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R222Q *SCN5A* Mutation Is Associated With Reversible Ventricular Ectopy and Dilated Cardiomyopathy

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Objectives The goal of this study was to characterize a variant in the *SCN5A* gene that encodes the alpha-subunit of the cardiac sodium channel, Nav1.5, which was identified in 1 large kindred with dilated cardiomyopathy (DCM) and multiple arrhythmias, including premature ventricular complexes (PVCs).

Background Treatment guidelines for familial DCM are based on conventional heart failure therapies, and no gene-based interventions have been established.

Methods Family members underwent clinical evaluation and screening of the *SCN5A* and *LMNA* genes. Cellular electrophysiology and computational modeling were used to determine the functional consequences of the mutant Nav1.5 protein.

Results An R222Q missense variant located in a Nav1.5 voltage-sensing domain was identified in affected family members. Patch-clamp studies showed that R222Q Nav1.5 did not alter sodium channel current density, but did left shift steady-state parameters of activation and inactivation. Using a voltage ramp protocol, normalized current responses of R222Q channels were of earlier onset and greater magnitude than wild-type channels. Action potential modeling using Purkinje fiber and ventricular cell models suggested that rate-dependent ectopy of Purkinje fiber origin is the predominant ventricular effect of the R222Q variant and a potential cause of DCM. In R222Q carriers, there were only modest responses to heart failure therapies, but PVCs and DCM were substantially reduced by amiodarone or flecainide, which are drugs that have sodium channel-blocking properties.

Conclusions The R222Q *SCN5A* variant has an activating effect on sodium channel function and is associated with reversible ventricular ectopy and DCM. Elucidation of the genetic basis of familial DCM can enable effective gene-targeted therapy to be implemented. (J Am Coll Cardiol 2012;60:1566–73) © 2012 by the American College of Cardiology Foundation

Dilated cardiomyopathy (DCM) is associated with significant morbidity and mortality, and is caused by inherited gene variants in a substantial proportion of cases. Familial DCM is clinically variable and genetically heterogeneous, with at least 40 disease genes reported to date (1). Identification of the genetic basis of DCM provides an opportunity for early diagnosis and preventative intervention in genotype-positive family members. However, the current reality is that mutations in most of the known disease genes are uncommon, and the molecular defects underpinning DCM in the majority of families (>70%) are unknown (1,2). Moreover, no effective disease gene-targeted therapies have been established for clinical use.

Mutations in the *SCN5A* gene that encodes the cardiac sodium (Na^+) channel alpha-subunit, Nav1.5, cause a variety of arrhythmic disorders, including long QT syndrome, Brugada syndrome, ventricular tachycardia, sick sinus syndrome, atrial standstill, conduction system abnormalities (CD), and atrial

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fibrillation (AF). *SCN5A* mutations have also been associated with DCM that is typically preceded by a prodrome of CD or AF, a similar phenotype to that observed with mutations in the *LMNA* gene, which encodes nuclear lamin A/C (3–5).

A large Caucasian kindred with a clinical diagnosis of DCM and CD was referred to our laboratory for molecular genetics analysis. Detailed phenotype evaluation demonstrated that multiple electrocardiographic (ECG) abnormalities were present. In particular, frequent polymorphic ventricular ectopy was a prominent and early manifestation, raising the possibility of a causal relationship with the development of DCM. We re-sequenced the *SCN5A* and *LMNA* genes and identified a heterozygous missense R222Q *SCN5A* variant that segregated with disease status in the kindred. Functional characterization of the R222Q variant showed an activating effect on cardiac Na^+ channel function, and in a ventricular cell model, these effects were predicted to induce premature ventricular complexes (PVCs) predominantly of Purkinje fiber cell origin. The availability of genotype results changed the medical management of affected family members, and administration of drugs with Na^+ channel-blocking properties enabled both ventricular ectopy and DCM to be reversed.

