

## Inherited Arrhythmias: A National Heart, Lung, and Blood Institute and Office of Rare Diseases Workshop Consensus Report About the Diagnosis, Phenotyping, Molecular Mechanisms, and Therapeutic Approaches for Primary Cardiomyopathies of Gene Mutations Affecting Ion Channel Function

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## Inherited Arrhythmias

### A National Heart, Lung, and Blood Institute and Office of Rare Diseases Workshop Consensus Report About the Diagnosis, Phenotyping, Molecular Mechanisms, and Therapeutic Approaches for Primary Cardiomyopathies of Gene Mutations Affecting Ion Channel Function

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**Abstract**—The National Heart, Lung, and Blood Institute and Office of Rare Diseases at the National Institutes of Health organized a workshop (September 14 to 15, 2006, in Bethesda, Md) to advise on new research directions needed for improved identification and treatment of rare inherited arrhythmias. These included the following: (1) Na<sup>+</sup> channelopathies; (2) arrhythmias due to K<sup>+</sup> channel mutations; and (3) arrhythmias due to other inherited arrhythmogenic mechanisms. Another major goal was to provide recommendations to support, enable, or facilitate research to improve future diagnosis and management of inherited arrhythmias. Classifications of electric heart diseases have proved to be exceedingly complex and in many respects contradictory. A new contemporary and rigorous classification of arrhythmogenic cardiomyopathies is proposed. This consensus report provides an important framework and overview to this increasingly heterogeneous group of primary cardiac membrane channel diseases. Of particular note, the present classification scheme recognizes the rapid evolution of molecular biology and novel therapeutic approaches in cardiology, as well as the introduction of many recently described diseases, and is unique in that it incorporates ion channelopathies as a primary cardiomyopathy in consensus with a recent American Heart Association Scientific Statement. (*Circulation*. 2007;116:2325-2345.)

**Key Words:** arrhythmia ■ cardiomyopathies ■ death, sudden ■ electrophysiology ■ genetics ■ ion channels ■ long-QT syndrome

The National Heart, Lung, and Blood Institute and Office of Rare Diseases at the National Institutes of Health organized a workshop to advise on new research directions needed for improved identification and treatment of rare

inherited arrhythmias. During the workshop, current levels of understanding and gaps in knowledge of “rare inherited arrhythmias” were presented in 3 areas: (1) inherited channelopathies; (2) other inherited arrhythmias; and (3) imple-

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tions for the future diagnosis and management of inherited arrhythmias. Elucidation of the basis of genetic diseases provides unique insights into the mechanisms responsible for more prevalent arrhythmias and sudden cardiac arrest (SCA) while also permitting the identification of therapeutic opportunities for treatment and prevention. From screening of larger patient cohorts, it has become clear that environmental and genetic modifiers play important roles for arrhythmia susceptibility and severity. Study of distinct forms of inherited arrhythmias requires evaluation in specific models to make accurate predictions of human pathophysiology. Appropriate *in vivo* arrhythmia models may vary from genetically engineered mouse or rabbit models to larger-animal models that more closely mimic human electrophysiological substrates. Workshop participants recommended multilevel translational approaches to identify and validate gene variations associated with arrhythmogenesis. Such approaches should span development of molecular and biophysical structure-function relationships and cellular mechanisms to examinations of organ and *in vivo* models. Integrated analysis by modeling approaches may aid in elucidation of arrhythmia mechanisms.

Opportunities to develop novel therapeutic approaches require comprehensive studies that characterize ion channel function and expression, regulation by intracellular signaling complexes, alterations in and cross talk of intracellular  $\text{Ca}^{2+}$  signals, and therapeutic interventions aimed at specific molecular defects. Future therapeutic and diagnostic approaches should include identification of novel surrogate clinical markers. It is also vital that training mechanisms be supported for young investigators in an integrated range of disciplines (including biology, medicine, computer modeling, bioengineering, information sciences) while providing scientific career opportunities.

Specific recommendations to the National Heart, Lung, and Blood Institute and Office of Rare Diseases to support, enable, or facilitate research in areas of key importance for the future understanding and treatment of inherited arrhythmic syndromes were as follows:

- Establish biological and computational models that appropriately reflect the molecular environment of the human heart and permit study of the functional effects of arrhythmogenic mutations. Biological models, including human stem cell–derived cardiomyocytes and genetically altered model organisms, should enhance the study of normal and mutant ion channel biosynthesis, assembly, macromolecular complexes, posttranslational regulation, trafficking, targeting (functional proteomics), and human arrhythmia mechanisms.
- Elucidate the molecular and physiological basis of arrhythmia triggers and substrates with the use of integrative animal, cell, and computational models.
- Identify new human arrhythmia-susceptibility genes, modifiers, and the mechanisms of their effects. Design and utilize more efficient (higher throughput) molecular, cellular, and integrative approaches to advance our understanding of genotype–phenotype interactions.
- Develop, test, and implement new therapeutic approaches to identify, treat, and prevent inherited arrhythmias based on genetic, molecular, and cellular mechanisms.
- Establish methods (including bioinformatics) to evaluate, integrate, and share structure-function genotype–phenotype relationships at the gene, protein, signaling complex, cell, organ, and *in vivo* levels to allow better prediction of the significance of specific genetic variants for arrhythmogenesis and to focus research efforts and therapy toward patient and family needs.

### Cardiac $\text{Na}^+$ Channelopathies

Mutations in *SCN5A*, the gene encoding the  $\text{Na}^+$  channel  $\alpha$ -subunit expressed in the human heart, cause inherited susceptibility to ventricular arrhythmias (congenital long-QT syndrome [LQTS] including prolongation of ventricular action potentials, dispersion of repolarization, QT-interval and T-wave abnormalities in surface ECG recordings [LQTS3]; idiopathic ventricular fibrillation [VF]),<sup>1–3</sup> cardiac conduction disease (CoD),<sup>4–6</sup> and dilated cardiomyopathy (DCM) with atrial arrhythmia (Genetic arrhythmia syndromes are numbered sequentially to identify and discriminate specific genotype–phenotype relationships; refer to Tables 1 through 3).<sup>7,8</sup> Mutations in *SCN5A* may also present with more complex phenotypes representing combinations of LQTS, CoD, and Brugada syndrome (BrS1). Examples of LQTS3 combined with either BrS1<sup>9</sup> or congenital heart block,<sup>10,11</sup> cases of BrS1 with impaired conduction,<sup>12</sup> or combinations of all 3 phenotypes have been documented.<sup>13</sup> Moreover, certain mutations may manifest different phenotypes in different individuals and families.

### Novel Clinical and Genetic Aspects of *SCN5A* Phenotypes

The voltage-gated  $\text{Na}^+$  channel ( $\text{Na}_v1.5$  encoded by *SCN5A*), responsible for the initial upstroke of the action potential, represents an important drug target for antiarrhythmic class Ia blockers. *SCN5A* was mapped to chromosome 3p21, identifying it as a candidate gene for LQTS3.<sup>27</sup> Subsequently, a mutation in *SCN5A* was found in families with 3p21-linked LQTS3 (Table 3).<sup>28</sup> After linkage of *SCN5A* to LQTS3 and abnormal cardiac repolarization, distinct disease phenotypes, including conduction slowing, have been linked to *SCN5A* mutations. A subgroup of idiopathic VF patients with a distinctive ECG pattern characterized by apparent right bundle-branch block, ST-segment elevation, and sudden death was described as a new clinical entity referred to as Brugada syndrome (BrS1) (Table 1).<sup>14</sup> Pharmacological  $\text{Na}^+$  channel block elicits or worsens the ECG features associated with BrS1.<sup>29</sup> Screening of families with BrS1 has revealed distinct mutations in the *SCN5A* gene.<sup>3</sup> In a large Dutch family with a history of sudden death, mostly occurring at night, living members demonstrated ECG features compatible with BrS1 and LQTS3 corresponding to a novel mutation in the C-terminus of *SCN5A* (1795insD).<sup>19</sup> Subsequently, a Y1795H mutation in a patient with BrS1 and a Y1795C mutation in a patient with LQTS3 were identified, providing evidence of the close interrelationship

Table 1. Brugada and Related Arrhythmia Syndromes

Brugada Syndrome (BrS), Conduction Disease (CoD), Atrial Arrhythmia (AA)						
Gene	Locus	Syndrome	Protein & subunit	Function & abnormality	Occurs In <sup>¶</sup>	Ref.
<i>SCN5A</i>	3p21	BrS1, CoD	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	20-30%	3,14
<i>GPD1L</i> <sup>+</sup>	3p24	BrS2, SIDS <sup>&amp;</sup>	G3PD1L	<i>I</i> <sub>Na</sub> ↓	< 1%	15,16,25,26
<i>SCN5A</i>	3p21	SUND <sup>†</sup>	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	common	17
<i>SCN5A</i>	3p21	Progressive CoD <sup>#</sup>	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	common	4
<i>SCN5A</i>	3p21	BrS1, CoD, AA	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	?	18
<i>SCN5A</i>	3p21	BrS1, LQTS3	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	?	9,19,20
<i>SCN5A</i>	3p21	BrS1, LQTS3, CoD	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	?	21
<i>SCN5A</i>	3p21	iVF <sup>§</sup> , CoD	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	?	22
<i>SCN5A</i>	3p21	DCM, CoD, AA (AF)	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	common	7,8,23
<i>SCN5A</i>	3p21	BrS1, SIDS <sup>&amp;</sup>	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	?	24
<i>SCN5A</i>	3p21	BrS1, CoD, SIDS <sup>&amp;</sup>	Nav1.5 α	<i>I</i> <sub>Na</sub> ↓	common	11

For a review of inherited conduction system abnormalities including mutations of genes not related to ion membrane transport, please see Wolf & Berul.<sup>183</sup> Genes contributing to distinct phenotypes are marked similarly for ease of comparison within and between tables 1 through 5. ↓ indicates loss of function; ?, unknown.

<sup>¶</sup>Relative syndromic occurrence for a given genetic syndrome (in %).

<sup>+</sup>Glycerol-3-phosphate dehydrogenase 1-like gene.

<sup>&</sup>Sudden infant death syndrome: An estimated 10 to 15% of SIDS stems from LQTS-, BrS-, and CPVT-causing mutations. Approximately 50% of ion channel-related SIDS involves defects in *SCN5A* or other components of the Na<sup>+</sup> channel macromolecular complex.

<sup>\*</sup>Sudden unexpected nocturnal death syndrome: estimated mortality rate 26 to 38/100 000 in young Thai men.

<sup>#</sup>Lenègre-Lev disease (fibrofatty atrophy of the His-Purkinje system).

