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Sodium Ion Channelopathies

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Introduction

Voltage-gated Sodium (Na^+) channels are sarcolemmal proteins that are responsible for the rapid upstroke of the cardiac action potential (AP), and for rapid impulse conduction through cardiac tissue. Therefore, Na^+ channel function plays a major role in initiation, propagation, and maintenance of the normal cardiac rhythm. Mutations in SCN5A, the gene encoding for the α-subunit of the cardiac Na^+ channel, the so-called "inherited sodium channelopathies," are known to evoke multiple life-threatening disorders of cardiac rhythm that can vary from tachyarrhythmias to bradyarrhythmias and may require implantation of pacemakers or implantable cardioverter/defibrillators (ICDs). However, recent studies have also linked Na^+ current (I_{Na}) dysfunction to structural cardiac defects, notably cardiac fibrosis, dilated cardiomyopathy, and, possibly, arrhythmogenic right ventricular cardiomyopathy. These structural changes may also be conducive to (reentrant) arrhythmias.

The identification of mutant Na^+ channels in inherited arrhythmia syndromes and the studies of their functional properties have significantly enhanced our knowledge of Na^+ channel function and our understanding of how Na^+ channel dysfunction may act as a major pathophysiological mechanism in various diseases, including common acquired disease (Table 9–1). Clearly, these observations highlight the cardiac Na^+ channel as an interesting target for novel therapy strategies. Accordingly, this chapter aims to provide an overview of presently identified disease entities with involvement of SCN5A variants, and concepts regarding arrhythmia susceptibility derived from studies of these conditions.

Na Channel Structure and Function

The cardiac Na^+ channel is a molecular complex consisting of various subunits: a main pore-forming subunit (α), encoded by SCN5A, and smaller accessory proteins, known as β-subunits.

The α-subunit consists of four homologous domains (DI–DIV), each composed of six membrane-spanning segments (S1–S6) linked by intracellular and extracellular loops (Figure 9–1). The linkers between S5 and S6 control ion selectivity and permeation[1] of the channel, while the positively charged segments S4 act as a voltage sensor.[2] Na^+ channels are dynamic molecules that undergo rapid structure rearrangements in response to changes in the electric field across the sarcolemma, a process known as "gating." Upon membrane depolarization, all four S4 segments move in a concerted way in an outward direction allowing the opening of the channel (activation).[2,3] This increase in Na^+ permeability causes the sudden membrane depolarization that characterizes the rapid upstroke of the AP. Activation of the channel lasts a few milliseconds and is followed by fast inactivation, a nonconducting state from which the channel cannot reopen. Finally, membrane repolarization is necessary to allow Na^+

TABLE 9–1. Sodium ion channelopathies.

Inherited primary electrical disease	Reported changes in I_{Na}
Brugada syndrome	↓
SUDS	↓
LQT3 syndrome	↑
SIDS	↑↓
Conduction disease	↓
Atrial standstill	↓
Sick sinus syndrome	↓
Structural disease	
Fibrosis	↓
Arrythmogenic right ventricular cardiomyopathy*	↓
Dilated cardiomyopathy	↓
Acquired disease	
Acquired Brugada syndrome	↓
Acquired long QT syndrome	↑
Congestive heart failure	↑↓
Ischemic heart disease	↑↓

↑ increased net I_{Na}; ↓ reduced net I_{Na}.
*Not fully resolved.

channels to recover from inactivation to the resting state (closed state).

