Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Arrhythmic Phenotypes Are a Defining Feature of Dilated Cardiomyopathy-Associated *SCN5A* Variants: A Systematic Review

Stacey Peters, MBBS; Bryony A. Thompson, PhD; Mark Perrin, MBBS, PhD; Paul James, MBChB, DPhil; Dominica Zentner, MBBS, PhD; Jonathan M. Kalman, MBBS, PhD; Jamie I. Vandenberg, MBBS, PhD; Diane Fatkin, MD

BACKGROUND: Variants in the *SCN5A* gene, that encodes the cardiac sodium channel, Nav1.5, are associated with a highly arrhythmogenic form of dilated cardiomyopathy (DCM). Our aim was to review the phenotypes, natural history, functional effects, and treatment outcomes of DCM-associated rare *SCN5A* variants.

METHODS: A systematic review of reported DCM-associated rare *SCN5A* variants was undertaken using PubMed and Embase.

RESULTS: Eighteen *SCN5A* rare variants in 29 families with DCM (173 affected individuals) were identified. Eleven variants had undergone experimental evaluation, with 7 of these resulting in increased sustained current flow during the action potential (eg, increased window current) and at resting membrane potentials (eg, creation of a new gating pore current). These variants were located in transmembrane voltage-sensing domains and had a consistent phenotype characterized by frequent multifocal narrow and broad complex ventricular premature beats (VPB; 72% of affected relatives), ventricular arrhythmias (33%), atrial arrhythmias (32%), sudden cardiac death (13%), and DCM (56%). This VPB-predominant phenotype was not seen with 1 variant that increased late sodium current, or with variants that reduced peak current density or had mixed effects. In the latter groups, affected individuals mainly showed sinus node dysfunction, conduction defects, and atrial arrhythmias, with infrequent VPB and ventricular arrhythmias. DCM did not occur in the absence of arrhythmias for any variant. Twelve studies (23 total patients) reported treatment success in the VPB-predominant cardiomyopathy using sodium channel-blocking drug therapy.

CONCLUSIONS: *SCN5A* variants can present with a diverse spectrum of primary arrhythmic features. A majority of DCM-associated variants cause a multifocal VPB-predominant cardiomyopathy that is reversible with sodium channel blocking drug therapy. Early recognition of the distinctive phenotype and prompt genetic testing to identify variant carriers are needed. Our findings have implications for interpretation and management of *SCN5A* variants found in DCM patients with and without arrhythmias.

Key Words: arrhythmias, cardiac ■ dilated cardiomyopathy ■ genomics ■ phenotype ■ sodium

Genetic testing is increasingly performed in the diagnostic work-up of patients with dilated cardiomyopathy (DCM).[1] The *SCN5A* gene, encoding the alpha subunit of the cardiac sodium channel, Nav1.5, is associated with an arrhythmic form of DCM and is widely regarded as one of the key genes to include on screening panels.[2,3] However, most protein-altering *SCN5A* variants are not deleterious. Moreover, pathogenic *SCN5A* variants have been associated with a range of different cardiac phenotypes including long QT (LQT) syndrome, Brugada syndrome, familial conduction disease, and atrial fibrillation.[3] Consequently, interpreting the clinical significance of rare *SCN5A* variants in patients with DCM can be challenging.

Bezzina et al[4] first described DCM in association with severe conduction defects (CD) and arrhythmias in

Correspondence to: Diane Fatkin, MD, Victor Chang Cardiac Research Institute, 405 Liverpool St, Darlinghurst NSW 2010, Australia. Email d.fatkin@victorchang.edu.au
This article was sent to Ruth McPherson, MD, PhD, Guest Editor, for review by expert referees, editorial decision, and final disposition.
Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.121.003432.
For Sources of Funding and Disclosures, see page 53.

Circulation: Genomic and Precision Medicine is available at www.ahajournals.org/journal/circgen

Nonstandard Abbreviations and Acronyms

AA	atrial arrhythmias
AVB	atrioventricular block
CD	conduction defects
DCM	dilated cardiomyopathy
LQT	long QT
SCD	sudden cardiac death
VPB	ventricular premature beats

a child who was a compound heterozygous carrier of 2 missense *SCN5A* variants. Subsequently, linkage analysis performed on a large multi-generational family with DCM and CD mapped a novel locus on chromosome 3p and a missense SCN5A variant, p.D1275N, was identified.[5] Since then, numerous reports of *SCN5A* variants in DCM kindreds have been published. However, it remains unclear whether myocardial contractile impairment results directly from Nav1.5 dysfunction or indirectly, via arrhythmia-mediated effects. A recent case-control cohort failed to show an excess of rare *SCN5A* variants in DCM patients.[6] Collectively, these observations call into question whether *SCN5A* is a bona fide DCM disease gene and hence, whether rare variants identified in patients with a DCM-predominant phenotype are likely to be disease-causing.

The aim of our study was to (1) systematically collate published reports of rare putative disease-causing *SCN5A* variants in DCM cases and document the spectrum of phenotypic features, (2) evaluate mechanistic defects associated with these *SCN5A* variants, (3) re-analyze variant-level and clinical evidence for disease causation, and (4) establish patterns of treatment success.

METHODS

An expanded Methods section is provided in the Supplemental Material. Study data are available from the corresponding author upon reasonable request.

RESULTS

Compilation of DCM-Associated *SCN5A* Variants

Three hundred eighty-nine reports were identified from the search strategy (Figure S1). After removal of duplicates, 303 citations underwent title and abstract screening, with 124 selected for full text screening. After exclusion of 98 studies, 26 studies underwent data extraction, yielding a total of 25 *SCN5A* variants in 35 kindreds. Four variants were excluded on the basis of minor allele frequencies in the gnomAD population database higher than expected (>0.0001) for roles in disease causation and benign/likely benign annotations in the ClinVar variant database (Table S2). Two compound heterozygous *SCN5A* variants were omitted due to the inability to independently evaluate these as causes of dominant disease,[4] and 1 variant did not segregate with cardiac disease in the family[7] (Table S3). The final analysis was based on 18 unique variants in 29 kindreds from 22 reports, including 15 missense variants, 2 in-frame deletions, and 1 frameshift duplication (Table 1).[5,8–28] Due to differences in the transcripts used for variant nomenclature across reported studies, variant locations were standardized to the ClinGen-recommended reference transcript, NM_000335.5 (Table S4).