<sup>§</sup>Idiopathic ventricular fibrillation without ECG signs of BrS1.

between BrS1 and LQTS3.<sup>20</sup> The effect of the 1795insD-*SCN5A* mutation during sinoatrial node pacemaking decreased the sinus rate by decreasing the diastolic depolarization rate, which may account for the bradycardia seen in LQTS3 patients, whereas sinoatrial node pauses or arrest may result from failure of sinoatrial nodal cells to repolarize.<sup>30</sup> In a large family with a Brugada-like phenotype, *SCN5A* could be excluded as a candidate gene indicating a distinct locus,<sup>15</sup> and BrS2 was subsequently linked to *GPD1L*, the glycerol-3-phosphate dehydrogenase 1-like gene.<sup>25</sup> Mutations in *GPD1L* have also been implicated in sudden infant death syndrome (SIDS).<sup>26</sup> Sudden unexplained nocturnal death syndrome, described in southeast Asia, resembles BrS1 and has been linked to mutations in the *SCN5A* gene.<sup>31</sup> It has also been suggested that BrS1 accounts for up to 60% of VF cases previously classified as idiopathic.<sup>32</sup> This seems to be particularly prevalent in Southeast Asia and Japan, with mortality rates as high as 38 per 100 000,<sup>17</sup> indicating that these arrhythmic syndromes may be much more common than previously thought.

Identification of *SCN5A* mutations in association with conduction system abnormalities has provided a major advance in our understanding of these disorders. Na<sup>+</sup> channel mutations have also been identified in patients with atrioventricular block and sick sinus syndrome (SSS). In a kindred with progressive conduction system disease (Lenègre-Lev disease), heterozygous *SCN5A* mutations were identified,<sup>4</sup> and additional *SCN5A* mutations have been associated with SSS (Table 2).<sup>5,33</sup>

#### Newly Recognized Phenotypes Associated With *SCN5A* Mutations

Genome-wide linkage analyses led to identification of an *SCN5A* missense mutation (D1275N) that cosegregated among 22 family members with a “novel” phenotype of DCM and atrial fibrillation (AF) (Table 1).<sup>7,8,23,53</sup> Variably expressed phenotypic traits included defects in impulse generation (SSS) and conduction (atrioventricular node and bundle-branch block), previously linked to loss-of-function mutations in *SCN5A*. Electric dysfunction (sinus bradycardia and SSS) typically preceded clinically apparent myocardial disease, and relatively slow ventricular rates in mutation carriers with AF ruled out tachycardia-induced cardiomyopathy. Subsequent mutation scanning in 156 probands with idiopathic DCM identified additional heterozygous missense (T220I, R814W, D1595H) and truncation (2550–2551insTG) *SCN5A* mutations, segregating with cardiac disease or arising de novo in 2.2% of the DCM cohort.<sup>7</sup> Among probands and their relatives with an *SCN5A* mutation, 27% had early features of DCM (mean age at diagnosis, 20.3 years), 38% had DCM (47.9 years), and 43% had AF (27.8 years). The same T220I and D1275N substitutions were previously reported as recessive loss-of-function alleles in SSS and familial atrial standstill, respectively.<sup>5,33</sup> The link between cardiac Na<sup>+</sup> channel loss of function and structural heart disease is supported by fibrosis and cardiac myocyte degeneration occurring both in aging heterozygous *SCN5A*<sup>+/-</sup> mice and in patients with BrS1.<sup>54,55</sup> These

Table 2. Atrial Arrhythmia Syndromes

Atrial Fibrillation (AF), Atrial Tachycardia (AT), and Sick Sinus Syndrome (SSS)						
Gene	Locus	Syndrome	Protein & subunit	Functional abnormality	Occurs In <sup>†</sup>	Ref.
<i>KCNQ1</i>	11p15.5	AF1	K <sub>v</sub> 7.1 $\alpha$	<i>I</i> <sub>Ks</sub> $\uparrow$ KvLQT1	?	34,35
<i>KCNE2</i>	21q22.1	AF2	MiRP1 $\beta$	<i>I</i> <sub>Ks</sub> $\uparrow$	?	36
<i>KCNJ2</i>	17q23	AF3	Kir2.1 $\alpha$	<i>I</i> <sub>K1</sub> $\uparrow$	?	37
<i>GJA5</i>	1q21.1	AF4	Connexin	Cell coupling	?	38,39
<i>KCNE1</i>	21q22.1	AF5	minK $\beta$	<i>I</i> <sub>Ks</sub> $\downarrow$	?	40,41
<i>KCNA5</i>	12p13	AF6	K <sub>v</sub> 1.5 $\alpha$	<i>I</i> <sub>Kur</sub> $\downarrow$	?	42
<i>ANK2</i>	4q25	AF7, Bradycardia, LQTS4	Ankyrin-B	<i>I</i> <sub>Na,K</sub> $\downarrow$ <i>I</i> <sub>NCX</sub> $\downarrow$	?	43-45
<i>KCNH2</i>	7q35	AF8, SQT52	K <sub>v</sub> 11.1 $\alpha$	<i>I</i> <sub>Kr</sub> $\uparrow$ HERG	?	46
<i>ABCC9</i>	12p12.1	AF9	SUR2A $\beta$	Ca <sup>2+</sup>	?	47
<i>SCN5A</i>	3p21	AF10	Na <sub>v</sub> 1.5 $\alpha$	<i>I</i> <sub>Na</sub> $\uparrow$ <sup><math>\Omega</math></sup>	?	
<i>HCN4</i>	15q24	SSS, AF, Bradycardia	HCN4 $\alpha$	<i>I</i> <sub>f</sub> $\downarrow$	?	48-50
<i>SCN5A</i>	3p21	SSS, congenital	Na <sub>v</sub> 1.5 $\alpha$	<i>I</i> <sub>Na</sub> $\downarrow$	?	5
<i>SCN5A</i>	3p21	SSS, atrial standstill	Na <sub>v</sub> 1.5 $\alpha$	<i>I</i> <sub>Na</sub> $\downarrow$	?	33
<i>GJA5</i>	1q21.1	(coinheritance)	Connexin	Coupling $\downarrow$		
<i>RyR2</i>	1q42	AT, CPVT1	RyR2 $\alpha$	SR Ca <sup>2+</sup> leak	?	51,52

Genes are highlighted by functional association and consistently throughout tables 1–5. AT indicates atrial tachycardia;  $\uparrow$ , gain-of-function;  $\downarrow$ , loss-of-function; and ?, unknown.

<sup>†</sup>Relative syndromic occurrence has not been determined.

<sup>$\Omega$</sup> Not determined for novel *SCN5A* variants.

observations implicate *SCN5A* in the pathogenesis of both electric and myopathic heart disease and support a recent American Heart Association Scientific Statement that classifies ion channelopathies as a primary cardiomyopathy.<sup>56</sup>

#### Unexplained Aspects: Variable Expressivity and Penetrance

Although mutations in Na<sup>+</sup> channels may lead to DCM and arrhythmias, many unanswered questions remain. For instance, is *SCN5A* also a disease gene for isolated DCM? Earlier studies in European BrS1 patients have excluded mutation carriers with cardiomyopathy. Histology analysis of cardiac tissue from human mutation carriers or heterozygous *SCN5A*<sup>+/-</sup> knockout mice has demonstrated age-dependent fibrosis in conjunction with CoD.<sup>54,57</sup> What are the mechanisms for development of DCM and myocardial fibrosis, and do other proteins that interact with the Na<sup>+</sup> channel complex modulate phenotypic expression? Factors that underlie the chamber-specific manifestation of Na<sup>+</sup> channel mutations may include interacting proteins. For instance, the dystrophin complex has been implicated in Na<sup>+</sup> channel interaction and plays a key role in preserving integrity of the myocellular membrane.<sup>58</sup> Moreover, common polymorphisms like H558R can (1) modify the phenotype of an existing *SCN5A* mutation and (2) produce a decrease in depolarization reserve through the differential function seen in the 2 ubiquitous alternatively spliced transcripts present in all humans. Thus, although not sufficient to cause BrS1 by itself, a quantitatively smaller

loss-of-function defect associated with positive H558R status has been shown to confer susceptibility for AF.<sup>59–61</sup> Future studies will need to characterize the relationship between the *SCN5A* genotype, polymorphisms, and the cardiac phenotype.

#### Biophysical Mechanisms of *SCN5A* Arrhythmias

Mechanisms contributing to arrhythmias include loss of function by synthesis of nonfunctional Na<sup>+</sup> channels and mutations in functionally expressed Na<sup>+</sup> channels that either increase or decrease Na<sup>+</sup> current. One existing challenge is to understand the structure-function relationship of Na<sup>+</sup> channels under physiological conditions and in appropriate disease models. An important property of Na<sup>+</sup> channels is intrinsic modal gating, defined by the probability of a single channel residing in and switching between multiple and distinct modes. Cardiac Na<sup>+</sup> channels regularly exhibit 2 modes of gating, distinguished by short and long channel open times. Whereas fast modes account for 99% of Na<sup>+</sup> channel gating, heart rate–dependent slow gating components are important in pathological states.<sup>62</sup> Models predict that maintained depolarization results in the passage of the channel into a series of inactivated states. In 1 in every 500 to 1000 depolarizations, inactivation fails, and channels open and shut by deactivation for several seconds. These opening bursts can contribute substantial inward current during the action potential plateau. Na<sup>+</sup> channel mutations may result in incomplete inactivation during maintained depolarization,<sup>28,63</sup>

Table 3. Long QT and Related Arrhythmia Syndromes

Long QT Syndrome (LQTS)* including Sudden Infant Death Syndrome (SIDS) <sup>£</sup>						
Gene	Locus	Syndrome	Protein & subunit	Function & abnormality	Occurs In <sup>¶</sup>	Ref.
<i>KCNQ1</i>	11p15.5	LQTS1, SIDS <sup>£</sup>	Kv7.1 $\alpha$	$I_{Ks}$ $\downarrow$ KvLQT1	30-35%	74,77,165
<i>KCNH2</i>	7q35	LQTS2, SIDS <sup>£</sup>	Kv11.1 $\alpha$	$I_{Kr}$ $\downarrow$ HERG	25-30%	75
<i>SCN5A</i>	3p21	LQTS3, SIDS <sup>£</sup>	Nav1.5 $\alpha$	$I_{Na}$ $\uparrow$	5-10%	1,12,28,150
<i>ANK2</i>	4q25	LQTS4, ABS <sup>\$</sup>	Ankyrin-B	$I_{Na,K}$ $\downarrow$ $I_{NCX}$ $\downarrow$	1-2%	43-45
<i>KCNE1</i>	21q22.1	LQTS5	minK $\beta$	$I_{Ks}$ $\downarrow$	1%	76,78
<i>KCNE2</i>	21q22.1	LQTS6, SIDS <sup>£</sup>	MiRP1 $\beta$	$I_{Kr}$ $\downarrow$	rare	79
<i>KCNJ2</i>	17q23	LQTS7, ATS <sup>#</sup>	Kir2.1 $\alpha$	$I_{K1}$ $\downarrow$	rare	80,81
<i>CACNA1C</i>	12p13.3	LQTS8, TS <sup>&amp;</sup>	Ca $v$ 1.2 $\alpha_{1c}$	$I_{Ca,L}$ $\uparrow$	rare	82,83
<i>CAV3</i>	3p25	LQTS9, SIDS <sup>£</sup>	Caveolin-3	$I_{Na}$ $\uparrow$	rare	84,85
<i>SCN4B</i>	11q23	LQTS10	Nav1.5 $\beta$ 4	$I_{Na}$ $\uparrow$	rare	86
<i>AKAP9</i>	7q21	LQTS11 <sup>Ω</sup>	Yotiao <sup>Ω</sup>	$I_{Ks}$ $\downarrow$ KvLQT1	rare	159a
<i>KCNQ1</i>	11p15.5	JLNS1 <sup>+</sup>	Kv7.1 $\alpha$	$I_{Ks}$ $\downarrow$ KvLQT1	rare	87,88
<i>KCNE1</i>	21q22.1	JLNS2 <sup>++</sup>	minK $\beta$	$I_{Ks}$ $\downarrow$	rare	78

Genes contributing to the same membrane currents and/or distinct phenotypes are marked similarly for ease of comparison within and between Tables 1 through 5.  $\uparrow$  indicates gain of function;  $\downarrow$ , loss of function.