Fast inactivation is primarily mediated by the intracellular linker between domains III and IV that acts as a lid, occluding the inner vestibule of the pore.[2,4] It was recently demonstrated that interactions between the C-terminus and the intracellular III–IV linker are required to stabilize channel inactivation. This interaction plays a critical role in the heart by preventing a small persistent inward Na^+ current (also called late I_{Na}) that would prolong the AP and render the heart susceptible to arrhythmias that are initiated by secondary depolarizations occurring before the

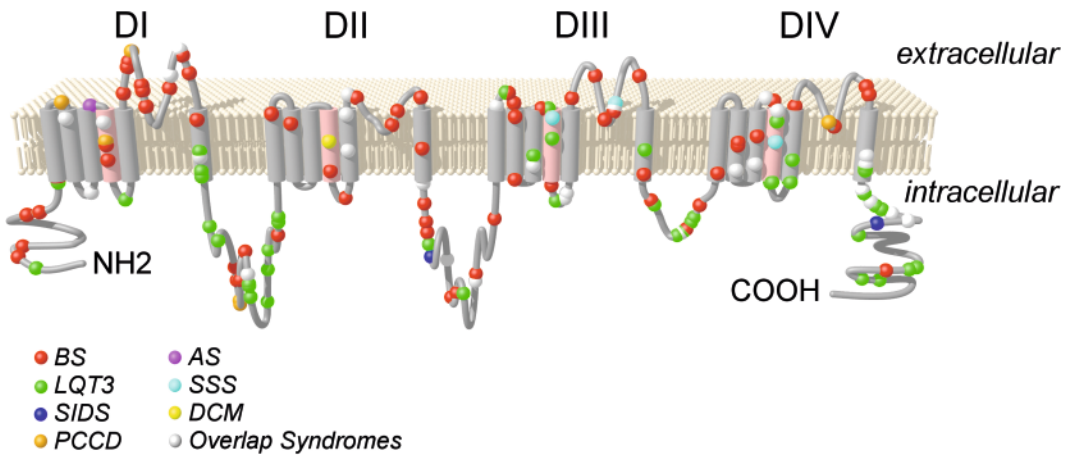

FIGURE 9–1. Schematic representation of the voltage-gated Na^+ channel α-subunit. The protein consists of four domains (D1–D4), each composed of six membrane-spanning segments (S1–S6) linked by intracellular and extracellular loops. The linkers between S5 and S6 control ion selectivity and permeation of the channel, while the positively charged segments S4 (in pink) act as a voltage sensor. Differently colored circles display the location of mutations associated with Brugada syndrome (BS), long QT syndrome 3 (LQT3), sudden infant death syndrome (SIDS), conduction disease (PCCD), atrial standstill (AS), sick sinus syndrome (SSS), dilated cardiomyopathy (DCM), and overlap syndromes.

cell has fully repolarized, called early afterdepolarizations (EADs).[5,6]

Beside this fast inactivation process (time frame: a few milliseconds), Na^+ channels can undergo a slower inactivation process (intermediate inactivation) when the membrane remains depolarized for a longer time. That more stable, nonconducting conformational state develops in cardiac Na^+ channels in about 50–100 msec and requires a prolonged period of hyperpolarization from which to recover.[7,8] Intermediate inactivation involves residues located in the outer pore and the C-terminus.[9,10] Finally, closed-state inactivation (inactivation from the closed-state without prior activation) may also occur[11] and can be clinically relevant.[12] The regions involved in closed-state inactivation await identification.

This spatial organization of gating functions suggests that single amino acid substitutions or deletions within the SCN5A coding region can evoke a broad spectrum of cardiac rhythm derangements by modulating this gating process. At the same time, common sequence variants (polymorphisms) in the Na^+ channel have also been implicated as risk factors in cardiac disease[13,14] and determinants of drug sensitivity.[15]

The α subunit interacts with smaller accessory proteins known as β-subunits. β-Subunits are glycoproteins with a single sarcolemma-spanning segment, a large immunoglobulin-like extracellular domain, and a small intracellular portion.[16] So far, four β-subunit isoforms (β_1–β_4) were identified and all are present in the heart.[17–19] Moreover, alternative RNA splicing has also been described for the β_1 subunit (β_{1A} and β_{1B}).[20,21] β_1 and β_3 share significant homology and are both not covalently associated with the α-subunit. On the other hand, β_2 and β_4 are similar and are linked to the α-subunit via disulfide bonds. β-subunits modulate the kinetic properties and the expression levels of the α subunit, and can play a role in cell adhesion.[22]