Variant Functional Subgroups

Variants underwent further classification based on experimental evidence of altered Nav1.5 function. In the normal cardiac action potential, there is an initial transient influx of sodium ions through Nav1.5 channels resulting in membrane depolarization (phase 0). This is followed by partial repolarization (phase 1) and a plateau phase maintained by influx of calcium ions and further sodium entry via the sodium-calcium exchanger (phase 2). Persistent opening or failure of inactivation can result in a persistent late sodium current during phase 2, as demonstrated for the p.Q1506_P1508del variant.[23,29] Inactivation of calcium channels together with outward potassium current results in membrane repolarization (phase 3). During phase 3, altered inactivation kinetics can give rise to premature reactivation of Nav1.5 channels often referred to as a window current (Figure 1). Of the 11 *SCN5A* variants for which functional data were available, an increased window current was the most common defect identified, being seen in 6 variants (Table 1).[5,8,10,16,17,21,24,25,30–33]

During the diastolic phase of the cardiac cycle the membrane potential is maintained at negative levels, primarily driven by activity of the inward rectifier potassium current (phase 4). Sodium channels are normally closed and do not pass current. However, amino acid substitution at 4 sites (p.R219H, p.R222Q, p.R225P, p.R814W) result in a new gating pore that allows the passage of cations (such as hydrogen, sodium, potassium) through the voltage-sensing domain.[13,30,31,33] This gating pore current can occur at resting membrane potentials, making these mutants distinct from those that show sustained inward currents only at more depolarized potentials.[33] Mutants that result in a sustained inward current, either at depolarized or resting membrane potentials, are often referred to as showing a gain-of-function as they allow passage of an inward current that is not seen in wild-type channels. We favour distinguishing between mutants that show increased current at resting membrane potentials

Table 1. Characteristics of DCM-Associated *SCN5A* Rare Variants

Genomic location	Protein change*	Location	Nav 1.5 effect	ClinVar annotation	ACMG classification	gnomAD MAF (total/NFE)	Ref
c.589G>C	p.D197H	D1 S3	Unknown	P	LP: PM2, PM5, PM6, PP3	Absent	14
c.611C>A	p.A204E	D1 S3	Increased WC	LP	LP: PS3, PM2, PP3	Absent	11
c.638G>A	p.G213D	D1 S3-S4 linker	Increased WC	NE	LP: PS3, PM2, PP1, PP3	Absent	10
c.656G>A	p.R219H	D1 S4	New GPC	Conflicting (LP-3, VUS-1)	LP: PS3, PM2, PP3	Absent	13,31
c.665G>A	p.R222Q	D1 S4	Increased WC, new GPC	P	P: PS3, PP1_Str, PM2, PP3,	Absent	16–21,26–28,30,33
c.674G>C	p.R225P	D1 S4	Increased WC, new GPC, increased LC	NE	P: PS3, PM2, PM5, PM6, PP3	Absent	8,33
c.1111C>G	p.Q371E	D1 S5-S6 linker	Unknown	NE	VUS: PM2, PP3	Absent	15
c.2184_2186del	p.L729del	D2 S1	Unknown	P	VUS: PM2, PM4	Absent	22
c.2440C>T	p.R814W	D2 S4	Increased WC, new GPC	LP/P	P: PS2, PS3, PM2, PP1, PP3	Absent	5,25,32,33
c.2482C>T	p.L828F	D2 S4-S5 linker	Increased WC	NE	LP: PS3, PM2, PP3	Absent	24
c.2550_2551dup	p.F851Cfs*19	D2 S5	Unknown	P	LP: PVS1, PM2	Absent	5
c.3820G>A	p.D1274N	D3 S3	Reduced PC, increased LC	P	P: PS3, PP1_Str, PM2, PS4_Supp	0.000008/0.000009	5,18,34,35
c.3832G>A	p.V1278I	D3 S3	Unknown	VUS	VUS: PM2, PP3	Absent	18
c.4024A>G	p.I1342V	D3 S5	Reduced PC, no change in LC	NE	LP: PM2, PS3	Absent	9
c.4516_4524del	p.Q1506_P1508del	D3-D4 linker	Increased LC	P	LP: PS3, PM2, PM4,	Absent	23,29
c.4557C>G	p.F1519L	D3-D4 linker	Unknown	NP	VUS: PM2	Absent	18
c.4639G>C	p.E1547Q	D4 S1	Unknown	NE	LP: PS2, PM2. PP3	Absent	12
c.4780G>C	p.D1594H	D4 S3	Mixed: increased steady state of inactivation, slowed onset of inactivation	LP	P: PS3, PM2, PM5, PP1, PP3	Absent	5,32

ACMG indicates American College of Medical Genetics and Genomics; GPC, gating pore current; LC, late current; LP, likely pathogenic; NE, no entry; NP, present in ClinVar but no classification provided; P, pathogenic; PC, peak current; PM, moderate evidence for pathogenicity; PP, supporting evidence for pathogenicity; PS, strong evidence for pathogenicity; VUS, variant of uncertain significance; and WC, window current.

*Variant nomenclature according to transcript NM_0003355.5.

from those that show inward currents at depolarized potentials with some mutants exhibiting both (p.R222Q, p.R225P, p.R814W).

Loss of peak sodium current, often referred to as loss of function, was relatively less frequent for DCM-associated *SCN5A* variants. Two variants (p.I1342V, p.D1274N), reduce peak Nav1.5 current,[9,34,35] with p.D1274N (commonly reported as D1275N; Table S4) additionally showing an increase in late sodium current. Mixed effects were also seen for the p.D1594H variant, with hyper-polarization of the steady state of inactivation (fewer Nav1.5 channels available for activation at normal resting membrane potentials) as well as slowed onset of inactivation (prolongs Nav1.5 current during the plateau phase of the action potential).[32] Functional consequences of the remaining 7 variants were unknown (Table 1).

Clinical Features of Study Cohort

Our genotype-phenotype analysis focused on 173 affected individuals (94 males, 68 females, 11 not stated) in 29 families with 18 unique *SCN5A* variants that were reported between 2005 and 2020 (Tables 2 and 3, Table S5). One hundred forty-three individuals were confirmed to be *SCN5A*-heterozygote, with the remaining 30 affected individuals not genetically tested (declined, deceased, or unavailable for testing). For the purposes of this review, we will discuss the total affected individuals rather than confirmed heterozygotes alone. Arrhythmias and CD were the presenting complaints in all probands, and 19 probands (66%) had DCM at presentation. In families where the proband did not present with DCM, it was detected later in the disease course, or in a relative during screening. Eighty-nine affected individuals (51%) had a diagnosis of DCM at some stage

