Novel *SCN5A* mutation in amiodarone-responsive multifocal ventricular ectopy-associated cardiomyopathy

Thomas M. Beckermann, PhD,[*] Karen McLeod, MD,[†] Victoria Murday, MD,[†] Franck Potet, PhD,[‡] Alfred L. George Jr., MD[*‡]

From the [*]Department of Pharmacology, Vanderbilt University, Nashville, Tennessee, [†]Royal Hospital for Sick Children, Yorkhill, Glasgow, Scotland, United Kingdom, and [‡]Division of Genetic Medicine, Department of Medicine, Vanderbilt University, Nashville, Tennessee.

BACKGROUND Mutations in *SCN5A*, which encodes the cardiac sodium channel $Na_V1.5$, typically cause ventricular arrhythmia or conduction slowing. Recently, *SCN5A* mutations have been associated with heart failure combined with variable atrial and ventricular arrhythmia.

OBJECTIVE The purpose of this study was to determine the clinical, genetic, and functional features of an amiodarone-responsive multifocal ventricular ectopy-related cardiomyopathy associated with a novel mutation in a $Na_V1.5$ voltage sensor domain.

METHODS A novel, *de novo SCN5A* mutation ($Na_V1.5$-R225P) was identified in a boy with prenatal arrhythmia and impaired cardiac contractility followed by postnatal multifocal ventricular ectopy suppressible by amiodarone. We investigated the functional consequences of $Na_V1.5$-R225P expressed heterologously in tsA201 cells.

RESULTS Mutant channels exhibited significant abnormalities in both activation and inactivation leading to large, hyperpolarized window and ramp currents that predict aberrant sodium influx at potentials near the cardiomyocyte resting membrane potential. Mutant channels also exhibited significantly increased persistent (late) sodium current. This profile of channel dysfunction shares features with other *SCN5A* voltage sensor mutations associated with cardiomyopathy and overlapped that of congenital long QT syndrome. Amiodarone stabilized fast inactivation, suppressed persistent sodium current, and caused frequency-dependent inhibition of channel availability.

CONCLUSION We determined the functional consequences and pharmacologic responses of a novel *SCN5A* mutation associated with an arrhythmia-associated cardiomyopathy. Comparisons with other cardiomyopathy-associated $Na_V1.5$ voltage sensor mutations revealed a pattern of abnormal voltage dependence of activation as a shared biophysical mechanism of the syndrome.

KEYWORDS SCN5A mutation; Cardiomyopathy; Electrophysiology; Amiodarone

ABBREVIATIONS **AV** = atrioventricular; **DMSO** = dimethylsulfoxide; **GFP** = green fluorescent protein; **LQTS** = long QT syndrome; **TTX** = tetrodotoxin; **WT** = wild-type

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Introduction

The voltage-gated cardiac sodium channel $Na_V1.5$ encoded by *SCN5A* is responsible for the initial upstroke of the cardiac action potential.[1] Mutations in *SCN5A* typically manifest as cardiac arrhythmias such as the congenital long QT syndrome (LQTS) or Brugada syndrome, or by variable degrees of impaired cardiac conduction. Importantly, some *SCN5A* mutations are associated with clinical features that overlap more than 1 disorder.[2] Additionally, a new genotype–phenotype correlation has emerged recently that has expanded the clinical spectrum of sodium channelopathies to include disorders which feature impaired cardiac contractility.

In 2004, McNair et al[3] described a multigenerational family segregating *SCN5A*-D1275N with a complex disorder featuring variable combinations of supraventricular arrhythmias, impaired atrioventricular (AV) conduction, and dilated cardiomyopathy. Shortly thereafter, Olson et al[4] screened a cohort of patients diagnosed with idiopathic dilated cardiomyopathy and identified 5 *SCN5A* mutations, including a novel voltage sensor mutation (R814W). Subsequent functional studies of R814W revealed a novel pattern of sodium channel dysfunction featuring a prominent defect in the voltage dependence of activation.[5] Two other SCN5A voltage sensor mutations associated with cardiomyopathy and variable arrhythmias have been identified (R219H, R222Q).[6–11] The R222Q mutation exhibits many of the same biophysical abnormalities as R814W, whereas

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R219H appears to have a distinct functional perturbation (gating pore leak current). Importantly, the clinical syndrome associated with some of these mutations, best illustrated for R222Q, exhibits reversibility of contractile dysfunction with antiarrhythmic therapy.[7] However, the pharmacologic mechanism responsible for this effect has not been explored.

Here we present a novel *SCN5A* mutation ($Na_V1.5$-R225P) associated with prenatal arrhythmias, impaired cardiac contractility, and postnatal multifocal ventricular ectopy-associated ventricular dysfunction reversed by amiodarone treatment. We elucidated the functional consequences of the mutation and demonstrated the likely mechanism for amiodarone efficacy. These findings extend the phenotypic spectrum of *SCN5A* mutations and reveal a plausible pharmacologic mechanism underlying the reversibility of arrhythmia-associated cardiomyopathy.