Inherited Primary Electrical Disease

Brugada Syndrome

Brugada syndrome (BS) is a cardiac disorder characterized by sudden death (especially at night and rest) due to ventricular tachyarrhythmias in the absence of structural heart disease as can be detected by routine cardiac examination. The electrocardiogram (ECG) of BS patients is characterized by ST-segment elevation in the right precordial leads (V1–V3), often in conjunction with signs of conduction slowing[23,24] (Figure 9–2). The ECG signs of the syndrome are dynamic and often concealed, but can be unmasked by Na^+ channel blockers, or during a febrile state.[25,26] Although the syndrome typically manifests during adulthood, with a peak around 40 years, arrhythmic events may occur at all ages. In western countries, the prevalence is estimated at 5–50 cases per 10,000 inhabitants.[27,28] In Southeast Asia, the disease is the leading cause of death in males under the age of 40, second only to car accidents.

Brugada syndrome exhibits an autosomal dominant pattern of inheritance, with incomplete penetrance and male predominance. Most drugs are not effective as a treatment in this syndrome

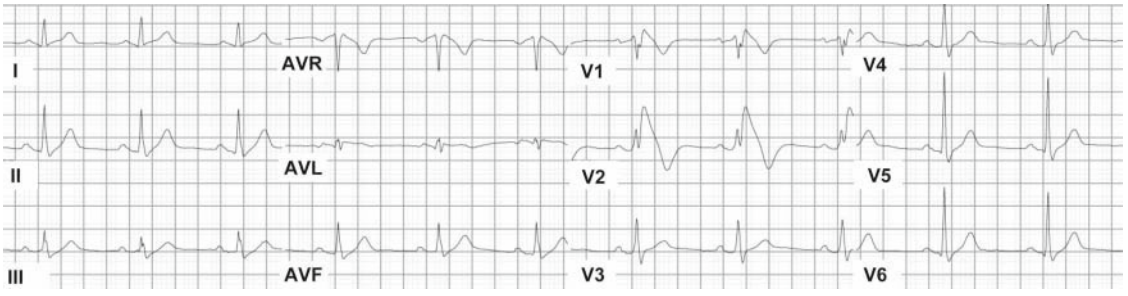

FIGURE 9–2. Representative electrocardiogram of Brugada syndrome. Note ST-segment elevation with high take-off (J point), and negative T waves, typically seen in right precordial leads V^1-V^2.

TABLE 9–2. Reported biophysical mechanisms of increase in net sodium current (gain-of-function) and reduction in net sodium current (loss-of-function).

Gain of function
Persistent current (disruption of fast inactivation)
Changes in voltage dependence of activation (hyperpolarizing shift) and inactivation (depolarizing shift)
Faster recovery from inactivation
Slower inactivation
Loss of function
Reduction in current density
Reduced number of functional sodium channels in sarcolemma
Truncated protein due to premature stop codon
Retention in endoplasmic reticulum (trafficking defect)
Mutation located in ion-conducting pore
Gating changes
Changes in voltage dependence of activation (depolarizing shift) and inactivation (hyperpolarizing shift)
Slower recovery from inactivation
Accelerated inactivation
Enhanced intermediate inactivation
Enhanced closed-state inactivation

and ICDs are the only recommended form of therapy to prevent sudden death.[29]

To date, a significant number of SCN5A gene mutations have been reported to contribute to BS. Functional analysis employing expression systems showed that all produce Na^4 channel loss of function. However, various mechanisms of I_{Na} reduction have been described (Table 9–2): (1) failure to express in the sarcolemma; (2) changes in the voltage and time dependence of activation, inactivation, and recovery from inactivation; (3) enhanced intermediate inactivation; (4) accelerated inactivation; and (5) trafficking defect. Of note, SCN5A mutations account for only 18–30% of BS cases, suggesting that other genes might be involved.[30] In 2002, a second locus on chromosome 3, close to but apart from the SCN5A locus, was linked to BS.[31] Here, an A280V mutation in the glycerol-3-phosphate dehydrogenase 1-like gene (GPD1L) was identified. This mutant, when transiently transfected into a HEK293 cell line that stably expressed SCN5A, resulted in reduction in I_{Na} when compared to wild type. Thus, GPD1L is a novel Na^+ channel modulator in the heart.[32] Another study showed a modulatory effect on the BS phenotype of the delayed rectifier potassium current encoded by KCNH2.[33]