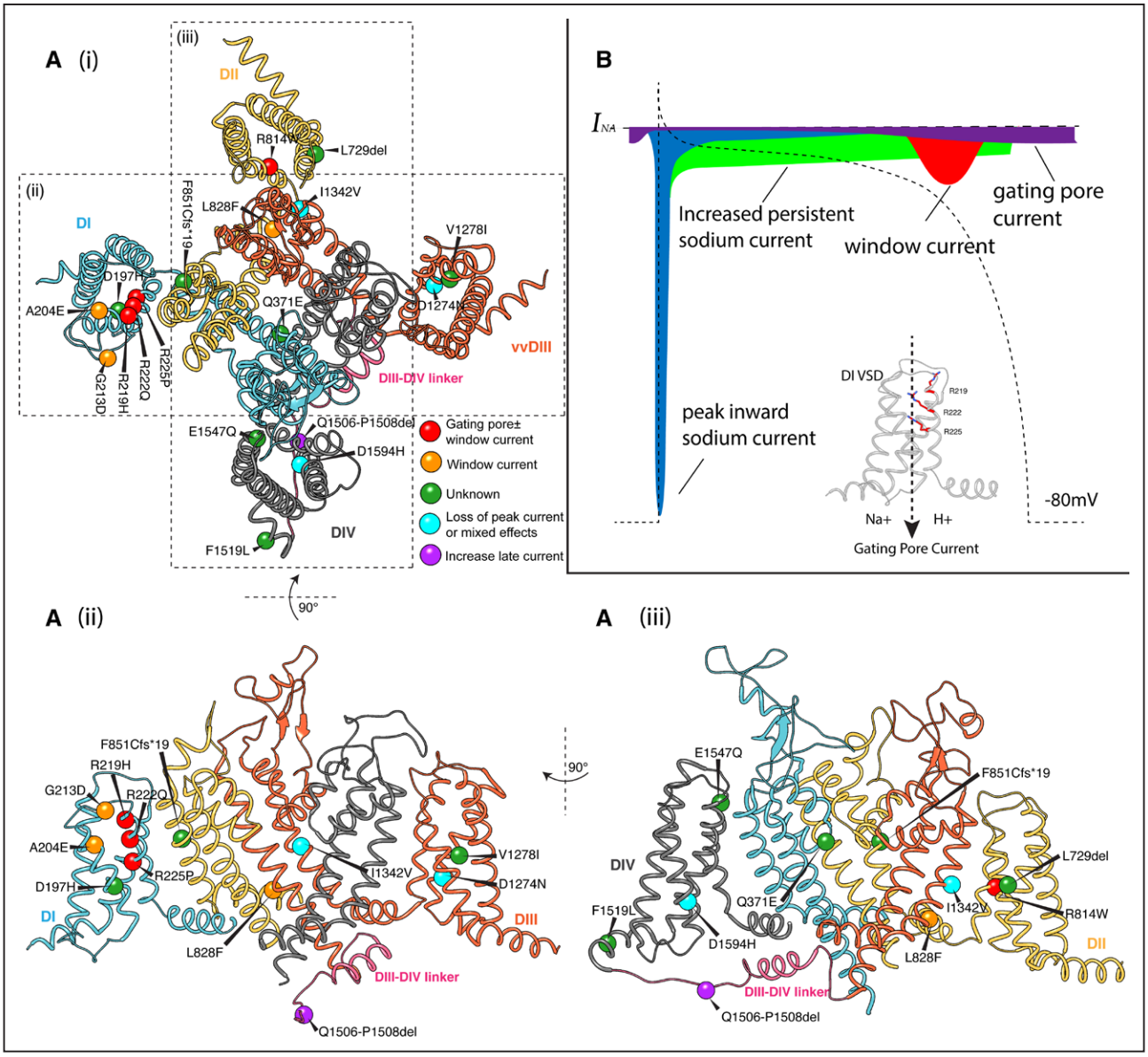

Figure 1. Location of SCN5A variants and their functional effects on Nav1.5 current.
A, Mapping of SCN5A variants on the Nav1.5 structure (Protein Data Bank:7DTC); Cα atoms of the disease-related residues are shown as spheres. (1) Variant positions viewed from the extracellular side of the channel. Side views of diagonal subunits (2) and (3) are shown below. **B**, Nav1.5 channels carry 3 time- and voltage-dependent currents: a large rapid inward current (blue) that causes cell depolarization; a persistent or late sodium current (blue, normal physiology; green, augmented with genetic variants) of small amplitude that flows throughout the plateau phase of the action potential; and a window current (red) that has a small amplitude in normal physiology due to the narrow voltage range over which it flows. Genetic variants in the S4 voltage sensor domain may create a gating pore current (purple and inset figure) that flows at depolarized or hyper-polarized potentials.

during their follow-up. Accounting for all phenotypic presentations there were only 5/143 (3%) individuals that were genotype-positive but truly phenotype-negative.

Phenotype Associated With Window and Gating Pore Currents

The most frequently reported single variant was p.R222Q SCN5A, being identified in 73 affected individuals from 11 geographically distinct families (Tables 2 and 3, Table S5).[16–21,26–28] Among the published families, the phenotype was characterized by a high burden of multifocal ventricular premature beats (VPB), together with ventricular arrhythmias, atrial premature beats, atrial arrhythmias (AA), and DCM. VPB were documented in 50 individuals (68%) and included both narrow complex (high septal) and wide complex (left or right bundle branch block) morphologies (Figure 2). Resolution of VPB during exercise was documented in individuals from 5 p.R222Q families.[16,17,27] Twenty-four individuals (33%) had a history of sustained ventricular arrhythmia. AA, including atrial fibrillation and accelerated junctional rhythms, were seen

Table 2. DCM-Associated *SCN5A* Variants Included in Systematic Analysis

Coding sequence	Protein change	No.G+ (P+)	DCM	VPB/ VA	AVB <70	CA/ SCD	AA	Sev HF	LQT	Complete or partial treatment success	Ref
New gating pore±increased window current											
c.656G>A	p.R219H	3 (6)	6	6	1	0	1	0	0		13
c.665G>A	p.R222Q	59 (73)	42	51	1	12	21	9	0	Flecainide, amiodarone, hydroquinidine, quinidine, disopyramide	16–21,26–28
c.674G>C	p.R225P	1 (1)	1	1	0	0	1	0	0	Amiodarone	8
c.2440C>T	p.R814W	4 (6)	4	3	0	2	3	1	0	Flecainide, mexilitene, quinidine	5,25
Increased window current											
c.611C>A	p.A204E	1 (1)	1	1	0	0	1	0	0	Hydroquinidine	11
c.638G>A	p.G213D	17 (20)	5	16	2	0	8	1	0	Flecainide, amiodarone, sotalol	10
c.2482C>T	p.L828F	2 (4)	3	3	0	1	0	0	0	Flecainide	24
Increased late current											
c.4508_4516del	p.Q1506_ P1508del	3 (4)	2	1	1	2	0	0	2		23
Loss of peak current or mixed effects											
c.3820G>A	p.D1274N	25 (25)	6	0	4	0	13	0	0		5
c.4024A>G	p.I1342V	1 (1)	1	1	0	0	1	0	0		9
c.4780G>C	p.D1594H	8 (7)	1	0	0	1	1	0	0		5
Unknown function											
c.589G>C	p.D197H	6 (6)	4	2	4	1	5	0	0		14
c.1111C>G	p.Q371E	4 (3)	3	0	0	0	0	2	2		15
c.2184_2186del	p.L729del	3 (3)	1	0	0	0	0	0	0		22
c.2550-2551insTG	p.F851Cfs*19	3 (4)	2	2	3	0	0	0	0		5
c.3832G>A	p.V1278I	1 (4)	2	1	0	0	1	0	0		18
c.4557C>G	p.F1519L	1 (4)	4	1	0	0	0	1	0		18
c.4639G>C	p.E1547Q	1 (1)	1	1	0	0	1	0	0		12

AVB indicates atrioventricular block; CA, cardiac arrest; DCM, dilated cardiomyopathy; G+, genotype-positive individuals; LQT, long QT syndrome; P+, phenotype-positive individuals; SCD, sudden cardiac death; sev HF, severe DCM (ejection fraction <20% or heart transplant/death from DCM); UF, unknown function; VA, ventricular arrhythmias; and VPB, ventricular premature beats.

in 21 individuals (29%). Forty-two affected individuals (58%; 24 male, 13 female, 5 unknown) had DCM and 9 (12%) had severe DCM. All DCM cases had documented arrhythmias. Twelve individuals from 7 families had a history of resuscitated cardiac arrest or sudden cardiac death (SCD). Six of these had a premorbid diagnosis of DCM and 6 had no premorbid cardiac assessment. There were no cases of resuscitated cardiac arrest or SCD where the left ventricular function was documented to be normal. CD was not a prominent feature in this group; 4 individuals had atrioventricular block (AVB) but only 1 was aged <70 years at diagnosis, while 1 one individual had sick sinus syndrome. Nineteen individuals had an implantable cardioverter defibrillator inserted for primary or secondary prevention and 3 had device-related complications including inappropriate shocks or lead placement issues. One individual died during attempted implantable cardioverter defibrillator device extraction.