\*LQTS indicates Romano-Ward (RW) syndrome resulting from autosomal-dominant heterozygous mutations, nomenclature is considered historical because of low average QTc penetrance of  $\approx$ 60%;<sup>148</sup> mechanism-based classification by protein dysfunction is preferable.

<sup>£</sup>LQTS- and CPVT1-causing mutations probably account for  $\approx$ 10% to  $\approx$ 15% of SIDS.<sup>10, 184, 185</sup>

<sup>\$</sup>Ankyrin-B syndrome (ABS) including sinus bradycardia, paroxysmal AF, VF, polyphasic T waves; see also Tables 2 and 5.

<sup>#</sup>Andersen Tawil syndrome (ATS):  $\approx$ 50% to  $\approx$ 60% are *KCNJ2* mutation carriers including periodic muscle paralysis and developmental abnormalities.

<sup>&</sup>See also Table 5.

<sup>Ω</sup>The A kinase anchoring protein (AKAP9) yotiao binds to a leucine zipper motif in the *KCNQ1* C-terminus and to protein kinase A (PKA) and protein phosphatase-1, which mediate cAMP-dependent channel modulation by the sympathetic nervous system.<sup>159a</sup>

<sup>+</sup>Jervelle and Lange-Nielsen syndrome type 1 (JLNS1) with autosomal-recessive inheritance resulting in homozygous loss-of-function mutations, congenital deafness, QTc prolongation, and ventricular tachyarrhythmias.

<sup>++</sup>Jervelle and Lange-Nielsen syndrome type 2 (JLNS2) with compound heterozygous mutations; asymmetric T waves with rapid terminal configuration.

<sup>¶</sup>Relative syndromic occurrence for a given genetic syndrome (in %).

a decrease in the level of channel expression, or acceleration of inactivation.<sup>63</sup> The resulting clinical phenotypes include LQTS3, BrS1, and heart block. LQTS3-associated Na<sup>+</sup> channel mutations enhance slow gating components and increase late persistent inward current as the basis for early afterdepolarizations as triggers of ventricular tachycardia (VT)/VF, especially at slow heart rates. A delK1500 mutation in the Na<sup>+</sup> channel III-IV interdomain linker identified in a family with LQTS3, BrS1, and CoD resulted in reduction of inactivation and an increased late current component.<sup>21</sup> Deletion of amino acid residues 1505 to 1507 ( $\Delta$ KPQ) in the cardiac Na<sup>+</sup> channel cause autosomal dominant LQTS3 and excessive prolongation of the action potential at low heart rates that predispose to development of fatal arrhythmias, typically at rest or during sleep. Mice heterozygous for a knockin  $\Delta$ KPQ deletion resemble the LQTS3 phenotype and show an increased late Na<sup>+</sup> current during the plateau phase of the action potential.<sup>64</sup> SSS was identified as the first recessive disorder of *SCN5A*, and biophysical characterization of the Na<sup>+</sup> channel mutants demonstrated loss of function or significant impairments in channel gating (inactivation) that predict reduced myocardial excitability.<sup>5</sup>

### Complex Biophysical Phenotypes

Examples of LQTS3 combined with either BrS1<sup>19</sup> or congenital heart block<sup>65,66</sup> and cases of BrS1 with impaired conduction have been documented.<sup>67</sup> In 1 unique family, all 3 clinical phenotypes occur together.<sup>21</sup> The increase in the late Na<sup>+</sup> current component shifts the current-voltage relation during repolarization such that reactivation of Ca $v$ 1.2 and early afterdepolarizations occur. The subgroup of patients with BrS1 Na<sup>+</sup> channel mutations has a conduction disturbance: Slow conduction delays endocardial-to-epicardial activation, resulting in paradoxical endocardial-to-epicardial repolarization (normal repolarization occurs in the epicardial-to-endocardial direction) and ST-segment and T-wave changes. This subgroup of patients also has HV-interval prolongation during electrophysiological study, confirming the presence of conduction slowing. In 2007, we do not fully understand the reasons for interfamilial and interindividual clinical variability association with *SCN5A* mutations. For some of the mixed arrhythmia phenotypes, the pattern of Na<sup>+</sup> channel dysfunction suggests plausible mechanisms responsible for multiple manifestations. However, for most of these mixed disorders, we cannot exclude a role for other genetic or

environmental factors in determining the type of clinical disease associated with particular mutations.

### ***Predicting Effects of Specific Na<sup>+</sup> Channel Kinetic Perturbations on Myocardial Dynamics***

Common single-scale approaches often fail to reveal the most sought after information: how disruptions in proteins due to mutations and consequently through complex interactions (behaviors of cells) lead to triggers that result in recurrent disorganized cardiac excitation, an arrhythmia hallmark. Computational approaches permit utilization and integration of experimentally and clinically obtained information gathered at individual system scales to understand arrhythmogenesis. Simulations can be undertaken to relate the integrated electrophysiological behavior of the cell to state-specific single-channel events. Such approaches may allow prediction of the effect of a mutation that alters a single voltage-dependent transition or, even more complex, multiple discrete transitions on the whole-cell behavior due to multiple nonlinear interactions within the cellular environment and cell-to-cell coupling in cardiac tissue.

An absolute requirement for using computational methods to make connections between Na<sup>+</sup> channel defects and disease is the development of sufficiently detailed models that accurately recapitulate all the basic features of Na<sup>+</sup> channel gating. Numerous experimental studies have shown that arrhythmia-linked mutations tend to affect single or specific multiple discrete transitions. Virtual transgenic cardiomyocytes have been used to demonstrate how specific defects disrupt channel-gating kinetics and underlie likely cellular arrhythmogenic mechanisms. Several investigations showed how modal gating of the Na<sup>+</sup> channel contributes to LQTS3.<sup>68,69</sup> Modeling approaches have also been used to investigate dynamics of channel gating under conditions of changing voltage, so-called nonequilibrium gating, to identify the mechanism of rate dependence of slow-gating, noninactivating current and to reveal a possible mechanism of conduction slowing.<sup>70–72</sup> Modeling studies have helped to explain the propensity to drug-induced arrhythmia in blacks who carry a common *SCN5A*-S1103Y polymorphism.<sup>12</sup>

### **Unanswered Questions and Opportunities for Future Research**

In addition to its role in impulse transmission in the specialized conduction system, atria, and ventricles, Na<sup>+</sup> channel function contributes to maintenance of the action potential plateau and excitation-contraction coupling. As a result of the complex physiological roles, Na<sup>+</sup> channelopathies may result in multiple distinct or overlapping phenotypes, including mixed arrhythmic phenotypes, primary CoD, and DCM. *SCN5A* mutations in CoD are characterized by depolarization/repolarization abnormalities and the extent and site of conduction block. The most profound phenotype is atrial standstill, with loss of atrial excitability and prolonged ventricular depolarization. Heterozygous mutation carriers exhibit mild conduction abnormalities and no evidence of sudden death or Brugada-like symptoms. In congenital SSS, compound heterozygous pairing of *SCN5A* mutations and biophysical characterization showed defects consistent with

impaired inactivation and slowed recovery from inactivation but no persistent late currents. The combination of *SCN5A*-D1275N with a Cx40 promoter polymorphism was associated with late-onset SSS (Table 2). The challenge of low-penetrance alleles is to identify silent carriers with loss-of-function *SCN5A* mutations that develop a phenotype later in life and to explain mechanisms that rescue heart rhythm in early childhood. It is important that future studies investigate associated fibrosis mechanisms in patient tissues and develop a rationale for SSS treatment in the elderly.

### ***Factors Accounting for Chamber-Specific Phenotype Expression***

Na<sup>+</sup> channels interact with multiple proteins, are targeted to specific membrane locations, and are regulated by a multitude of mechanisms. Therefore, genetic, functional, and modulatory mechanisms may all contribute to chamber-specific phenotype expression.<sup>43,73</sup> Mutations that affect any of these associated mechanisms may cause alterations in Na<sup>+</sup> channel expression within the atria, ventricles, or conduction system. An important future research question will be to identify factors that underlie chamber-specific manifestation of Na<sup>+</sup> channel mutations and their interaction with modulatory molecules.

An additional challenge is to understand the variable phenotype expression associated with the same or similar *SCN5A* mutations, the identification of Na<sup>+</sup> channel interactions with other molecular and cellular proteins, and genetic modifiers. For instance, the functional consequences of phosphorylation by protein kinases A and C are controversial. The controls of trafficking and channel expression localization are unresolved questions. Few high-resolution data are available to support structure-function correlations. Nuclear magnetic resonance and circular dichroism studies have been performed on small segments of the protein. The high structural resolution afforded by x-ray crystallography has not been applied to the Na<sup>+</sup> channel, and structural data from K<sup>+</sup> channels suggest that the motifs inferred from sequence data are simplistic.

The understanding of channel modulation by age-related factors and by sex hormones is limited, which may explain why some arrhythmia triggers are gender mediated. Therefore, it seems important to develop models of age- and environment-dependent factors to understand arrhythmia syndromes. Although Na<sup>+</sup> channel behavior appears to be consistent throughout species, the common mouse models show important phenotypic differences from human disease. Ideally, animal or computational models need to replicate all electrophysiological abnormalities seen in the human heart.

Understanding how *SCN5A* mutations confer arrhythmia susceptibility and other phenotypes should proceed to appropriate animal models. Such studies will be guided by predictions made with the use of computational modeling approaches and in vitro studies with recombinant Na<sup>+</sup> channels expressed in their native environment. Studies in transgenic models provide additional insights not readily predictable from heterologous expression. The LQTS3-associated Na<sup>+</sup> channel mutations enhance the slow components of gating observed in the normal phenotype. The resulting increase in

Table 4. Short-QT Syndromes

Short QT Syndrome (SQTS)						
Gene	Locus	Syndrome	Protein & subunit	Functional abnormality	Occurs In <sup>†</sup>	Ref.
<i>KCNH2</i>	7q35	SQTS1	Kv11.1 $\alpha$	$I_{Kr}$ $\uparrow$ HERG	?	46,91,92
<i>KCNQ1</i>	11p15.5	SQTS2	Kv7.1 $\alpha$	$I_{Ks}$ $\uparrow$ KvLQT1	?	35,93
<i>KCNJ2</i>	17q23	SQTS3	Kir2.1 $\alpha$	$I_{K1}$ $\uparrow$	?	94

$\uparrow$  indicates gain of function; ?, unknown.

<sup>†</sup>Relative syndromic occurrence for a given syndrome has not been determined.

the late current shifts the current-voltage trajectory in the inward direction and causes membrane oscillations that are the basis for early afterdepolarizations. The challenge will reside in developing novel therapeutic approaches that are tailored to precise genotypes or are capable of correcting specific Na<sup>+</sup> channel dysfunctions.

