syndrome (LQTS) is an arrhythmogenic disorder of QT interval prolongation that predisposes patients to life-threatening ventricular arrhythmias such as Torsades de pointes and sudden cardiac death. Clinical genetic testing has emerged as the standard of care to identify genetic variants in patients suspected of having LQTS. However, these results are often confounded by the discovery of variants of uncertain significance (VUS), for which there is insufficient evidence of pathogenicity.

OBJECTIVES The purpose of this study was to demonstrate that genome editing of patient-specific induced pluripotent stem cells (iPSCs) can be a valuable approach to delineate the pathogenicity of VUS in cardiac channelopathy.

METHODS Peripheral blood mononuclear cells were isolated from a carrier with a novel missense variant (T983I) in the KCNH2 (LQT2) gene and an unrelated healthy control subject. iPSCs were generated using an integration-free Sendai virus and differentiated to iPSC-derived cardiomyocytes (CMs).

RESULTS Whole-cell patch clamp recordings revealed significant prolongation of the action potential duration (APD) and reduced rapidly activating delayed rectifier K^+ current (I_{Kr}) density in VUS iPSC-CMs compared with healthy control iPSC-CMs. ICA-105574, a potent I_{Kr} activator, enhanced I_{Kr} magnitude and restored normal action potential duration in VUS iPSC-CMs. Notably, VUS iPSC-CMs exhibited greater propensity to proarrhythmia than healthy control cells in response to high-risk torsadogenic drugs (dofetilide, ibutilide, and azimilide), suggesting a compromised repolarization reserve. Finally, the selective correction of the causal variant in iPSC-CMs using CRISPR/Cas9 gene editing (isogenic control) normalized the aberrant cellular phenotype, whereas the introduction of the homozygous variant in healthy control cells recapitulated hallmark features of the LQTS disorder.

CONCLUSIONS The results suggest that the $KCNH2^{T983I}$ VUS may be classified as potentially pathogenic.
(J Am Coll Cardiol 2018;72:62–75) © 2018 by the American College of Cardiology Foundation.

Congenital long QT syndrome (LQTS) is a potentially lethal genetic disorder of cardiac repolarization that represents a leading cause of sudden cardiac death (SCD) in the young, with a prevalence of ~1 in 2,500 among the general population (1). Among 17 known LQTS subtypes, those associated with mutations in ion channel genes KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3) account for 90% of all genotype-positive cases (2). β-adrenergic blockers are the mainstay of treatment for symptomatic LQTS patients, but implantable cardioverter-defibrillator (ICD), left cardiac sympathetic denervation, and sodium-channel blocker therapy may also be recommended (3,4).

Listen to this manuscript's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.

From the [a]Stanford Cardiovascular Institute and Department of Medicine, Division of Cardiology, Stanford University, Stanford, California; [b]Center for Inherited Cardiovascular Disease, Division of Cardiovascular Medicine, Stanford University, Stanford, California; [c]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, Utah; and the [d]Division of Cardiovascular Medicine, Department of Internal Medicine, University of Utah, Salt Lake City, Utah. Funding support was received from the National Institutes of Health (NIH) through grants T32 EB009035 (to Dr. Garg) and NIH R01 HL113006, NIH R01 HL128170, R01 HL14131, R01 HL130020, and American Heart Association 17MERIT33610009 (to Dr. Wu). All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received December 8, 2017; revised manuscript received March 13, 2018, accepted April 12, 2018.

ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2018.04.041

Despite significant advances in the management of LQTS based on an improved understanding of implicated genes and underlying ion currents, the care of almost one-third of LQTS patients remains challenging largely due to low penetrance of clinical symptoms and high variability in phenotypic expression (5). Even multiple family members carrying the same mutation may have different QT intervals and clinical manifestations (6,7). Additionally, the prevalence of asymptomatic individuals with latent LQTS is higher than anticipated (8). This creates a management dilemma of committing patients to lifelong medical therapy that may be risky or unproven, with significant consequences for the patient's quality of life.