Other variants associated with window and gating pore currents were present in 7 families, with the p.R814W variant seen twice and the remaining variants being unique to single families (Tables 2 and 3, Table S5).[5,8,10,11,13,24,25] These families included 38 affected individuals, 20 of whom came from a single large family that carried the p.G213D variant.[10] The phenotype associated with these variants was almost identical to that reported for p.R222Q. Thirty (79%) individuals had frequent multifocal VPB, 13 (34%) had sustained ventricular arrhythmia, and 14 (37%) had AA. Reduction of VPB with exercise was observed in 2 reports,[10,11] however, ventricular trachycardia was induced on exercise in 1 report,[13] and exertional syncope (unmonitored) was documented in another.[24] Twenty individuals (53%; 8 males, 11 females, 1 unknown) had DCM and 2 (5%) had severe DCM. As with p.R222Q, there were no cases of DCM in the absence of arrhythmias. Three individuals died of SCD with no prior cardiac evaluation. Three individuals aged <70 years (8%) had AVB.[10,13] Fourteen had an implantable cardioverter defibrillator inserted, and there were no complications reported, though variable follow-up was recorded in these studies.

Table 3. Phenotype Characteristics Associated With Different Nav1.5 Functional Effects

Phenotype	Gating pore±window current (n=86)*	Increased window current only (n=25)*	Loss of peak current/ mixed effects (n=33)*	Unknown function (n=25)*
VPB	60 (70%)	20 (80%)	1 (3%)	3 (12%)
DCM	53 (62%)	9 (36%)	8 (24%)	17 (68%)
VA	26 (30%)	11 (44%)	0 (0%)	7 (28%)
AA	26 (30%)	9 (36%)	15 (45%)	7 (28%)
Sev HF	5 (6%)	1 (4%)	0 (0%)	3 (12%)
SCD	14 (16%)	1 (4%)	1 (3%)	2 (8%)
AVB < age 70	2 (2%)	2 (8%)	4 (12%)	7 (28%)
SSS	1 (1%)	0 (0%)	12 (36%)	2 (8%)
LQT	0 (0%)	0 (0%)	0 (0%)	2 (8%)

AA indicates atrial arrhythmias; AVB, atrioventricular block; DCM, dilated cardiomyopathy; LQT, long QT syndrome; SCD, sudden cardiac death; sev HF, severe DCM (ejection fraction <20% or heart transplant/death from DCM), SSS, sick sinus syndrome; VA, ventricular arrhythmias; and VPB, ventricular premature beats.

*Numbers denote all affected relatives in reported pedigrees, including those confirmed as heterozygote.

Among all reports of variants that increased window current and produced a gating pore current, cardiac magnetic resonance imaging was performed in 11 individuals; 10 had no late gadolinium enhancement,[10,13,16,17,21,24] while 1 had lateral infero-basal subepicardial and intraseptal fibrosis.[11]

Phenotype Associated With Increased Late Current

Variants that have sustained current during the action potential due to increased late sodium current are typically associated with LQT3 rather than DCM, and we identified only 1 small kindred in this subgroup.[23] Two of the 4 affected individuals had prolonged QT intervals and DCM, while the remaining 2 experienced SCD. The proband in this family had limited genetic evaluation and other causes of DCM cannot be excluded.

Phenotypes Associated With Loss of Peak Current

In the 3 families with variants confirmed to have loss of peak current or mixed effects, there was a total of 33 affected individuals (Tables 2 and 3, Table S5).[5,9] In 2 families with the p.D1274N and p.D1594H variants respectively, there was a predominance of CD, with 12 individuals having sick sinus syndrome and 4 diagnosed with AVB aged <70 years. Fourteen had AA. Only 1 individual had VPB,[9] and there was 1 individual with probable

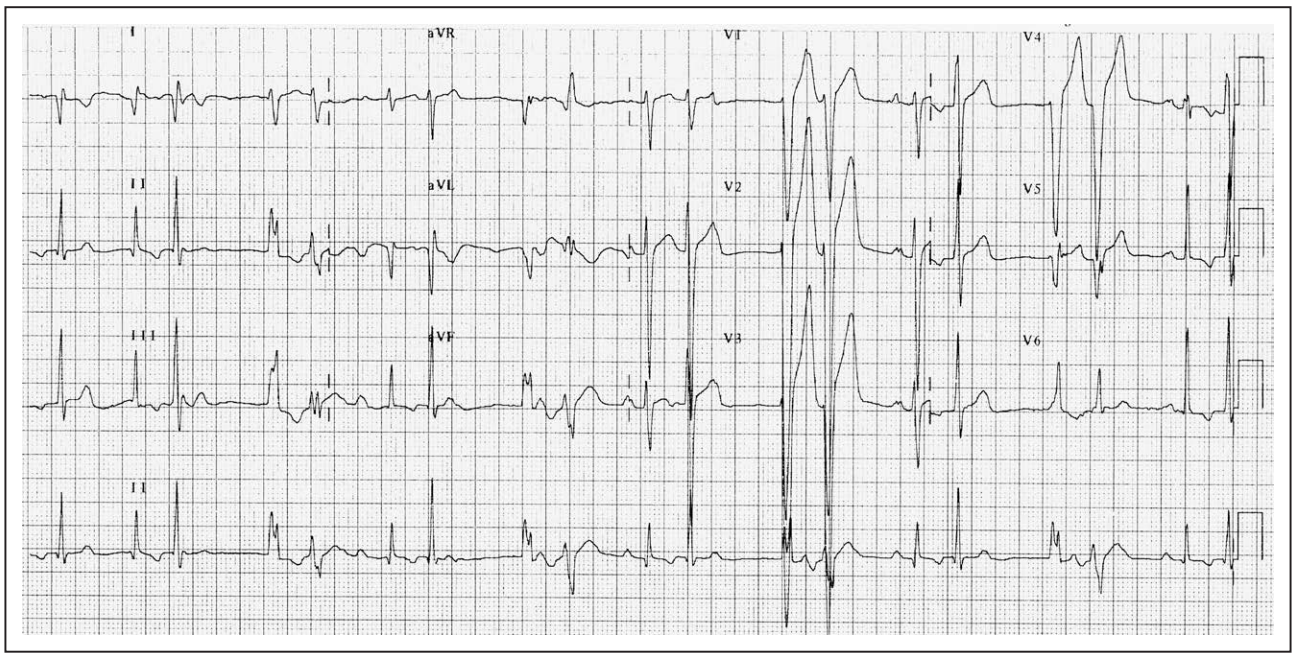

Figure 2. Characteristic ECG from an affected male p.R222Q SCN5A carrier.
Twelve-lead ECG shows sinus rhythm with frequent narrow junctional beats and broad complex premature beats with left and right bundle branch block patterns.