### Cardiac K<sup>+</sup> Channelopathies

#### Normal Cardiac K<sup>+</sup> Channel Function

Multiple K<sup>+</sup> channels, with distinct physiological roles, have been identified in the heart. Myocardial K<sup>+</sup> channels function, for example, to control resting membrane potentials, action potential plateau potentials, and both the initiation and the duration of membrane repolarization. Available evidence suggests that cardiac K<sup>+</sup> channels reflect the homomeric or heteromeric assembly of 4 voltage-gated (K<sub>v</sub>) or inward rectifier (Kir) pore-forming  $\alpha$ -subunits, together with accessory subunits and regulatory proteins. Heterologous expression of *KCNQ1* (KvLQT1) alone yields rapidly activating K<sub>v</sub> currents, whereas coexpression with *KCNE1* (minK) produces slowly activating currents that resemble the slow component of cardiac delayed rectification,  $I_{Ks}$ , suggesting that cardiac  $I_{Ks}$  channels reflect the coassembly of the KCNQ1 and minK proteins. Although expression of *KCNH2* (HERG) reveals currents that are similar to the rapid cardiac delayed rectifier,  $I_{Kr}$ , both *KCNE1* (minK) and *KCNE2* (MiRP1) have been suggested to function in the generation of cardiac  $I_{Kr}$  channels (Table 3).

#### Dysfunction in K<sup>+</sup> Channel–Based Cellular Pathways and LQTS

LQTS is associated with increased risk of syncope and sudden death from ventricular tachyarrhythmias, including torsades des pointes and VF. Several LQTS genes have been identified, and most encode the subunits of repolarizing voltage-gated K<sub>v</sub> channels. LQTS-linked mutations in the K<sup>+</sup> channel pore-forming  $\alpha$ -subunit genes *KCNQ1* (LQTS1) and *KCNH2* (LQTS2) account for the majority of inherited cases (multigenetic arrhythmia syndromes are numbered sequentially to identify and discriminate specific genotype–phenotype relationships; refer to Table 3). Mutations in K<sup>+</sup> channel accessory subunits *KCNE1* (LQTS5) and *KCNE2* (LQTS6) occur in <1%. Mutations in *KCNJ2*, encoding the inward rectifier K<sup>+</sup> channel  $\alpha$ -subunit Kir2.1, a component of cardiac  $I_{K1}$ , have been linked to LQTS7 or Andersen Tawil syndrome.

In  $\approx$ 25% of LQTS families, the genetic link remains to be established.

Heterozygous mutations in *KCNQ1* and *KCNH2* were first linked to the autosomal dominant inherited LQTS (Romano-Ward). Numerous *KCNQ1* (LQTS1) and *KCNH2* (LQTS2) mutations have been identified throughout the (KvLQT1 and HERG) protein sequences, and expression studies have suggested that all are loss-of-function mutations due to haploinsufficiency or to the generation of dominant negative subunits, which result in reduction in functional cell surface expression of  $I_{Ks}$  or  $I_{Kr}$  channels. In addition, many *KCNH2* mutations affect the processing of the channel proteins and the trafficking of assembled K<sup>+</sup> channels to the cell surface. LQTS7 mutations in *KCNJ2* are also loss-of-function mutations that result in reduced  $I_{K1}$  density. Computer simulations confirm that reduced  $I_{Ks}$ ,  $I_{Kr}$ , or  $I_{K1}$  densities will result in prolongation of ventricular action potentials. Given the intrinsic heterogeneities in channel densities and action potential waveforms in the ventricular myocardium, *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, and *KCNJ2* mutations result in heterogeneous dispersion of repolarization. Patients with autosomal recessive (Jervelle and Lange-Nielsen) LQTS are homozygous for loss-of-function mutations in either *KCNQ1* or *KCNE1* (Table 3). These patients have a more severe cardiac phenotype and complete loss of  $I_{Ks}$  in the hair cells and endolymph of the inner ear, resulting in congenital deafness.

#### Dysfunction in K<sup>+</sup> Channel–Based Cellular Pathways and Short-QT Syndrome

Short-QT syndrome (SQTS) is a more recently described syndrome characterized by ECG shortening of the QT interval and episodes of syncope, paroxysmal AF, and life-threatening cardiac arrhythmias. SQTS usually affects young and otherwise healthy individuals with no structural heart disease; it may be expressed as sporadic cases as well as in families. SQTS was originally identified in a family with short QT and AF, including a member who suffered SCA.<sup>89</sup> Families with a high incidence of SCA<sup>90</sup> and missense mutations in *KCNH2* were linked to SQTS1 (Table 4).<sup>46,91–94</sup>

The biophysical analysis showed a gain-of-function defect in *KCNH2* (HERG). Shortly thereafter, a mutation in *KCNQ1* responsible for SQTS2 was identified in a 70-year-old individual who suffered VF and had a QTc interval of 302 ms after resuscitation.<sup>93</sup> Similar to SQTS1, biophysical analysis showed a gain-of-function defect in *KCNQ1* (KvLQT1). A



second gain-of-function mutation in *KCNQ1* has been associated with SQT2 and AF in a newborn patient.<sup>35</sup> An additional form, SQT3, has been linked to mutations in *KCNJ2* underlying  $I_{K1}$ .<sup>94</sup> When expressed in CHO cells, the mutant Kir2.1 subunits generated  $I_{K1}$ -like channels that did not rectify as much as the wild-type channels, resulting in larger outward  $K^+$  currents in the physiological range of membrane potentials. Gain-of-function defects in 3 different  $K^+$  channel genes have therefore now been identified in SQTs; there will likely be more. Clinical and biophysical research efforts have enabled the investigation of possible therapeutic interventions, such as quinidine, which was found to improve (prolong) the QT interval in mutation carriers and which is presently recommended in patients who cannot receive a defibrillator or who have received multiple implantable cardioverter-defibrillator (ICD) shocks.<sup>90,95</sup>

#### **Metabolism-Sensing ATP-Sensitive $K^+$ Channel Variants**

Heart failure (HF) is a disease with a high prevalence of SCA and arrhythmias. The significance of the sarcolemmal ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channel for cardiac protection from HF is indicated by mutations that produce abnormal channel phenotypes with compromised metabolic signaling in patients with inherited cardiomyopathy.<sup>96</sup> A causal relationship between  $K_{ATP}$  channel dysfunction and the development of HF has been shown in models of experimental hypertension in which knockout of the *KCNJ11* gene, encoding the Kir6.2 pore-forming subunit of the  $K_{ATP}$  channel, predisposed to HF and sudden death.<sup>97</sup> In the heart,  $K_{ATP}$  channel activity has been linked to homeostatic shortening of the action potential under stress and a deficit in repolarization reserve, as demonstrated in Kir6.2-knockout hearts with an increased risk for triggered activity and ventricular arrhythmia.<sup>98</sup> Defects in hypertension-induced metabolic distress signals via the  $K_{ATP}$  channel may cause pathological  $Ca^{2+}$  overload, aggravated cardiac remodeling, and arrhythmias that could potentially be targeted by novel pharmacological approaches.

The significance of  $K_{ATP}$  channels in human disease is further underscored by an *ABCC9* missense mutation (T1547I) in the SUR2A nucleotide sensing channel subunit conferring risk for adrenergic-mediated AF originating from the vein of Marshall, a recognized source for adrenergic AF (Table 2).<sup>47</sup> Targeted knockout of the  $K_{ATP}$  channel verified the pathogenic link between channel dysfunction and predisposition to adrenergic AF. *ABCC9* mutation-induced AF susceptibility was cured by radiofrequency ablation disrupting the substrate for arrhythmia conferred by  $K_{ATP}$  channelopathy.<sup>47</sup>

#### **Current Challenges and Important Issues for Future Studies**

Since identification of the LQTS-causing genes *KCNQ1* and *KCNH2*, considerable progress has been made in the characterization of specific gene mutations in affected families, the discovery of additional LQTS genes, and linkage of novel phenotypes such as SIDS (Table 3). Refined clinical phenotyping of LQTS families has led to improved, disease-specific, therapeutic paradigms. Nevertheless, important problems remain such as the identification of the genetic and environmental factors that affect the well-documented indi-

vidual variations in disease susceptibility, induction (onset), presentation, and severity. Studies focused on the identification and characterization of gene modifiers and proteins will provide new insights into cellular and systemic disease mechanisms while facilitating the development of improved paradigms for risk assessment and therapeutic intervention. Although AF is the most common arrhythmia, genetic defects responsible for AF and their molecular mechanisms remain incompletely understood (Table 2).

Phenotypic characterization of SQTs is in its early stages (Table 4), although it is now well accepted that a QT interval  $<320$  ms indicates increased SCA risk. It also appears that a gradient of phenotypic severity likely exists according to QT-interval length similar to LQTS.<sup>99</sup> From a therapeutic point of view, the first line of therapy in individuals recovered from SCA or with a history of syncope is the implantation of an ICD.<sup>90</sup> Although some of the class III antiarrhythmic agents appear suitable for SQTs,<sup>91,95,100</sup> no specific pharmacological treatment for SQT2 and SQT3 is yet known. Similar to LQTS, SQTs is a polygenetic disease highlighting the need to characterize each individual form to specifically direct therapeutic approaches.

Apart from the identification of novel LQTS genes, mutations, modifier genes, and proteins, delineation of protein trafficking mechanisms and regulatory pathways has emerged as an important area. Proteomics of ion channel macromolecular complexes is increasingly recognized as important. Genetically engineered large-animal models of LQTS will be necessary to help to translate basic sciences into the human context. An improved transitioning process into clinical medicine will be necessary to translate complex genotype-phenotype relations from the basic sciences. Renaming and realignment of disease entities based on improved genotype-phenotype characterization according to channel and modifier dysfunction are important to facilitate comprehensive characterization beyond preliminary or early phenotype description. Current recommendations suggest that "inclusive" phenotype classification systems such as "cardiomyopathy" are preferable to more exclusive ones such as "electric heart disease," which tend to underestimate phenotype expression.<sup>56</sup> Finally, continuous improvement of risk assessment and stratification in mutation carriers together with development of targeted, gene, or mutation-specific therapies is necessary to provide more effective and specific therapeutic options.