Methods

Subject ascertainment

The mother of the proband volunteered her son's clinical history and genetic information without solicitation. Subsequently, informed consent was obtained to evaluate medical record information including genetic testing data. The informed consent procedure was approved by the Vanderbilt University Institutional Review Board. Genetic testing for mutations in *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2,* and *KCNJ2* was performed by The Scottish Genetics Laboratory, Aberdeen.

Cloning and expression of $Na_V1.5$

A cDNA encoding human $Na_V1.5$ was cloned into the bicistronic vector pRc-CMV_IRES2-CD8, and mutations were created using site-directed mutagenesis. Human-derived tsA201 cells (HEK293 cell line expressing SV40 large T antigen) were transiently transfected with 1.0 μg of wild-type (WT) or mutant $Na_V1.5$ plasmid using FuGeneHD (Roche Diagnostics, Indianapolis, IN) in combination with 0.6 μg of a bicistronic plasmid (pIRES-EGFP-hβ1) encoding enhanced green fluorescent protein (GFP) and the human β_1 subunit (hβ1) under the control of the CMV immediate early promoter. Positively transfected cells were determined by GFP emission and the binding of beads conjugated with anti-CD8 antibodies.

Electrophysiology

Sodium currents were recorded at room temperature (22°–23°C) 48 to 72 hours after transfection using the whole-cell patch-clamp technique as previously described.[5] All data were analyzed using pCLAMP 10.0 or Microsoft Excel 2007 and plotted using SigmaPlot 10.0 (Systat Software, San Jose, CA). Statistical analysis was performed using Student *t* test.

Experiments examining persistent I_{Na} and ramp currents used tetrodotoxin (TTX; Tocris Bioscience, Bristol, United Kingdom) to allow for the determination of TTX-sensitive sodium current. TTX was added to the bath solution from a stock solution (3 mM in water) to a final concentration of 30 μM. TTX-sensitive current was determined by offline digital subtraction.

Pharmacology

Amiodarone hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich) to create a stock concentration of 30 mM. For experiments, amiodarone stock was diluted into bath solution for use at 3 μM. Fresh dilutions were made on the day of experiments. Amiodarone or DMSO was continually present in the superfusate during experiments examining the biophysical effects of amiodarone. DMSO concentration never exceeded 0.01% in control or test conditions.

Results

Case presentation

A Caucasian male was delivered at 37 weeks' gestation after an eventful 15-week antenatal course. Fetal tachycardia accompanied by poor ventricular function was noted at 22 weeks. Maternal flecainide administration was initiated at 27 weeks. At 28 weeks, fetal tachycardia with 2:1 AV conduction was noted and suggested a supraventricular origin of the arrhythmia. Tachycardia persisted despite escalating the dosage of flecainide. Propranolol was added at 31 weeks, and fetal heart rate normalized with concomitant improvement in ventricular function. A maternal flecainide/propranolol regimen was maintained through parturition.

Immediately after birth, a wandering atrial rhythm at 137 bpm with frequent, multifocal ventricular ectopic beats was noted (Figure 1A, Online Supplemental Figure S1A). Treatment with amiodarone (5 mg/kg initially 3 times daily for 1 week, then twice daily for 1 week, followed by a maintenance dose of amiodarone 5 mg/kg daily) in combination with propranolol (1 mg/kg 4 times daily; 3 mg 4 times daily absolute dose) was initiated. Continued atrial and ventricular ectopy was evident at age 1 month, and an ECG obtained at 7 weeks showed atrial tachycardia (atrial rate 260 bpm) with 2:1 AV block (Figure 1B and Online Supplemental Figure S1B). The antiarrhythmic regimen was not changed. By 3 months of age, ECG was reported to show predominantly sinus rhythm with some ectopy (trace not available). Because of persistent sinus rhythm at age 6 months, amiodarone was discontinued while propranolol was continued (3 mg 4 times daily). Two weeks after stopping amiodarone, ECG showed sinus rhythm but with a prolonged QTc interval of 480 ms (Figure 1C and Online Supplemental Figure S1C). Propranolol was discontinued at that time. However, the patient was hospitalized 6 weeks later with supraventricular tachycardia (200 bpm) and multifocal ventricular ectopy (Figure 1D and Online Supplemental Figure S1D) Echocardiography demonstrated impaired ventricular function (ejection fraction 43%, fractional shortening 17%; Online Supplemental Figure S2). Amiodarone was restarted at an initial loading dose, followed by a maintenance dose of 5 mg/kg/day. Propranolol was