SCD though details were scarce.[5] Of the 25 affected individuals in the p.D1274N family, only 6 had DCM and 5 of these had a history of atrial fibrillation with/without CD. In the p.D1594H family, DCM was only diagnosed in 1 individual post-mortem. One additional case with a p.I1342V variant, had VPB, DCM, and atrial fibrillation. This mixed phenotype was consistent with the underlying electrophysiological findings of loss of peak current and gain of a sustained inward current.

Variants of Unknown Function

There were 25 affected individuals in 7 families that had *SCN5A* variants of unknown function (Tables 2 and 3, Table S5).[5,12,14,15,18,22] Two families had a phenotype suggestive of sustained inward current effects, 4 with loss of peak current density effects and 1 had DCM with LQT.

American College of Medical Genetics and Genomics Variant Classification

To determine how reported disease-causing mutations might be annotated using the current criteria employed in clinical genetic testing, we re-classified variants using the American College of Medical Genetics and Genomics framework with criteria specification for cardiomyopathy (Table 1).[36] Of the 18 included variants, 5 were scored as pathogenic and 9 were scored as likely pathogenic. Three of the latter variants had unknown functional effects but achieved likely pathogenic status on the basis of: suspected de novo case and another pathogenic missense variant at same site (p.D197H), predicted loss of function in a gene where loss of function is a known mechanism of disease (p.F851Cfs*19), and confirmed de novo case (p.E1547Q). The remaining 4 variants of unknown function were scored as variants of uncertain significance.

Efficacy of Targeted Pharmacological Therapy

Responses to sodium channel -altering drug therapy were mainly documented for variants associated with window and gating pore currents. Data were available for 23 individuals from 12 studies, including 14 individuals with p.R222Q (Table 4).[8,10,11,16,17,20,24–28] In total, there were 20 instances of complete treatment success (87%) with a sodium channel blocking agent, either as a first or second line. Treatment success constituted relief of arrhythmic burden and complete reversal of left ventricular systolic dysfunction (ejection fraction >50%). There were 5 instances of partial treatment success (improvement in arrhythmic burden and ejection fraction but not to >50% at time of publication or change to second line). There were 8 instances of treatment failure with a first line agent, and in 5 of those cases, treatment success occurred with a second line agent. In 2 cases, the patient died or was transplanted before a second agent was tried. In 1 case, there was no report of a follow-up agent. Flecainide was the most commonly used agent, with 8 cases of treatment success and 1 case of treatment failure after 1 month of therapy before the patient was changed to hydroquinidine.

There were 5 adverse events to sodium channel blockade reported. Amiodarone caused severe thyroiditis in 1 patient[2] and LQT with Torsades de Pointes in another.[24] Flecainide caused severe AVB in one patient who was subsequently treated with disopyramide with partial success.[27] In another patient, flecainide caused rapidly conducted atrial flutter.[2] Quinidine caused mild diarrhea and dry mucus membranes in 1 patient. Most studies reported a lack of treatment success with standard heart failure drugs, including beta-blockers.

DISCUSSION

In a systematic review of reported DCM-associated *SCN5A* variants, we identified 18 rare variants with suitable supportive genetic evidence in 29 kindreds. These variants had differing effects on Nav1.5 current and resulted in a spectrum of arrhythmic phenotypic features with variable presence and severity of DCM. Taken together, our findings raise interesting questions about how Nav1.5 defects impair myocardial function and have clinical implications.

Table 4. Efficacy of Antiarrhythmic Therapy in Patients With Window and Gating Pore Currents

	Amiodarone	Beta-blockers	Disopyramide	Flecainide	Hydroquinidine	Mexilitene	Propafenone	Quinidine	Sotalol
Success as first line	4			7	2			1	
Success as second line				1	1			3	1
Partial success	1	1	1			2			
Unsuccessful	1	1		1		1	1	1	2
Total success	4			8	3			4	1
Total all use	6	2	1	9	3	3	1	5	3
Adverse events	2			2				1	
Intolerance				1					

Success as first or second line if improvement in EF to >50% during documented treatment period. Partial success if improvement in EF <50% at time of publication. Unsuccessful if no change to EF documented at time of publication or before switching to second line agent. Adverse events, recognized treatment sequelae. Intolerance, reported side effects but no adverse events. EF indicates ejection fraction.

Most (64%) of the 11 variants for which functional data were available showed a gain of sustained inward currents either at depolarized potentials (due to increased window current or reduced inactivation) or at resting membrane potentials (due to generation of a new gating pore current). Remarkably, affected carriers had a similar phenotype, with a distinctive feature being frequent multifocal narrow and broad complex VPB, which has been attributed to premature activity of the high septal and distal Purkinje fibres, respectively. The presence of very frequent multifocal VPB with DCM and the reversibility of this phenotype with sodium channel blocking therapy help to distinguish *SCN5A*-related cardiomyopathy from other highly arrhythmogenic forms of DCM associated with genes such as *LMNA*, *FLNC*, *DSP*, *RBM20*, *PLN*, and *DES*. Furthermore, while only 11 individuals had cardiac magnetic resonance imaging, myocardial fibrosis appears rare in this group, while it is common in other forms of genetic arrhythmogenic DCM.[1] The reversible nature of this DCM should not indicate that it is a relatively more benign cardiomyopathy. Eleven percent of individuals with the VPB-predominant phenotype had severe heart failure leading to transplant or death. Of the 15 individuals with resuscitated cardiac arrest or SCD, half were known to have DCM while the remainder had no premorbid assessment, and there were no cases of resuscitated cardiac arrest/SCD in individuals with normal left ventricular function. This suggests that DCM is a risk factor for more severe arrhythmic events and underscores the importance of treatment. Individuals who carried variants that had loss of peak current or mixed effects had a very different clinical presentation, being less likely to have VPB, ventricular arrhythmia, or SCD, and presenting mainly with AA, sinus node dysfunction, and CD.

In the context of clinical genetic testing for DCM, a key question is whether *SCN5A* is truly a DCM disease gene or whether myocardial contractile dysfunction is a secondary consequence of a high arrhythmia burden or other factors. Frequent VPB (>16%–24%/d) and dyssynchronous myocardial activation have been associated with an increased risk of cardiomyopathy, and improved contractile function can be induced by effective arrhythmia management.[37] Variants that increase sustained sodium current, either during the action potential or at resting membrane potentials will have disrupted intracellular sodium-calcium and sodium-hydrogen homeostasis, and the consequent deleterious effects on contractile function may provide additional vulnerability to DCM in the setting of frequent arrhythmias.[38]

Four SCN5A variants (p.R219H, p.R222Q, p.R225P, p.R814W) found in families with high VPB burden introduced a new gating pore. Gosselin-Badaroudine et al[13] showed that p.R219H did not alter the biophysical properties of Nav1.5, but instead allowed a proton-selective gating pore through the voltage sensor domain during diastole. The direction of flow through this pore could be inward or outward depending on ionic concentrations and voltage gradients. A sustained inward cation current during diastole would depolarize the cell causing membrane instability, and through intracellular acidification directly depress cardiac contraction.[13] An inward proton leak current would also promote sodium and calcium loading of myocardial cells, subsequent to activation of the sodium proton exchanger, with pro-arrhythmogenic consequences. Studies of p.R219H in human induced pluripotent stem cell-derived cardiomyocytes showed reductions of cell contractility in the absence of arrhythmic stimuli, suggesting that these features can be independent.[31] The p.R222Q, p.R225P, and p.R814W variants have been found to create a cation-selective gating pore.[30,33] A common feature of these 4 gating pore variants is their location in S4 segments of voltage-sensing domains.[33] This cation current appears to be enhanced in low potassium and acidic environments, suggesting that a combination of channel disruption and downstream intracellular effects would be more likely to promote DCM under certain environmental conditions, and this may vary uniquely in individual variant carriers.[30,31] The extent to which gating pore currents are a key mechanistic explanation for DCM development is unclear. Currently, gating pore currents have only been studied for variants located in the S4 segments and whether variants located near the S4 segment (such as p.A204E, p.G213D, p.L828F) produce a similar current is unknown.