How I_{Na} reduction causes the characteristic features of BS is unresolved. One hypothesis (repolarization disorder hypothesis) proposes that reduced I_{Na} may exacerbate the effects of the intrinsic difference in density of the transient outward current, I_{to}, between epicardial (high I_{to} density) and endocardial (low I_{to} density) layers.[34] According to this hypothesis, this strong repolarizing current renders epicardial cells more sensitive than endocardial cells to reductions in Na^+ current. An alternative hypothesis revolves around slowing of impulse propagation in the right ventricle, particularly the right ventricular outflow tract (depolarization disorder hypothesis).[35,36] Both hypotheses are supported by clinical and experimental data, rendering it likely that BS is not fully explained by one single mechanism. Yet evidence of conduction abnormalities in patients with BS is accumulating. Widening of P-wave and QRS duration and prolongation of the PQ and HV intervals, all of which represent conduction abnormalities, are often observed in BS patients. Smits *et al.* showed that greater prolongation of PQ and HV intervals at baseline and excessive QRS interval prolongation after Na^+ channel blockade are more likely to be found in BS patients who carry a SCN5A mutation than in those who do not.[37] Moreover, in a study of 78 individuals carrying a SCN5A mutation associated with BS, resting ECGs showed a spontaneous BS ECG pattern in 28 of 78 (36%) gene carriers, while 59 of 78 (76%) exhibited conduction defects. Moreover, the conduction defects worsened with age in mutation carriers, leading in five cases to pacemaker implantation.[38] This study showed that the most common phenotype of gene carriers of a BS-type SCN5A mutation is a progressive cardiac conduction defect similar to the Lenègre disease phenotype (see the section on "Conduction Disease" below).

Finally, recent studies highlight the role of other pathophysiological derangements, e.g., fibrosis[39,40] (see the section on "Cardiac Fibrosis" below), thereby contributing to the emerging notion that BS is not a monofactorial disease.

Sudden Unexplained Death Syndrome

Sudden unexplained death syndrome (SUDS) is characterized by sudden death, typically during

sleep, in young- and middle-aged males in South East Asian countries. The syndrome is the leading natural cause of death in young Thai men.[41] The patients, almost exclusively men, have structurally normal hearts and ECG patterns similar to BS. SCN5A mutations were identified in SUDS patients, which resulted in "loss-of-function" alterations such as in BS. These data suggest that SUDS and BS are phenotypically, genetically, and functionally the same disorder.[42]

Long QT Syndrome

The congenital long QT syndrome (LQTS) is an inherited disorder estimated to affect 1/5000 individuals, with the onset of symptoms typically occurring within the first two decades of life. The syndrome is mostly transmitted in an autosomal dominant fashion (Romano–Ward syndrome) and rarely as an autosomal recessive disease associated with congenital deafness (Jervell and Lange–Nielsen syndrome). The syndrome derives its name from the characteristic prolongation of the QT interval on the surface ECG (Figure 9–3), which is coupled at the cellular level to prolonged AP duration and delay in myocardial repolarization. AP prolongation can lead to EADs, which may initiate a life-threatening form of polymorphic ventricular tachycardia called torsade de pointes (TdP).[43] LQTS is a heterogeneous disorder with different genotypes and corresponding phenotypes. So far, mutations in eight different genes were associated with LQTS. Mutations in SCN5A characterize LQT3. LQT3 accounts for 8% of LQTS patients.[44]