SEE PAGE 76

An emerging standard of care for LQTS patients uses clinical genetic testing to identify causal variants in the LQTS susceptibility genes. However, such testing can identify >100 novel nonsynonymous coding variants in any given individual. Ascertaining which, if any, of these is a true causal variant is currently one of the major challenges in the treatment of heritable disorders, especially when the variant is of uncertain significance (VUS) due to inadequate evidence of pathogenicity (9,10). In the era of next-generation sequencing, interpretation of growing numbers of discovered uncertain variants will represent an even greater challenge to therapy (9).

Currently, there are no reliable platforms to predict a priori whether a given variant predisposes an individual to a disease or whether the coding change is benign. The launch of the Precision Medicine Initiative in 2015 has facilitated rapid advances in technologies such as induced pluripotent stem cells (iPSCs) and clustered regularly interspaced short palindromic repeats (CRISPR) genome editing. The creation of isogenic iPSC lines with single-variant changes using CRISPR allows a direct comparison of phenotype at a cellular level. This novel approach of combining these methods can be applied to test the precise phenotypic effect of VUS. Such a strategy may aid in deciphering VUS pathogenicity in LQTS and in tailoring drug treatment and ICD therapy, and could potentially be applied to other inherited cardiac disorders.

By combining patient-specific iPSCs and genome editing, we aimed to develop and validate a human-based platform for elucidating VUS pathogenicity "in a dish" for inherited arrhythmia syndromes and channelopathies. To this end, iPSC lines were derived from a patient carrying the novel variant T983I in the C-terminus of the KCNH2 gene ($KCNH2^{T983I}$), which encodes a channel that mediates the rapidly activating component of the delayed rectifying potassium current (I_{Kr}). Consistent with an LQT2 phenotype, we observed a prolongation of the action potential duration (APD) and reduced I_{Kr} density in VUS-induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) compared with cells derived from a healthy control subject. VUS iPSC-CMs were more susceptible to proarrhythmic effects of torsadogenic drugs compared with healthy control iPSC-CMs, whereas treatment of cells with an I_{Kr} activator restored normal APD. Finally, CRISPR/Cas9 genome editing of VUS iPSCs rescued the observed electrophysiological abnormalities, and the introduction of the homozygous variant in a healthy control line recapitulated hallmark LQTS phenotype. Our findings provide important insights into the pathophysiological mechanisms of this previously uncharacterized variant and suggest that the presence of this mutation could be sufficient to induce LQT2.

METHODS

An extended methods section is available in the Online Appendix.

GENERATION OF iPSC LINES. Somatic reprogramming was used to generate iPSC lines from peripheral blood mononuclear cells of the patient with the $KCNH2^{T983I}$ variant as confirmed by RT-PCR and from a healthy subject using the Sendai virus reprogramming protocol, as described previously (11). iPSCs were also derived from an affected patient with a verified LQT2 ($KCNH2^{A561V}$) mutation (12), as described in the previous text. At least 3 colonies were generated from both patients and healthy control subjects. All recruitment and consenting procedures conformed to the Stanford Institutional Review Board–approved protocol.

DIFFERENTIATION OF iPSC-CMs. iPSC-CMs were generated using a 2-dimensional monolayer differentiation protocol and maintained in a 5% CO_2/air environment as previously published (13). Briefly, iPSC colonies were dissociated with 0.5 mmol/l ethylenediaminetetraacetic acid (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts) into single-cell suspension and resuspended in E8 media containing 10 μmol/l Rho-associated protein kinase inhibitor (Sigma-Aldrich, St. Louis, Missouri). Approximately 100,000 cells were replated into Matrigel-coated

ABBREVIATIONS AND ACRONYMS

AP = action potential

APD = action potential duration

Cas9 = CRISPR-associated protein 9

CRISPR = clustered regularly interspaced short palindromic repeats

EAD = early afterdepolarization

hERG = human ether-a-go-go related gene

ICD = implantable cardioverter-defibrillator

I_{Kr} = rapidly activating delayed rectifier K^+ current

iPSC = induced pluripotent stem cell

iPSC-CM = induced pluripotent stem cell–derived cardiomyocyte

LQTS = long QT syndrome

MEA = multielectrode array

SCD = sudden cardiac death

VUS = variant of uncertain significance

VUS^{corr} = genome-edited corrected variant of uncertain significance

VUS^{hom} = genome-edited homozygous variant of uncertain significance