#### **Arrhythmias Linked to $Ca^{2+}$ Transport Mechanisms**

##### **Dynamic Roles of $Ca^{2+}$ in Arrhythmogenesis**

Changes in intracellular  $Ca^{2+}$  cycling within macroscopic cardiac regions contribute to an unstable conduction substrate and the development of ectopic electric activity.<sup>101,102</sup> Underlying this change in electric activity are characteristic changes in intracellular  $Ca^{2+}$  signals and  $Ca^{2+}$  storage organelle (sarcoplasmic reticulum [SR]) content occurring in subcellular compartments in a tissue-specific manner.<sup>103–105</sup> The primary feature of  $Ca^{2+}$ -dependent arrhythmias is an unstable state of SR  $Ca^{2+}$  storage, which may be rate dependent and sensitive to hormonal and pharmacological modulation. Thus, cellular and molecular

Table 5. Ca<sup>2+</sup>-Dependent Arrhythmia Syndromes

Timothy Syndrome (TS) and Autism Spectrum Disorder (ASD)						
Gene	Locus	Syndrome	Protein & subunit	Functional abnormality	Occurs In <sup>¶</sup>	Ref.
<b>CACNA1CA</b>	12p13.3	TS1, ASD <sup>*</sup>	Ca <sub>v</sub> 1.2 α <sub>1C</sub>	I <sub>Ca,L</sub> ↑	?	82
<b>CACNA1C</b>	12p13.3	TS1, ASD <sup>*</sup>	Ca <sub>v</sub> 1.2 α <sub>1C</sub>	I <sub>Ca,L</sub> ↑	?	83
Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)						
Gene	Locus	Syndrome	Protein & subunit	Functional abnormality	Occurs In <sup>¶</sup>	Ref.
<b>RyR2</b>	1q42	CPVT1, SIDS <sup>£</sup>	RyR2 α	SR Ca <sup>2+</sup> leak ↑	50-60%	115-117
<b>RyR2</b>	1q42	CPVT1, LQTS <sup>#</sup>	RyR2 α	SR Ca <sup>2+</sup> leak ↑	?	51,118
<b>RyR2</b>	1q42	CPVT1, ARVC2	RyR2 α	SR Ca <sup>2+</sup> leak ↑	?	119
<b>CASQ2</b>	1p13.3	CPVT2	Calsequestrin	SR Ca <sup>2+</sup> leak ↑	< 5%	120,121
<b>KCNJ2</b>	17q23	CPVT ? <sup>Ω</sup>	Kir2.1 α	I <sub>K1</sub> ↓	?	80,123,156
<b>ANK2</b>	4q25	CPVT ? <sup>†</sup>	Ankyrin-B	SR Ca <sup>2+</sup> leak ↑	?	43,44,45,122
Dilated Cardiomyopathy (DCM)						
Gene	Locus	Syndrome	Protein & subunit	Functional abnormality	Occurs In <sup>¶</sup>	Ref.
<b>ABCC9</b>	12p12.1	DCM, VT	SUR2A β	Ca <sup>2+</sup> overload ↑	?	47,96
<b>PLN</b>	6q22.1	DCM, HF, LVH	PLN β	Ca <sup>2+</sup> overload ↑	?	124,132

AT indicates atrial tachycardia; LVH, left ventricular hypertrophy; black boxes, genes directly involved in Ca<sup>2+</sup> transport function; ↑, gain of function; ↓, loss of function; ARVC2, Arrhythmogenic Right Ventricular Cardiomyopathy type 2 (atypical form of ARVC); and ATS, Andersen Tawil syndrome.  
<sup>¶</sup>Estimated occurrence within a given syndrome.  
<sup>\*</sup>Timothy syndrome (TS): multisystem disorder including congenital heart disease, AF, VT, autism, syndactyly in 100%, musculoskeletal disease, immune dysfunction; *CACNA1C* has been associated with more severe arrhythmia risk, absence of syndactyly, and nemaline rod myopathy.  
<sup>£</sup>LQTS- and CPVT1-causing mutations may account for ~10% to ~15% of SIDS.  
<sup>#</sup>QTc intervals of select groups of *RyR2* mutation carriers were reported as slightly but significantly longer (for comparison to LTQS, see Tester et al<sup>186</sup>).  
<sup>Ω</sup>Some *KCNJ2* mutation carriers exhibit CPVT-like symptoms including bidirectional VT and/or normal QT intervals.  
<sup>†</sup>Some *ANK2* mutation carriers exhibit CPVT-like symptoms, including stress-induced VT, syncope, sudden cardiac arrest, and/or normal-to-borderline QT intervals.

studies have linked inherited arrhythmias (eg, LQTS4 or CPVT1) to arrhythmogenic mechanisms that are similar to those seen in more common disease forms such as HF.<sup>43,105–109</sup> Changes in the subcellular spatial organization of heart cells may further contribute to abnormal Ca<sup>2+</sup> signaling.<sup>109–111</sup> Additionally, changes in the Ca<sup>2+</sup> sensitivity of excitation-contraction coupling and Ca<sup>2+</sup> cycling proteins can directly contribute to arrhythmogenesis.<sup>107,112,113</sup>

Under resting, diastolic conditions, spontaneous elementary Ca<sup>2+</sup> release events (sparks) occur at a rate of ≈100 s<sup>-1</sup> per cardiomyocyte, each representing the activity of a single functional SR Ca<sup>2+</sup> release complex containing tens to hundreds of cardiac RyR2 channels. Each Ca<sup>2+</sup> spark produces a local SR Ca<sup>2+</sup> depletion within the SR lumen visualized as Ca<sup>2+</sup> “blinks.”<sup>114</sup> SR Ca<sup>2+</sup> overload produces higher spark rates that may form abnormal intracellular Ca<sup>2+</sup> waves that alter the electric properties of cardiomyocytes. In HF, T-tubule remodeling results in signaling changes affecting SR Ca<sup>2+</sup> release structures. The consequence of T-tubule remod-

eling is dyssynchronous SR Ca<sup>2+</sup> release that may disrupt Ca<sub>v</sub>1.2-RyR2 signaling and contribute to arrhythmogenesis.

**Timothy Syndrome and Ca<sub>v</sub>1.2 Mutations**

Timothy syndrome (TS) is a multisystem disorder characterized by congenital heart disease including LQTS8 (Tables 3 and 5) and cardiac arrhythmias including bradycardia, atrioventricular block, torsades de pointes, VT, and VF contributing to early mortality.<sup>82</sup>

Common features include syndactyly, dysmorphic facial features, myopia, immunodeficiency with recurrent infections, intermittent hypoglycemia, and hypothermia. Children with TS show developmental delay, autism, and generalized cognitive impairment. TS results from a de novo, gain-of-function missense mutation in splice exon 8A of *CACNA1C* that encodes the pore-forming α-subunit (Ca<sub>v</sub>1.2) of the cardiac L-type Ca<sup>2+</sup> channel, a key protein in excitation-contraction coupling in the heart that is also expressed in the brain, smooth muscle, immune system, teeth, and testes.<sup>125</sup> Heterologous expression of mutant and wild-type channels

demonstrated that the causative mutation, G406R, results in loss of voltage-dependent channel inactivation likely to induce intracellular  $\text{Ca}^{2+}$  overload in multiple cell types. In an atypical case of TS with severe QT-interval prolongation, skeletal nemaline myopathy, but no syndactyly, a de novo missense mutation was found that was affecting the dominant cardiac splice exon 8. One analogous TS-associated mutation, G406R, and another, G402S, both cause loss of voltage-dependent channel inactivation, leading to maintained inward  $\text{Ca}^{2+}$  currents possibly by affecting a gating hinge mechanism.<sup>83</sup> *CACNA1C* mutations in TS include cardiac and autism spectrum phenotypes, indicating the importance of including the central nervous system in the disease characterization (Table 5).

#### **Catecholaminergic Polymorphic VT Resulting From RYR2 and CASQ2 Mutations**

Catecholaminergic polymorphic VT (CPVT) is a familial cardiomyopathy characterized by stress-induced ventricular arrhythmias that result in syncope and sudden death in children or young adults. Two major genetic variants have been identified: The majority of cases have been linked to missense mutations in the *RYR2* gene encoding the ryanodine receptor, the principal intracellular  $\text{Ca}^{2+}$  release channel of the heart (CPVT1)<sup>115,116</sup>; additionally, unrelated cases have been linked to a recessive form caused by homozygous mutations in the *CASQ2* gene encoding calsequestrin2 (CPVT2), a low-affinity, high-capacity  $\text{Ca}^{2+}$  buffering protein of the SR  $\text{Ca}^{2+}$  storage organelle.<sup>120,126</sup> Recently, mutations in *RYR2* associated with a CPVT1-like phenotype were discovered as an uncommon cause of SIDS.<sup>117</sup>

Different *RYR2* missense mutations resulted in a gain-of-function defect characterized by high channel open probability during sympathetic stimulation consistent with a “leaky” channel phenotype.<sup>107,112</sup> The inability of CPVT-mutant RyR2 to achieve stable channel closure is amplified by protein kinase A phosphorylation of RyR2-Serine-2808 (S2808) and was associated with abnormally decreased calstabin2 (FKBP12.6) binding compared with wild-type RyR2 channels.<sup>107,112</sup> Accordingly, RyR2-S2808A knockin prevented VT and sudden death, indicating that protein kinase A phosphorylation of Ser2808 is a key mediator of RyR2 dysfunction during catecholaminergic VT.<sup>127</sup> The mechanism of stress-induced arrhythmias in the calstabin2-deficient background appears to be triggered activity, as evidenced by intracellular  $\text{Ca}^{2+}$  leak causing a transient inward current ( $I_{\text{leak}}$ ) and delayed afterdepolarizations.<sup>107,108</sup> Intracellular  $\text{Ca}^{2+}$  leak and delayed afterdepolarizations have been confirmed in HL-1 cells expressing mutant RyR2 and in a CPVT1 knockin mouse model.<sup>128,129</sup> Neutralizing a charge in a mutant calstabin2-D37S increases binding to and rescues mutant RyR2 channel function and prevents SR  $\text{Ca}^{2+}$  leak in vitro and in vivo.<sup>107,130</sup> JTV519, a 1,4-benzothiazepine derivative, normalized mutant RyR2 channel function.<sup>112</sup> Because all CPVT mutant RyR2 channels showed a reduced calstabin2 binding affinity, the arrhythmogenic consequences of calstabin2 deficiency were investigated in a knockout mouse. Stress testing by treadmill exercise followed by epinephrine injection resulted in polymorphic sustained VT in

*calstabin2*<sup>-/-</sup>-deficient mice, which could not be prevented by JTV519 treatment.<sup>107,131</sup> However, JTV519 prevented sustained VT in haploinsufficient *calstabin2*<sup>+/-</sup> mice, consistent with in vivo rebinding of calstabin2 to RyR2 and normalization of single-channel function.<sup>131</sup>

#### **Phospholamban Mutations**

Heart muscle relaxation occurs from SR  $\text{Ca}^{2+}$  reuptake mediated by SERCA2a  $\text{Ca}^{2+}$  pumps. Phospholamban (PLN), a 52-amino acid transmembrane SR protein, inhibits SERCA2a in its dephosphorylated state. SERCA2a activity is decreased in human HF, potentially contributing to intracellular  $\text{Ca}^{2+}$  overload and arrhythmias. A PLN-R9C mutation in the cytosolic PLN domain occurs in inherited, rapidly progressive DCM (Table 5), and transgenic mice expressing the mutant PLN<sup>R9C</sup> protein resemble the human phenotype.<sup>132</sup> PLN<sup>R9C</sup> traps protein kinase A, preventing PLN phosphorylation and resulting in chronic SERCA2a inhibition. A homozygous PLN mutation (L39stop) results in PLN deficiency and progressive DCM, indicating that lack of PLN expression is detrimental to humans.<sup>124</sup> The PLN-Arg14del mutation in the human *PLN* gene results in superinhibition of SERCA2a activity, indicating that a gain of inhibitory PLN function predisposes to DCM and potentially early death from arrhythmias.<sup>133</sup> Whether PLN mutations and SERCA2a dysregulation directly cause cardiac arrhythmias needs to be addressed by future research.