In general, Na^+ channel mutations linked to LQT3 are associated with disrupted inactivation, as was originally identified in the ΔKPQ mutation,[45] a three amino acid deletion in the DIII–DIV linker. This intracellular segment is critically involved in Na^+ channel fast inactivation. Accordingly, this mutation results in a noninactivating persistent Na^+ current (gain-of-function) during the AP plateau that prolongs the AP duration and may account for the development of arrhythmogenic triggered activity, such as EADs. Although most of the mutations associated with LQT3 show a persistent inward current, some mutations exhibited other gating disorders that also led to the prolongation of the QT interval. These include faster recovery from inactivation, shift in voltage dependence of activation and inactivation, and a slower inactivation[46–49] (Table 9–2). Because a very delicate balance of inward and outward currents maintains the AP plateau, these subtle current alterations during repolarization may provoke QT prolongation and TdP.

Genotype–phenotype studies have shown that the risk of cardiac events differs among the different LQTS genotypes. In the case of LQT3, symptoms occur especially at rest or during sleep, when sympathetic nerve activity is expected to be low. These differences must be taken into account during diagnosis and treatment.

β-Adrenergic blockers remain the cornerstone of therapy in LQTS, although this treatment may be less efficacious in SCN5A mutation carriers.[50] Clinical and *in vitro* evidence suggests that mexiletine may reduce persistent Na^+ current and shorten the QT interval in SCN5A mutation

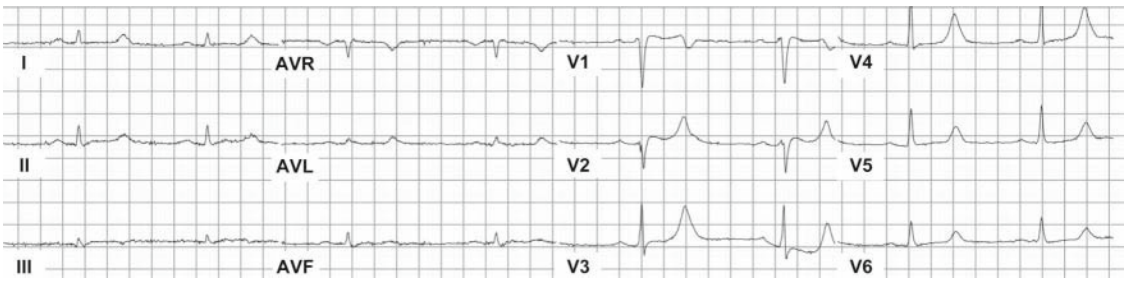

FIGURE 9–3. Representative electrocardiogram of long QT syndrome type 3. Note the marked QT interval prolongation with late peaked T waves.

carriers, although there are no data to indicate a reduction in mortality.[51,52] Flecainide has also been observed to shorten QT intervals,[53,54] but some have argued over the safety of this therapy.[55]

Sudden Infant Death Syndrome

Sudden infant death syndrome (SIDS) continues to be the most common cause of postneonatal infant death, accounting for about 25% of all deaths between 1 month and 1 year of age. It is a complex, multifactorial disorder, the cause of which is still not fully understood. However, much is now known about environmental risk factors, some of which are modifiable. These include maternal and antenatal risk factors, such as smoking during pregnancy, the use of alcohol and drugs, and infant-related risk factors, such as nonsupine sleeping position and soft bedding. Emerging evidence also shows an increased number of genetic risk factors responsible for SIDS. Overall, it is estimated that 5–10% of SIDS are associated with a defective cardiac ion channel and, therefore, an increased potential for lethal arrhythmias.[56]

Schwartz *et al.* provided the first molecular proof that LQTS may be involved in SIDS by studying an infant who experienced cardiac arrest from ventricular fibrillation (VF). Genetic analysis demonstrated a sporadic *de novo* SCN5A mutation (S941N).[57] Subsequent studies reported other mutations linked to LQT3.[49,58] Other studies demonstrated that BS can also be considered as a cause of sudden death in children.[59,60] In conclusion, complex interactions between genetic and environmental risk factors have to be taken into account to determine the risk of SIDS in individual infants.