Methods

Clinical evaluation. Informed written consent was obtained from all participants, and the study protocol was approved by the institutional Human Research Ethics Committee. The proband and participating family members >16 years of age were evaluated by history and physical examination, 12-lead ECG, and transthoracic echocardiography. The results of additional cardiac investigations performed for clinical indications, including Holter monitor and electrophysiology studies (EPS), were obtained from medical records. One hundred unrelated healthy Caucasian volunteers comprised the control group.

Genetic analysis. Genomic DNA was isolated from peripheral blood samples. Protein-coding sequences of the *SCN5A* and *LMNA* genes were amplified by polymerase chain reaction and re-sequenced using an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, California). The R222Q *SCN5A* substitution results in loss of a *Hinf1* site and was independently confirmed by restriction enzyme digestion.

Cellular electrophysiology and modeling. Chinese hamster ovary (CHO) cells were transfected with cDNA clones encoding wild-type (WT) and R222Q Nav1.5 plus WT Navβ1. Cardiac Na^+ channel currents (I_{Na}) were recorded using conventional patch-clamp techniques. To investigate the functional consequences of mutant channels, WT and R222Q Nav1.5 channels were modeled using Hodgkin-Huxley formalism as described (6), and WT and mutant models were incorporated into Purkinje cell (6) and ventricular cell (7) models (Online Appendix).

Results

Family phenotype. Forty-two individuals in 2 related large kindreds (Family HY) underwent clinical evaluation and genetic testing (Fig. 1, Table 1). The family phenotype was characterized by a high prevalence of atrial and ventricular arrhythmias, with DCM occurring mainly in males. A striking feature of the ECG tracings of family members studied in sinus rhythm was the relative paucity of normally conducted sinus beats, with the majority of beats being PVCs, including narrow PVCs of probably high septal origin that had varying morphology and axis, as well as wide PVCs of left and right bundle branch type (Figs. 1B to 1D). Premature atrial complexes (PACs) and accelerated junctional rhythms were also seen. Six individuals had documented AF, and 3 individuals received pacemakers for symptomatic bradycardia or complete heart block in later adult life. EPS results were available in 4 cases and uniformly showed multiple PVC foci in the left and right ventricles, with no inducible ventricular arrhythmias. Five individuals had prophylactic implantation of cardioverter-defibrillators (ICDs).

Abbreviations and Acronyms

- **AF** = atrial fibrillation
- **CD** = conduction system abnormalities
- **CHO** = Chinese hamster ovary
- **DCM** = dilated cardiomyopathy
- **ECG** = electrocardiogram
- **EPS** = electrophysiology studies
- **ICD** = implantable cardioverter-defibrillator
- **I_{Na}** = Na^+ channel current
- **Na^+** = sodium
- **PAC** = premature atrial complex
- **PVC** = premature ventricular complex
- **WT** = wild-type

Eight individuals had a diagnosis of DCM (Table 1). In 2 asymptomatic young males, DCM was detected only as a result of family screening. A history of palpitations that predated DCM diagnosis was elicited in all other cases. DCM was present in 7 of the 10 genotype-positive males but in only 1 of the 7 genotype-positive females. Myocardial fibrosis was excluded in 2 individuals with severe DCM by magnetic resonance imaging (IV-27) and cardiac biopsy (IV-34), respectively.

Identification of R222A *SCN5A* variant. The coding regions of the *SCN5A* and *LMNA* genes were re-sequenced in the proband's DNA (III-10; Fig. 1A) and a heterozygous 665G>A change in the *SCN5A* gene was identified that alters the amino acid at codon 222 from arginine (R) to glutamine (Q). The R222 residue is located in a voltage-sensing S4 region of Nav1.5 and has high homology across different members of the human Na^+ channel gene family and different species (Online Fig. 1). Fourteen individuals were genotype-positive, and 3 deceased individuals (II-5, III-5, III-7) were obligate carriers. Sixteen of these 17 gene variant carriers were clinically affected, with the exception being a 56-year-old male (III-12) who had a normal ECG and echocardiogram. R222Q is a recurrent rare variant identified in 4 DCM families (8–11). This variant was not found in 200 control chromosomes (this study), in 506 control chromosomes in a previous report (8), or in the 1000 Genomes and