#### **Arrhythmias Linked to Accessory, Regulatory, and Scaffolding Proteins**

##### *Dysfunction in Ankyrin-B*

How do defects in proteins not comprising membrane ion transporters result in arrhythmias? Ion channels and transporters in higher vertebrates function within specialized cellular microdomains (compartments) and depend on local regulation by protein complexes. Ankyrins are membrane-adaptor proteins that link structurally unrelated ion channels, transporters, and cell adhesion molecules with the spectrin- and actin-based cytoskeleton<sup>134</sup> in many cell types.<sup>135</sup> Two unique ankyrin gene products, ankyrin-B (*ANK2*) and ankyrin-G (*ANK3*), target ion channels and transporters to distinct cardiomyocyte membranes. Human *ANK2* variants resulting in ankyrin-B loss of function cause the “ankyrin-B syndrome” (including LQTS4) originally identified in E1425G carriers (prolonged QTc not common in all variant carriers), including bradycardia, AF, CoD, and increased risk for catecholaminergic sudden death (Tables 3 and 5).<sup>43,122</sup> Heterozygous mice with reduced ankyrin-B expression resemble the LQTS4 phenotype and implicate aberrant intracellular  $\text{Ca}^{2+}$  homeostasis in the generation of stress-induced arrhythmias.<sup>43</sup> Loss-of-function ankyrin-B mutations result in loss of cellular targeting and expression of the ankyrin-binding proteins  $\text{Na}^+/\text{K}^+$  ATPase,  $\text{IP}_3$  receptor, and  $\text{Na}^+/\text{Ca}^{2+}$  exchanger to their appropriate membrane microdomains.<sup>43,136</sup> With the use of a combined neonatal cardiomyocyte overexpression and rescue strategy, distinct functional classes of *ANK2* loss-of-function variants have been identified that correspond with the severity of arrhythmia expression in the respective mutation carriers.<sup>45</sup> Importantly, variants with less

severe in vitro phenotypes (eg, L1622I) appear to be present in a small percentage of the population.<sup>44,45</sup> The principal voltage-gated Na<sup>+</sup> channel in the heart, Na<sub>v</sub>1.5, is directly associated with ankyrin-G (encoded by *ANK3*) required for correct Na<sub>v</sub>1.5 targeting and expression.<sup>137</sup> Human variants in *SCN5A* that disrupt interaction between ankyrin-G and Na<sub>v</sub>1.5 result in decreased Na<sub>v</sub>1.5 expression at the cardiomyocyte intercalated disc and cause BrS1.<sup>137</sup> Dysfunction in ankyrin-based pathways in human arrhythmias clearly demonstrates the importance of precise ion channel and transporter targeting and localization pathways.

#### *Caveolin-3 Mutations Cause LQTS9*

Caveolin-3 (Cav-3) is a muscle-specific isoform and principal protein component of caveolae, 50 to 100 nm invaginations that represent subcompartments of the plasma membrane. Cav-3 contains a 20–amino acid scaffolding domain (residues 54 to 73) that is critical for interaction with associated signaling molecules. Cav-3 copurifies with dystrophin and dysferlin, and mutations in these 3 proteins have been linked to inherited muscular dystrophies. In Japanese brothers with hypertrophic cardiomyopathy whose father with hypertrophic cardiomyopathy had died suddenly, a T63S substitution in *CAV3* was identified.<sup>138</sup> Four novel mutations in *CAV3* were found recently that result in increased late Na<sup>+</sup> current and LQTS9 arrhythmias<sup>84</sup> similar to LQTS3-associated *SCN5A* mutations (Table 3). Cav-3 and Na<sub>v</sub>1.5 colocalize to caveolae in human myocardium. More recently, missense mutations in Cav-3 were discovered in a small subset of black infants dying from apparent SIDS. Similar to the patients with LQTS, the SIDS-associated Cav-3 mutations accentuated the late Na<sup>+</sup> current attributed to the otherwise intact Na<sup>+</sup> channel  $\alpha$ -subunit.<sup>85</sup> Future studies are needed to address the molecular mechanisms that result in the gain-of-function defects associated with these mutations.

#### *SCN4B Mutations Cause LQTS10*

Akin to the K<sup>+</sup> channel  $\beta$ -subunits responsible for LQTS5 and LQTS6, the Na<sup>+</sup> channel  $\beta$ 4 subunit encoded by *SCN4B* has been established as a novel, albeit rare, LQTS-susceptibility gene (LQTS10; Table 3). Identified in a multigenerational Mexican-mestizo family, the missense mutation conferred a secondary gain of function on the Na<sup>+</sup> channel such that the accentuated late Na<sup>+</sup> current mimicked that of classic LQTS3-associated mutations in *SCN5A*.<sup>86</sup>

#### *Intracellular Targeting and Signaling Defects Indicate Common Mechanisms*

Overlap between genetic defects responsible for cardiomyopathies and arrhythmias is important in the understanding of complex disease phenotypes.<sup>56</sup> Interactions between ion channels and structural proteins of the cardiomyocyte cytoskeleton and sarcomere, as well as nuclear proteins, need to be evaluated. For instance, DCM may occur because of disruption of the link between the sarcolemma and sarcomere, and young male dystrophin mutation carriers and their mothers develop both cardiomyopathy and arrhythmias. Interactions between cytoskeletal proteins, such as dystrophin, with Na<sup>+</sup> (and other ion) channels may provide an explanation for how structural defects translate into arrhythmic mechanisms.

Interactions with the dystrophin-sarcoglycan complex indicate that a monogenetic disruption may cause a multitude of distinct defects.<sup>139</sup> In arrhythmogenic right ventricular cardiomyopathy, desmosome function is disturbed, leading to disruption of intercalated discs and fibrofatty replacement of the myocardium. Arrhythmias may result after disruption of membrane-bound channels from the desmosome apparatus. At least 6 of the LQTS-susceptibility genes (*ANK2*, *KCNE1*, *KCNE2*, *CAV3*, *SCN4B*, and *AKAP9*) do not encode pore-forming ion channel  $\alpha$ -subunits, and these findings strongly support the notion that defective protein-protein interactions or targeting mechanisms contribute to arrhythmogenesis (Table 3). Specific animal models are required along with cellular studies to elucidate mechanisms and dissect protein-protein interactions and their specific regulation.

#### *Summary and Future Directions*

Arrhythmia mechanisms caused by defective targeting and expression of ion transporters (eg, ankyrin mutations) may depend critically on the native cardiac cellular environment, which differs from heterologous expression systems because of proteome- and cell type-specific expression characteristics. Moreover, specific genetic defects in ion transporters may cause structural pathology (eg, fibrosis), implicating an important role of fibroblasts or other cell types that may directly contribute to the arrhythmogenic substrate. Important unresolved questions include the following: How does spatial remodeling influence Ca<sup>2+</sup>-dependent arrhythmogenesis? How does microdomain signaling influence cellwide function? How do ion channels and transporters interact with cytoskeletal, regulatory, and other effector proteins? How do changes in the spatial organization of heart cells influence the ability of the conductive medium to generate and support altered signal propagation? In addition, how does this cellular architecture differ among the various types of electrically excitable cell types, as well as with the nonexcitable cells of the heart?

Arrhythmias are a common cause of death and morbidity in cardiomyopathies associated with HF and abnormalities of the conduction system. Intracellular Ca<sup>2+</sup> leak from protein kinase A hyperphosphorylated RyR2 channels may represent an important defect contributing to arrhythmias and disease progression.<sup>140,141</sup> Current evidence supports stress-induced intracellular Ca<sup>2+</sup> leak as the trigger mechanism of CPVT.<sup>107,108,142</sup> In CPVT1, mortality in *RYR2* mutation carriers at 35 years of age reaches 35% to 50%,<sup>112,115</sup> indicating that stress-induced RyR2 Ca<sup>2+</sup> leak represents a very aggressive arrhythmia mechanism. Molecular determinants of the RyR2 channel closed state instability include RyR2 protein kinase A phosphorylation, calstabin2 depletion, and decreased sensitivity to Mg<sup>2+</sup> inhibition.<sup>107,112</sup> Future studies are needed to characterize the mechanisms of RyR2 channel leak and cytoplasmic regulation at the structural level. Intracellular Ca<sup>2+</sup> leak has been recognized as a pathogenic mechanism that activates a Ca<sup>2+</sup>-dependent transient inward current (*I<sub>ti</sub>*) and delayed afterdepolarizations, leading to triggered activity.<sup>108,143,144</sup> Overlap between intracellular Ca<sup>2+</sup> changes and altered plasma membrane ion currents occurs also in other genetic forms of arrhythmias. Thus, characterization of Ca<sup>2+</sup>-

dependent mechanisms contributing to arrhythmogenic membrane instabilities will aid in conceptualizing arrhythmia triggers versus substrates, as well as in developing rational therapeutic interventions.

### Implications for Diagnosis and Management of Inherited Arrhythmias

More than 400 different mutations have been reported among the 11 LQTS-susceptibility genes, yet only a small amount of patient-related data exists on the relationship of specific ion channel mutations, their coding characteristics, and the influence of the biophysical dysfunction on patients' clinical course.<sup>145</sup>

#### State of Genetic Testing for Cardiac Channelopathies

##### Arrhythmia Susceptibility Genes

LQTS is a potentially lethal, heritable arrhythmia syndrome affecting  $\approx 1$  in 2500 people. To date, 11 LQTS-susceptibility genes have been identified (Table 3); 5 of these genes encode critical ion channel pore-forming  $\alpha$ -subunits, and 3 of them encode  $\beta$ -subunits. The other 3 genes encode the adapter proteins ankyrin-B, caveolin-3, and AKAP9 (yotiao). Mutations in the genes responsible for LQTS1–3 account for  $\approx 75\%$ , and the rest of the known susceptibility genes collectively account for  $<3\%$  of LQTS. The concept that inherited arrhythmia syndromes like LQTS may originate from defects in scaffolding or adaptor proteins points to a new class of candidate genes possibly responsible for the  $\approx 20\%$  to  $25\%$  of unexplained LQTS cases. Tables 1 to 5 summarize genetic arrhythmia syndromes, susceptibility genes, and their relative frequency within a syndrome and provide an overview of concealed versus overlapping or complex phenotypes.