Conduction Disease

Progressive cardiac conduction defect (PCCD), also known as Lenègre disease, is characterized by progressive slowing of conduction velocity through the His–Purkinje system with right or left bundle branch block, manifesting on the surface ECG as PR interval prolongation and QRS widening, usually in older individuals (Figure 9–4). Complete atrioventricular (AV) block can occur, resulting in syncope or sudden death. Implantation of a pacemaker is the treatment of choice. The disorder has been linked to mutations in SCN5A and in another chromosomal locus (19q13.2–q13.3)[61,62] at which the involved gene is yet unknown. The first description of a SCN5A mutation was in a large French family. In this family, P-wave width, PR, and QRS intervals prolonged with age.[63] However, in a Dutch family, where the disease was caused by another SCN5A mutation, the proband was a child.[63] This suggests that the resulting phenotype may be progressive or immediate. The first study on the biophysical properties of a PCCD-associated SCN5A mutation (G514C) showed reduced I_{Na}.[12] Patch-clamp studies revealed opposing gating changes that resulted in attenuated I_{Na} reduction. As confirmed by modeling studies, this decrease in Na^+ current was sufficient to cause conduction disease, but insufficient to cause BS. Although

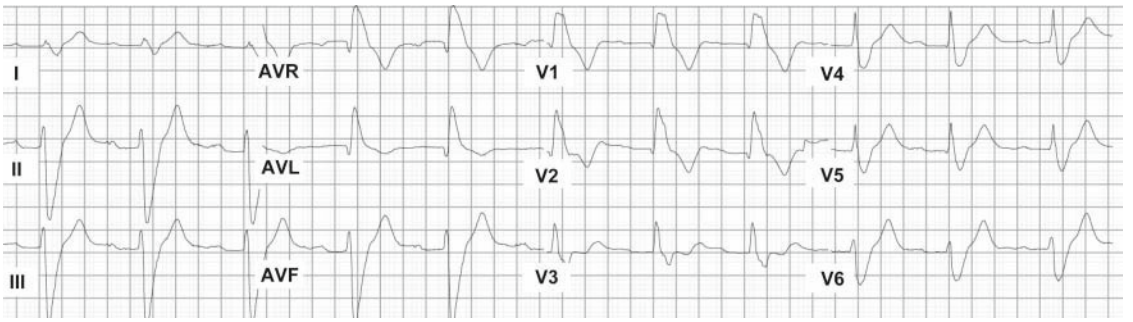

Figure 9–4. Representative electrocardiogram of conduction disease. Note the marked QRS widening, PQ interval prolongation, and left QRS axis deviation.

through different mechanisms, all subsequent SCN5A mutants studied were characterized by a loss of Na^+ channel function.[14,64–67] The decreased I_{Na} would result in a reduction of the AP upstroke velocity, thereby slowing the conduction velocity. Recently, in a large Finnish family, the loss-of-function mutation D1275N was identified. The affected individuals showed cardiac conduction defects and atrial arrhythmias.[68] Of note, the same mutation was found in families with atrial standstill[69] and with dilated cadiomyopathy.[70,71]

Atrial Standstill

Atrial standstill (AS) is a rare arrhythmia that consists of loss in electrical and mechanical activity of the atria. It is characterized by bradycardia, absence of P-waves, and junctional escape rhythm.[72] Whereas in most reports, AS is secondary to other diseases, familial AS without underlying cardiac disorder is extremely rare and identification of genetic factors for AS is hampered by the small number of affected individuals in each family. The first mutation associated with AS was found in SCN5A (D1275N) and resulted in a loss-of-function Na^+ channel. Of interest, the affected individuals carried the mutation and were homozygous for two linked polymorphisms in Cx40, the gene encoding the atrium-specific gap junction protein connexin 40.[69] These Cx40 polymorphisms were localized in the promoter region and resulted in reduced Cx40 expression. This was predicted to hamper cell-to-cell electrical coupling in the atrium, thereby reducing atrial excitability. At the same time, the requirement of this Cx40 variant was used to explain why the phenotype in this family was restricted to the atrium. Recently, another SCN5A mutation (L212P) linked to AS was identified.[73] When expressed in a heterologous system, this mutation showed opposing gating changes, resulting in a net loss-of-function of I_{Na}. Further screening for genetic variations revealed that the affected individual carried the same Cx40 polymorphisms as described previously[69] but was heterozygous for them. Taken together, these studies indicate that genetic defects in SCN5A most likely underlie AS, but that additional genetic factors that modulate atrial electrical coupling are relevant to the clinical manifestation of this inherited arrhythmia.