SCN5A variants that lengthen the plateau and repolarization phases of the cardiac action potential are an established cause of LQT3. Reported QTc intervals were generally normal, however, in families with window and gating pore current variants. While this might reflect a Purkinje-specific effect of these *SCN5A* variants that is not evident on the surface ECG, it is notable that paradoxical shortening of the action potential duration in p.R222Q Purkinje cells was reported in one study.[30] This action potential duration shortening might be explained by an outward potassium current flowing through the cation-specific gating pore. In another study, p.R222Q lengthened the action potential duration in isolated Purkinje fiber cells but did not change action potential duration in ventricular cells.[16] The model used did not include the gating pore current. In contrast, carriers of the p.Q1506_P1508del variant, that increased late sodium current, had marked prolongation of QTc intervals (up to 750 ms), together with Torsades de Pointes and SCD. The p.Q371E variant, although functionally uncharacterized, had a similar clinical picture with LQT3 and DCM. The overall low prevalence of DCM in most LQT3 families is notable and argues in favour of additional patient-related genetic and acquired factors being involved in the families reported here.

The reported effects of loss of peak sodium current on myocardial function have varied with different models

used. For p.D1274N, although heterologous expression systems showed normal Nav1.5 channel activity, animal models recapitulated the human phenotype showing significant CD.[34,35] It was proposed that the mutant channels had dysfunctional post-translational regulation that was only evident in vivo. Whether loss of peak sodium current density causes structural heart disease remains contentious. A transgenic p.D1274N zebrafish model showed significant CD but no change in contractile function.[34] A heterozygote *SCN5A* knockout mouse had normal ventricular function although the presence of myocardial fibrosis suggested that cardiomyopathic changes were present.[39] Watanabe et al[35] demonstrated reduced fractional shortening in p.D1274N knock-in mice but acknowledged that mechanical dyssynchrony might have caused this phenotype.

There are several important clinical implications of our study. First, it is vital that physicians recognize the distinctive phenotype of *SCN5A* variants associated with window and gating pore currents and undertake genetic testing in patients and their families. Second, we found no cases of DCM without arrhythmias and all probands presented first with arrhythmic symptoms. Consequently, the index of suspicion that a rare *SCN5A* variant is responsible for disease in patients with a DCM-only phenotype is relatively low, and other causes need to be sought. Third, is the exquisite sensitivity of window and gating pore current variants to sodium channel-blocking therapy, with treatment of affected carriers representing one of the most impressive success stories to date for precision medicine in inherited cardiomyopathies. Twenty of 23 (87%) individuals treated with sodium channel blockade had complete resolution of VPB and normalization of left ventricular ejection fraction. In a minority of individuals, there were incomplete responses or adverse effects to specific drugs that necessitated alternative therapeutic agents. We recently published longitudinal follow-up of a large p.R222Q kindred and demonstrated sustained reversibility of VPB and DCM out to 10 years with flecainide.[2] In patients with severe DCM, several individuals were rescued from heart transplantation by timely institution of sodium channel blockade. However, there were 2 reported cases of death or heart transplant before treatment taking effect. Thus, it is unknown whether complete phenotypic reversibility can be expected in those with extensive myocardial damage and end-stage DCM. Rather, early intervention before the development of marked structural changes is likely to be much more effective.

For patients with novel *SCN5A* variants and variants of unknown function, optimal management strategies are less clear. Some of these variants can be expected to have an increased sustained sodium current, but without clear experimental data, use of sodium channel blockade cannot be advocated, especially given the potential for these drugs to worsen CD. Our findings suggest that functional evaluation should be prioritized if possible, particularly when the multifocal VPB-predominant typical phenotype is suspected. Further, if a previously undescribed variant is located in an S4 transmembrane segment, suspicion of a reversible type of *SCN5A*-related DCM should be high. Comprehensive electrophysiological evaluation of all new variants using patch clamping technique is ideal but can be time-consuming. The development of new techniques for high throughput mutational scanning, such as recently described by Glazer et al[40] holds enormous promise for rapid identification of function-altering *SCN5A* variants. The reliability of the multifocal VPB-predominant cardiomyopathy phenotype in this study indicates that this could be incorporated into *SCN5A* variant interpretation, though further study into the specificity of this phenotype is needed.

CONCLUSIONS

SCN5A-related DCM is primarily a disease that affects the voltage-sensing domains and results in an increased sustained sodium current. Variants that increase window current and generate new gating pore current appear to be associated with a high risk of ventricular ± atrial arrhythmic events and can progress to severe heart failure. Nonetheless, multiple studies have now highlighted the exquisite reversibility of this phenotype with sodium channel blockade, among which flecainide may be the most reliable. *SCN5A*-related DCM is an important condition to diagnose promptly, as initiation of sodium channel blockade may prevent significant morbidity and mortality.

ARTICLE INFORMATION

Received April 9, 2021; accepted November 24, 2021.

Affiliations

Department of Cardiology (S.P., M.P., D.Z., J.M.K.), Department of Genomic Medicine (S.P., B.A.T., M.P., P.J., D.Z.), Department of Pathology (B.A.T.), Royal Melbourne Hospital. Department of Medicine, University of Melbourne (S.P., P.J., D.Z., J.M.K.). Familial Cancer Centre, Peter MacCallum Centre, Melbourne, Victoria (P.J.). Molecular Cardiology Division, Victor Chang Cardiac Research Institute (J.I.V., D.F.). St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney (J.I.V., D.F.). Cardiology Department, St. Vincent's Hospital, Sydney, New South Wales, Australia (D.F.).

Acknowledgments

We thank Dr Carus Lau for assistance with preparation of the figure.

Sources of Funding

Dr Peters is supported by a Commonwealth Research Training Program Scholarship and a Melbourne Health Research Fellowship grant. Dr Kalman is funded by a Practitioner Fellowship of the National Health and Medical Research Council of Australia (NHMRC). Drs Vandenberg and Fatkin receive support from the Victor Chang Cardiac Research Institute, NSW Health, NHMRC, and Australian Genomics.

Disclosures

None.