Identification of mutations in the *KCNQ1*-, *KCNH2*-, and *SCN5A*-encoded  $K^+$  and  $Na^+$  channel  $\alpha$ -subunits as the cause of LQTS1–3, respectively (Table 3), in concert with a growing body of evidence implicating ion channels in episodic syndromes of excitable tissues, defined a new class of diseases, channelopathies.<sup>146</sup> Genetic testing has indicated that LQTS is a common risk factor in arrhythmogenesis. Over the past decade, LQTS genetic testing was performed in research laboratories while genotype–phenotype relationships were being determined.<sup>147</sup> Today, genetic testing is commercially available, and clinical diagnostic screening consists of comprehensive open reading frame sequence analysis (60 translated exons) of 5 LQTS-susceptibility genes: *KCNQ1* (LQTS1), *KCNH2* (LQTS2), *SCN5A* (LQTS3), *KCNE1* (LQTS5), and *KCNE2* (LQTS6).<sup>148</sup> Hundreds of mutations have been reported within these 5 genes, with the vast majority yielding LQTS1–3 (Table 3).<sup>149,150</sup> Among patients with definitive clinical evidence of LQTS, the yield of genetic testing is  $\approx 75\%$ : LQTS1 (30% to 35%), LQTS2 (25% to 30%), LQTS3 (5% to 10%), LQTS5 (1%), and LQTS6 (1%).<sup>151,152</sup> The majority of known mutations are missense mutations, and approximately half are unique, novel mutations. Thus, conversion from a sequencing-based genetic test to a chip-based genetic test containing prespecified mutations is not yet possible. Besides these 5 LQTS genes, there are now 6 additional susceptibility genes for LQTS-related syn-

dromes, including the following: *ANK2* (LQTS4,  $<1\%$ ), *KCNJ2* (LQTS7,  $<1\%$  and 50% to 65% of Andersen Tawil syndrome), *CACNA1C* (LQTS8 or TS1  $<1\%$ ), *CAV3* (LQTS9,  $<1\%$ ), *SCN4B* (LQTS10,  $<1\%$ ), and *AKAP9* (LQTS11,  $<1\%$ ). Clinical genetic testing for the rare subtypes of LQTS is not yet available. Still, the pathogenesis for 20% to 25% of LQTS remains unexplained. Possible reasons for this include (1) missed regions and false-negative results within the currently explored gene regions of interest, (2) disease-causing mutations in noncoding sequence (eg, promoters, introns) of the known LQTS-susceptibility genes, and (3) novel or unknown LQTS genes. “Allelic dropout” is a mechanism responsible for some previous false-negative genetic test results,<sup>153</sup> and the new discovery of *CAV3*-LQTS9, *SCN4B*-LQTS10, and *AKAP9*-LQTS11 indicates that all 3 reasons are likely.

Genetic testing has to be combined with clinical evaluation and management of patients and is now most available for LQTS. In contrast to LQTS loss-of-function  $K^+$  channel mutations, gain-of-function mutations in *KCNH2*, *KCNQ1*, and *KCNJ2* confer susceptibility for SQTs (Table 4). It is unclear what percentage of SQTs is explained by mutations in these 3 genes because a specific genetic test is not currently available. In contrast to gain-of-function, LQTS3-causing  $Na^+$  channel mutations, loss-of-function mutations in *SCN5A* cause BrS1 (Table 1). A *SCN5A*-only gene test is available clinically.<sup>154</sup> However, mutations in *SCN5A* account for only 20% to 30% of BrS1. Recently, a mutation in *GPD1L*-encoded glycerol-3-phosphate dehydrogenase 1-like gene has been implicated as a novel cause of BrS2 and SIDS (Table 1).<sup>25,26</sup> Finally, mutations in *RYR2* and *CASQ2* cause  $\approx 50\%$  to 60% of CPVT (Table 5). A targeted examination of 38 of the 105 *RYR2* translated exons has been released recently as a commercially available clinical genetic test for CPVT.

#### Genotype–Phenotype Relationships

Understanding the pathogenetic link between genotype and clinical phenotype is vital to the diagnosis and treatment of inherited arrhythmias. This includes the biophysical phenotype of abnormal protein function (eg, effects on currents), the cellular phenotype caused by this abnormal function (eg, effects on action potential,  $Ca^{2+}$  loading), and the tissue and organ phenotype (eg, ECG change, type of arrhythmia) that characterizes the clinical phenotype (eg, syncope, sudden death) (Figure). At each phenotype level, environmental factors (eg, acidosis, autonomic nerve activity, ion concentrations) and other genetic factors (genetic background and modifier genes) may affect the phenotypes.<sup>155</sup> Better understanding of these complex interrelations is especially important for inherited arrhythmia syndromes and has immediate implications for the discovery of new mutations and for cost-effective screening. Most importantly, insights into these relationships may point the way to improved treatment.

In inherited arrhythmia, the relationship between a genotype and a clinical phenotype is not necessarily linear. As an example, mutations in the cardiac  $Na^+$  channel gene *SCN5A* may lead to different arrhythmia syndromes, including BrS1 and/or LQTS3 (Tables 1 and 3). Even if one restricts consideration to loss-of-function mutations for *SCN5A*, mul-



**Figure.** Integrative study of arrhythmic cardiomyopathies (refer to Implications for the Diagnosis and Management of Inherited Arrhythmias for conceptual discussion).

multiple clinical phenotypes such as BrS1, idiopathic VF without BrS1, AF, SSS, and CoD can result (Table 2). Finally, even single *SCN5A* mutations can exhibit multiple phenotypes.<sup>5,21</sup> Incomplete penetrance and variability of the clinical phenotype present additional challenges, as seen commonly with Andersen-Tawil syndrome mutations in *KCNJ2*.<sup>156</sup> Conversely, an apparently single clinical phenotype can result from >1 genotype. For example, CPVT may result from mutations in the different  $Ca^{2+}$ -handling genes including *RYR2*<sup>116,157,158</sup> or *CASQ2*,<sup>120</sup> and perhaps *ANK2*<sup>43</sup> or *KCNJ2*.<sup>80,123</sup>

Several approaches are used to study the link between genotype and clinical phenotype (Figure). The first is the careful definition of the clinical syndrome and classic genetic linkage study. This makes the connection but does not fill in the pathogenetic gaps of the biophysical phenotype, cellular phenotype, and tissue phenotype. In addition, the large families required for linkage analysis are not always available. Cohort analyses depend more heavily on defining the biophysical phenotype of mutations to make the pathogenetic claim. When the specific gene and mutation are located, a second model and standard way to study an arrhythmia mutation is to take the gene of interest and express it in heterologous systems for functional characterization. This technique has yielded important insights into genotype–phenotype pathogenesis. However, the characterization is inherently limited to the molecular level (eg, currents, trafficking). In addition, these systems lack constituents of cardiac ion channel macromolecular complexes that might be required to accurately show the molecular “cardiac-specific” phenotype of the mutation.<sup>159</sup> This is important because mutations causing inherited arrhythmia syndromes may occur in genes encoding non-pore-forming channel subunits such as *SCN4B* as well as in genes encoding more general structural proteins such as *ANK2*,<sup>43</sup> *CAV3*,<sup>84,85</sup> and *AKAP9*.<sup>159a</sup>

A third model is overexpression of the mutation of interest *in vivo* (Figure), for example, in the transgenic mouse as successfully used for the LQTS3  $\Delta$ KPQ mutation.<sup>64</sup> Expressing the mutation in a transgenic mouse has the major advantage of allowing studies at all phenotypic levels. Nevertheless, in noninducible transgenic systems the gene of interest is overexpressed throughout organ development, and phenotypic distinction between overexpression effects on heart development and specific defects causing arrhythmias in the mature heart can be difficult. Mouse models are also costly and time consuming and are not practical for high-throughput screening of rare inherited arrhythmia mutations. Furthermore, the mouse may be of limited usefulness for the development of arrhythmia models for mechanistic studies given the short-duration action potentials and the high resting heart rates. A fourth approach is computer simulations that can help to integrate, predict, and project to the cellular and tissue level on the basis of biophysical data (Figure). Such simulations may be valuable heuristic, integrative, and predictive devices but are not a substitute for experimental data and need to be confirmed, validated, and informed by experimental data. Moreover, the LQTS phenotype in older patients may be influenced by variable expression rather than by genotype, indicating that nongenetic variables may play important roles.

A fifth approach, overexpression of mutations in native cardiomyocytes, allows for the investigation of a cellular phenotype in an environment in which the components of the full macromolecular complex are present and in which interventions such as adrenergic stimulation may be evaluated. No suitable immortalized ventricular cell culture lines are available, and freshly isolated adult cells dedifferentiate significantly during day-long culture. Neonatal cells are easier to culture and transfect but differ significantly from adult electrophysiology. Rodent cells are relatively inexpensive but have a different electrophysiology, and ideally one would use a human cell model. Human embryonic stem cell–derived cardiomyocytes may provide suitable cells for such studies if issues of differentiation and culturing conditions are overcome.<sup>160</sup>

### Therapy of Arrhythmias

#### Current Treatment Options and Limitations

$\beta$ -Blockers represent the mainstay of current drug therapy for most patients with either LQTS or CPVT. The rationale, to inhibit catecholaminergic stimulation, seeks to inhibit arrhythmia trigger mechanisms. However, although  $\beta$ -blocker treatment is effective for syncope prevention,<sup>161</sup> SCA prevention with  $\beta$ -blockers is not universal or complete throughout all LQTS genotypes or patient subpopulations.<sup>162</sup> A recent study found that risk reduction from  $\beta$ -blocker therapy may be most successful in high-risk adolescent LQTS patients who have experienced recent syncope.<sup>162</sup> The limitations of  $\beta$ -blocker treatment are due to nonspecific mechanisms, and significant side effects may result in noncompliance. ICD therapy is considered an effective primary and secondary therapy in high-risk patients with a strong personal history of syncope and marked QT prolongation. However, significant ICD device–specific risks exist, and arrhythmia treatment

may not be effective in some cases, eg, in CPVT1-mutation carriers.<sup>163</sup> Moreover, nonspecific resuscitation pharmacotherapy in SCA victims and ICD discharge causing patient emotional distress may aggravate catecholamine-dependent arrhythmias. Although ICD therapy in high-risk patients must be considered, many LQTS patients are at relatively low risk for sudden death and require careful risk stratification to justify ICD treatment.<sup>164</sup> Thus, it is highly desirable to develop novel mechanism-based therapeutic strategies that do not interfere with adrenergic regulation of the heart and avoid the risks and limitations of ICD device therapy.

#### *Future Mechanism- and Risk-Based Antiarrhythmic Treatment Strategies: General Considerations*

Genetic information contributes increasingly to the understanding of channelopathy, phenotype expression, and arrhythmia severity. For example, a particular mutation may result in a <50% reduction of ion channel function from a trafficking defect (haploinsufficiency) or in a >50% reduction because of a dominant negative mechanism affecting the function of a multimeric ion channel complex. Dominant negative defects cause approximately a doubling of arrhythmogenic risk, and mutations in transmembrane portions of KCNQ1 constitute an additional, independent risk factor.<sup>165</sup> A combined approach of genetic and biophysical electrophysiological characterization of ion-channel mutations for channelopathy-related inherited disorders may greatly aid therapy orientation for patients.<sup>166,167</sup> It is important to identify the patient and family members at high risk for tachyarrhythmias resulting in syncope or SCA, particularly when they are young. Although treating asymptomatic mutation carriers is controversial, LQTS mutation carriers require a primary or secondary prevention therapy based on a 10% risk of a major cardiac event by age 40 years.<sup>168</sup> On the other hand, 40% of LQTS mutation carriers are not detected by clinical testing, arguing strongly for the need of more comprehensive approaches including genotyping, risk assessment, and evaluation of preventive therapeutic strategies.<sup>148</sup> In other words, variable penetrance of the LQTS or CPVT phenotype may result in false-negative diagnosis, and genotyping can add predictive information needed to implement specific SCA prevention strategies.