Supplemental Materials

Supplemental Methods
Tables S1–S5
Figure S1
References [41–44]

REFERENCES

1. Fatkin D, Calkins H, Elliott P, James CA, Peters S, Kovacic JC. Contemporary and future approaches to precision medicine in inherited cardiomyopathies: JACC focus seminar 3/5. *J Am Coll Cardiol*. 2021;77:2551–2572. doi: 10.1016/j.jacc.2020.12.072
2. Peters S, Johnson R, Zentner D, James P, Kalman JM, Fatkin D. Long-term efficacy and safety of sodium channel antagonists in patients with p.R222Q SCN5A-related arrhythmic dilated cardiomyopathy. *JACC Clin Electrophysiol*. 2021;7:126–128. doi: 10.1016/j.jacep.2020.09.023
3. Wilde AAM, Amin AS. Clinical spectrum of SCN5A mutations: long QT syndrome, brugada syndrome, and cardiomyopathy. *JACC Clin Electrophysiol*. 2018;4:569–579. doi: 10.1016/j.jacep.2018.03.006
4. Bezzina CR, Rook MB, Groenewegen WA, Herfst LJ, van der Wal AC, Lam J, Jongsma HJ, Wilde AA, Mannens MM. Compound heterozygosity for mutations (W156X and R225W) in SCN5A associated with severe cardiac conduction disturbances and degenerative changes in the conduction system. *Circ Res*. 2003;92:159–168. doi: 10.1161/01.res.0000052672.97759.36
5. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, Horton SC, Rodeheffer RJ, Anderson JL. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. *JAMA*. 2005;293:447–454. doi: 10.1001/jama.293.4.447
6. Mazzarotto F, Tayal U, Buchan RJ, Midwinter W, Wilk A, Whiffin N, Govind R, Mazaika E, de Marvao A, Dawes TJW, et al. Reevaluating the genetic contribution of monogenic dilated cardiomyopathy. *Circulation*. 2020;141:387–398. doi: 10.1161/CIRCULATIONAHA.119.037661
7. Bodian DL, Vilboux T, Hourigan SK, Jenevein CL, Mani H, Kent KC, Khromykh A, Solomon BD, Hauser NS. Genomic analysis of an infant with intractable diarrhea and dilated cardiomyopathy. *Cold Spring Harb Mol Case Stud*. 2017;3:a002055. doi: 10.1101/mcs.a002055
8. Beckermann TM, McLeod K, Murday V, Potet F, George AL Jr. Novel SCN5A mutation in amiodarone-responsive multifocal ventricular ectopy-associated cardiomyopathy. *Heart Rhythm*. 2014;11:1446–1453. doi: 10.1016/j.hrthm.2014.04.042
9. Boddum K, Saljic A, Jespersen T, Christensen AH. A novel SCN5A variant associated with abnormal repolarization, atrial fibrillation, and reversible cardiomyopathy. *Cardiology*. 2018;140:8–13. doi: 10.1159/000487475
10. Calloe K, Broendberg AK, Christensen AH, Pedersen LN, Olesen MS, de Los Angeles Tejada M, Friis S, Thomsen MB, Bundgaard H, Jensen HK. Multifocal atrial and ventricular premature contractions with an increased risk of dilated cardiomyopathy caused by a Nav1.5 gain-of-function mutation (G213D). *Int J Cardiol*. 2018;257:160–167. doi: 10.1016/j.ijcard.2017.11.095
11. Doisne N, Waldmann V, Redheuil A, Waintraub X, Fressart V, Ader F, Fossé L, Hidden-Lucet F, Gandjbakhch E, Neyroud N. A novel gain-of-function mutation in SCN5A responsible for multifocal ectopic Purkinje-related premature contractions. *Hum Mutat*. 2020;41:850–859. doi: 10.1002/humu.23981.
12. Franaszczyk M, Truszkowska G, Chmielewski P, Rydzanicz M, Kosinska J, Rywik T, Biernacka A, Spiewak M, Kostrzewa G, Stepien-Wojno M, et al. Analysis of de novo mutations in sporadic cardiomyopathies emphasizes their clinical relevance and points to novel candidate genes. *J Clin Med*. 2020;9:E370. doi: 10.3390/jcm9020370
13. Gosselin-Badaroudine P, Keller DI, Huang H, Pouliot V, Chatelier A, Osswald S, Brink M, Chahine M. A proton leak current through the cardiac sodium channel is linked to mixed arrhythmia and the dilated cardiomyopathy phenotype. *PLoS One*. 2012;7:e38331. doi: 10.1371/journal.pone.0038331
14. Kean AC, Helm BM, Vatta M, Ayers MD, Parent JJ, Darragh RK. Clinical characterisation of a novel SCN5A variant associated with progressive malignant arrhythmia and dilated cardiomyopathy. *Cardiol Young*. 2019;29:1257–1263. doi: 10.1017/S1047951119001860
15. Kimura M, Kohno T, Aizawa Y, Inohara T, Shiraishi Y, Katsumata Y, Egashira T, Fukushima H, Kosaki K, Fukuda K. A novel SCN5A mutation found in a familial case of long QT syndrome complicated by severe left ventricular dysfunction. *Can J Cardiol*. 2017;33:554.e5–554.e7. doi: 10.1016/j.cjca.2016.10.010
16. Laurent G, Saal S, Amarouch MY, Béziau DM, Marsman RF, Faivre L, Barc J, Dina C, Bertaux G, Barthez O, et al. Multifocal ectopic Purkinje-related premature contractions: a new SCN5A-related cardiac channelopathy. *J Am Coll Cardiol*. 2012;60:144–156. doi: 10.1016/j.jacc.2012.02.052
17. Mann SA, Castro ML, Ohanian M, Guo G, Zodgekar P, Sheu A, Stockhammer K, Thompson T, Playford D, Subbiah R, et al. R222Q SCN5A mutation is associated with reversible ventricular ectopy and dilated cardiomyopathy. *J Am Coll Cardiol*. 2012;60:1566–1573. doi: 10.1016/j.jacc.2012.05.050
18. McNair WP, Sinagra G, Taylor MR, Di Lenarda A, Ferguson DA, Salcedo EE, Slavov D, Zhu X, Caldwell JH, Mestroni L; Familial Cardiomyopathy Registry Research Group. SCN5A mutations associate with arrhythmic dilated cardiomyopathy and commonly localize to the voltage-sensing mechanism. *J Am Coll Cardiol*. 2011;57:2160–2168. doi: 10.1016/j.jacc.2010.09.084.
19. Morales A, Painter T, Li R, Siegfried JD, Li D, Norton N, Hershberger RE. Rare variant mutations in pregnancy-associated or peripartum cardiomyopathy. *Circulation*. 2010;121:2176–2182. doi: 10.1161/CIRCULATIONAHA.109.931220
20. Morini E, Mango R, Rizzacasa B, Maletta S, Marsili L, Morgagni R, Novelli G, Sangiuolo F, Romeo F, Amati F. Complex inherited cardiac diseases inheritance in a family harbouring compound SCN5A and MYBPC3 mutations. *Eur Heart J*. 2016;37(Supplement 1):682–683.
21. Nair K, Pekhletski R, Harris L, Care M, Morel C, Farid T, Backx PH, Szabo E, Nanthakumar K. Escape capture bigeminy: phenotypic marker of cardiac sodium channel voltage sensor mutation R222Q. *Heart Rhythm*. 2012;9:1681–1688.e1. doi: 10.1016/j.hrthm.2012.06.029
22. Roudijk R, Loh P, Van Der Heijden J, Scholten M, Wilde A, Asselbergs F, Hauer R, Te Riele A, Van Tintelen P. Variation of phenotype in carriers of a (P.LEU729DEL) SCN5A founder mutation. *J Interventi Cardiac Electrophysiology*. 2019;55:S28.
23. Shi R, Zhang Y, Yang C, Huang C, Zhou X, Qiang H, Grace AA, Huang CL, Ma A. The cardiac sodium channel mutation delQKP 1507-1509 is associated with the expanding phenotypic spectrum of LQT3, conduction disorder, dilated cardiomyopathy, and high incidence of youth sudden death. *Europace*. 2008;10:1329–1335. doi: 10.1093/europace/eun202
24. Ter Bekke RMA, David M, Krapels IPC, Crijns HJGM, Volders PGA. Beauty and the beat: a complicated case of multifocal ectopic Purkinje-related premature contractions. *HeartRhythm Case Rep*. 2018;4:429–433. doi: 10.1016/j.hrcr.2018.05.010
25. Zakrzewska-Koperska J, Bilinska ZT, Truszkowska TG, Franszczyk M, Kalin K, Guzek K, Hasiec A, Stepien-Wojno M, Orczykowski M, Bodalski R, et al. Successful antiarrhythmic therapy with combination of quinidine and mexiletine in Purkinje-related ventricular arrhythmia, persistent atrial tachyarrhythmia and DCM associated with R814W SCN5A mutation. *Eur J Heart Fail*. 2019;21:300–301.
26. Zakrzewska-Koperska J, Franaszczyk M, Bilińska Z, Truszkowska G, Karczmarz M, Szumowski Ł, Zieliński T, Płoski R, Bilińska M. Rapid and effective response of the R222Q SCN5A to quinidine treatment in a patient with Purkinje-related ventricular arrhythmia and familial dilated cardiomyopathy: a case report. *BMC Med Genet*. 2018;19:94. doi: 10.1186/s12881-018-0599-4
27. Robinson VM, Asghar O, Venetucci L, Muhyaldeen S. A case of familial dilated cardiomyopathy with a reversible phenotype: the utility of genetic testing and genotype-specific treatment. *J Am Coll Cardiol*. 2017;69(11 Supplement 1):2128
28. Saeed Y, Temple IP, Borbas Z, Atkinson A, Yanni J, Maczewski M, Mackiewicz U, Aly M, Logantha SJRJ, Garratt CJ, et al. Structural and functional remodeling of the atrioventricular node with aging in rats: the role of hyperpolarization-activated cyclic nucleotide-gated and ryanodine 2 channels. *Heart Rhythm*. 2018;15:752–760. doi: 10.1016/j.hrthm.2017.12.027.
29. Keller DI, Acharfi S, Delacrétaz E, Benammar N, Rotter M, Pfammatter JP, Fressart V, Guicheney P, Chahine M. A novel mutation in SCN5A, delQKP 1507-1509, causing long QT syndrome: role of Q1507 residue in sodium channel inactivation. *J Mol Cell Cardiol*. 2003;35:1513–1521. doi: 10.1016/j.yjmcc.2003.08.007
30. Daniel LL, Yang T, Kroncke B, Hall L, Stroud D, Roden DM. SCN5A variant R222Q generated abnormal changes in cardiac sodium current and action potentials in murine myocytes and Purkinje cells. *Heart Rhythm*. 2019;16:1676–1685. doi: 10.1016/j.hrthm.2019.05.017
31. Moreau A, Gosselin-Badaroudine P, Mercier A, Burger B, Keller DI, Chahine M. A leaky voltage sensor domain of cardiac sodium channels causes arrhythmias associated with dilated cardiomyopathy. *Sci Rep*. 2018;8:13804. doi: 10.1038/s41598-018-31772-0
32. Nguyen TP, Wang DW, Rhodes TH, George AL Jr. Divergent biophysical defects caused by mutant sodium channels in dilated

cardiomyopathy with arrhythmia. *Circ Res*. 2008;102:364–371. doi: 10.1161/CIRCRESAHA.107.164673
33. Moreau A, Chahine M. A new cardiac channelopathy: from clinical phenotypes to molecular mechanisms associated with Nav1.5 gating pores. *Front Cardiovasc Med*. 2018;5:139. doi: 10.3389/fcvm.2018.00139
34. Huttner IG, Trivedi G, Jacoby A, Mann SA, Vandenberg JI, Fatkin D. A transgenic zebrafish model of a human cardiac sodium channel mutation exhibits bradycardia, conduction-system abnormalities and early death. *J Mol Cell Cardiol*. 2013;61:123–132. doi: 10.1016/j.yjmcc.2013.06.005
35. Watanabe H, Yang T, Stroud DM, Lowe JS, Harris L, Atack TC, Wang DW, Hipkens SB, Leake B, Hall L, et al. Striking in vivo phenotype of a disease-associated human SCN5A mutation producing minimal changes in vitro. *Circulation*. 2011;124:1001–1011. doi: 10.1161/CIRCULATIONAHA.110.987248
36. Kelly MA, Caleshu C, Morales A, Buchan J, Wolf Z, Harrison SM, Cook S, Dillon MW, Garcia J, Haverfield E, et al. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. *Genet Med*. 2018;20:351–359. doi: 10.1038/gim.2017.218
37. Baman TS, Lange DC, Ilg KJ, Gupta SK, Liu TY, Alguire C, Armstrong W, Good E, Chugh A, Jongnarangsin K, et al. Relationship between burden of premature ventricular complexes and left ventricular function. *Heart Rhythm*. 2010;7:865–869. doi: 10.1016/j.hrthm.2010.03.036
38. Kohmoto O, Spitzer KW, Movsesian MA, Barry WH. Effects of intracellular acidosis on [Ca2+]i transients, transsarcolemmal Ca2+ fluxes, and contraction in ventricular myocytes. *Circ Res*. 1990;66:622–632. doi: 10.1161/01.res.66.3.622
39. Royer A, van Veen TA, Le Bouter S, Marionneau C, Griol-Charhbili V, Léoni AL, Steenman M, van Rijen HV, Demolombe S, Goddard CA, et al. Mouse model of SCN5A-linked hereditary Lenègre's disease: age-related conduction slowing and myocardial fibrosis. *Circulation*. 2005;111:1738–1746. doi: 10.1161/01.CIR.0000160853.19867.61
40. Glazer AM, Kroncke BM, Matreyek KA, Yang T, Wada Y, Shields T, Salem JE, Fowler DM, Roden DM. Deep mutational scan of an SCN5A voltage sensor. *Circ Genom Precis Med*. 2020;13:e002786. doi: 10.1161/CIRCGEN.119.002786
41. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. doi: 10.1038/gim.2015.30.
42. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F. The ensembl variant effect predictor. *Genome Biol*. 2016;17:122. doi: 10.1186/s13059-016-0974-4
43. Kwon HW, Lee SY, Kwon BS, Kim GB, Bae EJ, Kim WH, Noh CI, Cho SI, Park SS. Long QT syndrome and dilated cardiomyopathy with SCN5A p.R1193Q polymorphism: cardioverter-defibrillator implantation at 27 months. *Pacing Clin Electrophysiol*. 2012;35:e243–e246. doi: 10.1111/j.1540-8159.2012.03409.x
44. Ge J, Sun A, Paajanen V, Wang S, Su C, Yang Z, Li Y, Wang S, Jia J, Wang K, et al. Molecular and clinical characterization of a novel SCN5A mutation associated with atrioventricular block and dilated cardiomyopathy. *Circ Arrhythm Electrophysiol*. 2008;1:83–92. doi: 10.1161/CIRCEP.107.750752