#### *Pharmacological Options for BrS1 and SQTS*

The ECG manifestation of BrS1 may be transient or concealed but can be unmasked with Na<sup>+</sup> channel blockers (ajmaline, flecainide, procainamide), with vagotonic stimulation, or during fever. For the high-risk patient with BrS1, the current treatment of choice is ICD therapy. However, pharmacotherapy with quinidine has been tried.<sup>169,170</sup> Other pharmacological approaches use ranolazine or derivatives that selectively inhibit late I<sub>Na</sub>, reduce [Na<sup>+</sup>]<sub>i</sub>-dependent intracellular Ca<sup>2+</sup> overload, and attenuate the abnormalities of ventricular repolarization and contractility that are associated with ischemia/reperfusion injury and/or HF. Future studies will have to determine whether inhibition of late I<sub>Na</sub> reduces proarrhythmic Ca<sup>2+</sup> overload.

SQTS1 was treated initially with the class III antiarrhythmic sotalol or with sotalol and ibutilide, both of which were ineffective at prolonging the QT interval in SQTS1 patients.

The class Ia antiarrhythmic drug quinidine normalized the QT interval in SQTS1 and rendered arrhythmias noninducible. A N588K mutation was later identified in *KCNH2* that results in decreased HERG channel blocking potency for quinidine in SQTS1. At present, however, quinidine is the only drug shown to have the potential to restore normal QT intervals in SQTS1 patients, and disopyramide has been suggested as an inhibitor of N588K-HERG channels. The challenge will be to develop mechanism-based therapeutic options in the future. Additionally, *KCNA5* mutations have been linked to reduced quinidine sensitivity in the I<sub>Kur</sub> current,<sup>171</sup> and therefore future studies will need to address mechanisms of altered drug sensitivity in other genetic forms of arrhythmic cardiomyopathies.

#### *Proteome-Based Therapy*

For several of the inherited arrhythmia syndromes, the central arrhythmogenic principle for arrhythmia generation is a loss of the normal current. The mechanisms proposed for loss-of-function phenotypes are (1) abnormalities in gene transcription or protein translation, (2) abnormalities in protein maturation and trafficking, (3) abnormalities in channel gating, and (4) abnormalities in channel permeation. Thus, potential therapeutic approaches may aim at normalizing any one or combinations of these defects. In the last decade, abnormalities in protein maturation/trafficking have been linked to several diseases (cystic fibrosis, hereditary childhood emphysema, type 2 diabetes mellitus, familial amyloidosis, LQTS, BrS). These abnormalities can be classified broadly as membrane protein trafficking diseases because a defect in protein folding at some stage of the cell secretory pathway may result in intracellular protein retention, causing a loss of activity or, in some cases, protein aggregation. A key goal of future work is to determine compartments and components of exocytic and endocytic pathways that provide the chemical landscapes in which protein function and folding are modulated in eukaryotic cells as part of a "quality control system" that prevents mutant ion channel expression.<sup>172,173</sup>

Transport of ion channel proteins through the secretory pathway involves a selective mechanism in which cargo molecules are concentrated into carrier vesicles. Adding complexity to the importance of protein trafficking abnormalities is the recognition that this can be corrected. An early example was the observation that culturing cells expressing the key trafficking-deficient cystic fibrosis mutation ΔF508 at reduced temperature could restore the cellular processing of the CFTR protein and Cl<sup>-</sup> transport. This has resulted in an intense search for chemical chaperones that can act as ligands to cause pharmacological correction of trafficking-deficient CFTR mutations. It is estimated that as little as a 10% to 20% improvement in protein trafficking would ameliorate most of the clinical syndrome. Compared with a monogenetic defect in cystic fibrosis (1:2000) that occurs at a frequency similar to that in LQTS (1:2500), LQTS is a multigenetic syndrome (>10 genes), each LQTS form has been associated with multiple mutations (Table 3), and, probably because of the complexity of mechanisms and defects, industry interest in LQTS mechanism-specific drug development has been limited. However, recent studies have indicated that channel

trafficking defects are common in LQTS2,<sup>173</sup> and therefore pharmacological or other trafficking rescue approaches are promising therapeutic rationales.

Specific to LQTS2, most human mutations in *KCNH2* (*HERG*) are single nucleotide changes that typically result in single amino acid substitutions (missense mutations). These *KCNH2* mutations result in the generation of full-length *HERG* protein subunits that can assemble with wild-type subunits to form trafficking-deficient heteromeric channels that are retained in the endoplasmic reticulum. Correction of the trafficking defect is possible for many, but not all, *KCNH2* missense mutations.<sup>173</sup> Several approaches to improving trafficking have now been demonstrated, including (1) cell culture at reduced temperature; (2) culture in drugs that cause high-affinity block of the *HERG* channel pore (E4031, astemizole, cisapride, quinidine); (3) culture with drug metabolites that cause high-affinity block of *HERG* (terfenadine carboxylate); (4) culture with drugs that alter intracellular  $Ca^{2+}$  (thapsigargin); and (5) introduction of a second mutation into the drug-binding region (intragenic suppression).<sup>173</sup> These “rescued” channels increase *HERG* current by exhibiting either normal or abnormal biophysical function, and this has led to the concept that for most LQTS2 mutations the biogenic impact dominates biophysical changes in channel function. Conversely, in SQT1 a small number of drugs have been identified that appear to reduce protein trafficking of the gain-of-function *HERG* channel into the cell membrane. A *KCNA5* mutation has been successfully corrected in culture with drugs that promote translational read-through (aminoglycosides).<sup>42</sup> Thus, the development of drugs that modulate protein trafficking may have clinical utility in LQTS, SQT1, and AF. However, pharmacological rescue of channel trafficking defects by channel blockers in *SCN5A* mutation carriers may result in drug-induced LQTS and torsades de pointes VT.<sup>174</sup> Thus, careful evaluation of the consequences after correction of abnormal channel expression will be necessary.

#### *Gene and Cell Therapies*

Gene and cell-based therapies depend greatly on successful delivery strategies and comprehensive knowledge of potentially therapeutic cell or protein function. Delivery to myocardial targets may employ local injection, coronary perfusion, or epicardial painting methods. Gene expression can be targeted (eg, to certain cell types). Gene therapy approaches have been used in proof-of-principle experiments to suppress atrioventricular nodal conduction,<sup>175,176</sup> to enhance myocyte automaticity,<sup>177</sup> and to alter myocyte repolarization.<sup>178–180</sup> Cell therapy approaches for altering automaticity and repolarization have been described.<sup>181,182</sup> Certain therapeutic approaches (atrioventricular node modification, focal alterations in repolarization, automaticity) are currently developed and require preclinical evaluation of efficacy and toxicity.

#### *Need for Novel Disease Gene and Modifier Discovery*

The current challenges for genetic testing for cardiac channelopathies include discovery of novel disease susceptibility genes, translation to a clinical diagnostic test, and distinguishing disease-causing mutations from innocuous genetic variants. The difficulty in distinguishing pathogenic mutations

from rare background variants has thus far prevented the incorporation of *ANK2*-LQTS4 into genetic tests. Currently, ≈25% of LQTS, 35% of Andersen Tawil syndrome, 75% of BrS, and 40% of CPVT elude genetic explanation. Approximately 3% to 5% of healthy subjects have rare genetic variants in the genes that underlie the 3 major subtypes LQTS1–3. Consequently, some of the presumed LQTS/BrS-causing mutations could be false-positive test results. This argues for the need for ongoing functional analyses of “yet another” mutation in a known LQTS-susceptibility gene. In contrast, except for 1 common nonsynonymous single nucleotide polymorphism in *RYR2*, genetic variation in the gene responsible for CPVT1 is extremely uncommon. As such, a high probability exists that a rare genetic variant in *RYR2* confers susceptibility for CPVT1. The size of *RYR2* poses a significant challenge, and efforts are needed to determine the best targeted examination toward a clinical diagnostic test. An important issue is to determine the influence of modifier genes (polymorphic variants) on the biophysical electrophysiological dysfunction of specific ion-channel mutations. This information should permit the development and/or direction of more effective electrophysiologically targeted therapy. The anticipated advances include (1) new fundamental insights into arrhythmogenesis, (2) new insights into channel structure-function domains, (3) new antiarrhythmic therapeutic targets, (4) expanded genotype–phenotype relationships, and (5) more comprehensive clinical genetic tests.

#### *Need for New Therapeutic Approaches*

In the field of symptomatic and life-threatening cardiac arrhythmias, over the last 20 years, drug therapy has moved from primary intervention to mainly an adjunctive role and has been replaced by device therapy (ICD and pacemaker) and procedural interventions (ablation). The recognition that protein trafficking disorders may be common has led to attempts in several diseases to develop novel therapies utilizing correcting strategies, in addition to gene therapy (either gene augmentation or replacement). Limitations in the protein trafficking correction are evident: Not every disease-causing mutation results in a trafficking defect, not all trafficking-deficient mutations are correctable, and for some mutations (eg, truncation near the channel pore), correction would be predicted to result in a nonfunctional channel. The extent of correction may be difficult to regulate, and overcorrection (to shorten the QT interval) could produce new arrhythmias. Some “corrected” channels will still have biophysical abnormalities. Some diseases are likely to be better candidates for these approaches than others. Finally, the funding of new therapies for uncommon and/or very complex inherited arrhythmias will be an added challenge. Nonetheless, in diseases in which gene mutation produces protein trafficking abnormalities, the correction or improvement of protein trafficking may offer an approach to novel human therapies.

#### *Summary: Need for Integration Back to Bedside for Treatment and Management of Patients With Heritable Arrhythmia Syndromes*

Arrhythmias are responsible for the majority of deaths in patients with heart disease. Although we face an increasing



number of genetic defects, the molecular mechanisms of arrhythmia initiation and important modifiers of these events are incompletely understood. Thus, the concept of a channelopathy leading to electric heart disease has been extended to defective macromolecular signaling complex dysfunction leading to a specific cardiomyopathy with a dominant arrhythmogenic phenotype expression. We would like to emphasize that integrative and systematic approaches involving several disciplines and experimental approaches will be necessary to understand complex arrhythmogenic syndromes (Figure). For instance, a monogenetic defect can be conceptualized into a multigenetic, cell-specific, and tissue-specific context at an increasing number of investigative levels. Although population studies try to identify genetic defects, their occurrence, environmental modulators, and specific risk, experimental studies aim to characterize biophysical defects, arrhythmogenic mechanisms, and targeted therapeutic approaches. Thus, continuous integration, review, discussion, and refinement of information through a collaborative approach focused on molecular pathophysiological mechanisms, including careful mutation carrier phenotyping, is needed (Figure). Modeling studies may integrate information at all levels and predict and test specific mechanisms, which may be verified by experiments and vice versa. The complexity of inherited arrhythmic cardiomyopathies will continue to be increasingly challenging at both the basic sciences and clinical levels, and progress toward understanding and treatment rationales will depend greatly on information exchange within interdisciplinary networks and continuity of research groups.

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### Disclosures

Dr Ackerman is a consultant for PGxHealth with respect to its FAMILION genetic test for cardiac channel mutations. Dr Donahue has stock, a consulting agreement, and a board position in Excigen, Inc, a start-up company that is developing gene therapy for clinical use in arrhythmias. Dr January is a cofounder of Cellular Dynamics International, Inc, a start-up biotech company providing ion channel and human embryonic stem cell cardiomyocyte toxicology screening services and related products. Dr Marks is on the scientific advisory board of and owns shares in ARMGO Pharma, Inc, a start-up company that is developing RyR2-targeted drugs for clinical use in the treatment of HF and sudden death. The other authors report no conflicts.

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