Sick Sinus Syndrome

Idiopathic sick sinus syndrome (SSS) is characterized by sinus bradycardia and sinus arrest in the absence of any structural heart disease. In three independent studies, mutations in HCN4, the gene encoding the α-subunit of the pacemaker current, I_f, were identified.[74–76] These HCN4 mutations resulted in loss-of-function of I_f. Although the role of I_{Na} in sinoatrial node depolarization is less clear, SCN5A mutations were also identified in 5 of 10 children (three families) with sinus node disease.[77] Compound heterozygosity was, however, necessary, as members of the studied families who carried mutations in only one allele were clinically unaffected. The SCN5A mutations resulted in loss-of-function of I_{Na}. The phenotype in these individuals included bradycardia that progressed to atrial inexcitability. Thus, there may be substantial overlap with the atrial standstill phenotype. The available data suggest, similar to other channelopathies, that idiopathic SSS is genetically heterogeneous.

Variant Na^+ Channels in Structural Defects

Cardiac Fibrosis

The first association between reduced I_{Na} and structural defects was derived from a study of two infants of asymptomatic parents who exhibited prolonged conduction intervals (PR, QRS) associated with episodes of wide complex tachycardia.[64] Genetic analysis revealed compound heterozygosity for two SCN5A mutations that both caused I_{Na} reduction (due to a truncated protein resulting from a premature stop codon in W156X, in conjunction with severely reduced I_{Na} density of R225W channels). The postmortem examination of the heart of one of the children who died from severe reduction in cardiac excitability and ventricular tachyarrhythmias showed fibrosis and necrosis in the left and right ventricle, and fibrosis of the AV node and specialized conduction system, providing the first evidence that a monogenic channel defect can progressively lead to myocardial structural abnormalities in humans.

Similarly, studies of the explanted heart of a BS patient with a loss-of-function SCN5A mutation

(G1935S) revealed severe fibrosis in the right ventricular midmyocardial free wall.[39] Of note, this region was critically involved in tachyarrhythmias elicited by programmed electrical stimulation. Finally, in a study of 18 unrelated patients with the clinical phenotype of BS and normal cardiac structure and function on noninvasive examinations, endomyocardial biopsies revealed structural derangements in all. Mutations in SCN5A were identified in 4 of 18 patients.[40] All mutations resulted in loss-of-function I_{Na}, characteristic of BS. One mutation carrier showed fibrofatty myocardial replacement, suggestive of arrhythmogenic right ventricular cardiomyopathy (ARVC). Of note, in the BS patients with SCN5A mutations, myocyte apoptosis in both the left and the right ventricle was significantly higher than in control.

The association between reduced I_{Na} and structural defects is supported by experimental studies. In a SCN5A knockout mouse model, mice that were homozygous for the null allele died before birth, with profound derangements in cardiac development. Heterozygous mice survived, and exhibited severe conduction abnormalities, resembling the PCCD phenotype, along with a 50% reduction in I_{Na}, as found in patch-clamp studies.[78] In a follow-up study, it was found that old (but not young) heterozygous mice had prominent cardiac fibrosis in the left and right ventricular free walls and the interventricular septum. In addition to fibrosis, altered expression of connexin was also identified.[79] Finally, in aged heterozygous mice, reduced I_{Na} in combination with interstitial fibrosis and disarrangement of gap junctions resulted in severe conduction impairment.[80]

Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart muscle disease characterized pathologically by specific derangements of the right ventricle (RV) (fibrosis, fibrofatty replacement of myocardium) and clinically by ventricular tachycardia that may culminate in ventricular fibrillation and sudden death. Of interest, the clinical features of ARVC may exihibit significant overlap with those of BS.[81–83] Most notably, the hallmark ST elevations of BS have also been reported in ARVC, and may be provoked by Na^+ channel blockers, similar to BS.[84] Conversely, autopsy studies of BS patients revealed fatty tissue deposition in the RV.[85,86] Moreover, in biopsies from the RV, fatty tissue infiltration mimicking ARVC was observed in 8 of 22 BS cases (36%).[86] Most recently, in a study of 18 unrelated patients with the clinical phenotype of BS, endomyocardial biopsies revealed structural derangements in all, with histopathological findings suggestive of ARVC in a patient who carried a SCN5A mutation.[40] Although overlap may exist between ARVC and BS, at present, ARVC has been linked to genes that are different from those responsible for BS. Only the ARVC5 locus has been mapped to a region on the same chromosome that contains SCN5A and GPD1L, the two genes associated with BS, but the gene has not been identified yet.[87] These data not only substantiate an overlap between ARVC and BS[88] but may also exemplify the potential of SCN5A mutations to be causally involved in structural cardiac defects.

Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is an idiopathic, genetically heterogeneous disorder characterized by heart failure and an enhanced incidence of arrhythmias. The majority of the identified genes encode structural proteins of the contractile apparatus and cytoskeleton. In 1996, a locus for DCM was identified on chromosome 3 (3p22–p25), which contains SCN5A.[89] Subsequent screening of SCN5A revealed the missense mutation D1275N, as reported in studies by McNair *et al.*[70] and Olson *et al.*[71] In these studies, affected members had dilated cardiomyopathy and signs of conduction disease, along with atrial fibrillation. Of interest, this phenotype is distinct from a previous study by Groenewegen *et al.*, in which the D1275N mutation caused familial AS, when combined with a rare Cx40 polymorphism (in the study of McNair *et al.*, DCM did not correlate with the presence of Cx40 polymorphisms).[69] A more recent study by Laitinen-Forsblom *et al.* also linked the D1275N mutation with cardiac conduction defects and atrial arrhythmias, while no contractile dysfunction was observed.[68] Why the D1275N mutation caused DCM in the family

reported by Olson *et al.* and McNair *et al.*, but not in the families reported by Groenewegen *et al.* and Laitinen-Forsblom *et al.*, remains a matter of debate.[90] It was proposed that contractile dysfunction and DCM may be secondary to chronically increased heart rates from atrial fibrillation. Still, the possible role of SCN5A mutations in causing DCM was supported by the presentation by Olson *et al.* of loss-of-function SCN5A mutations in four other DCM families (T220I, R814W, D1595H, and the basepair insertion 2550–2551insTG, leading to a stop codon), with histopathological derangements in two of four mutation carriers (in the T220I and R814W families).[71] In conclusion, while this study[71] supports the proposed link between reduced I_{Na} and structural derangements, it is unclear how identical loss-of function mutations in SCN5A may lead to different phenotypes.

Overlap Syndromes and Modulating Factors

Several SCN5A mutations have been identified, which resulted in overlapping phenotypes characterized by a combination of various features of the above described disorders. The 1795insD mutant was the first to be reported. Carriers of this mutation exhibited features of LQT3 and BS[7]. Patch-clamp studies revealed that these opposing phenotypes (gain of function in LQT3, and loss-of-function in BS) may be explained by distinct gating changes. Thus, enhanced persistent I_{Na} accounted for AP prolongation and LQT3, while, concurrently, enhanced intermediate inactivation resulted in reduced I_{Na} availability and ST elevation at fast heart rates. A subsequent study revealed that this SCN5A variant is also associated with sinus rate slowing, and that this phenomenon may equally be explained by altered biophysical properties, notably prolongation of the sinus node AP by persistent I_{Na}.[91] Additionally, mutation of the same residue to a histidine (Y1795H) or cysteine (Y1795C) resulted in BS and LQTS, respectively.[92] Kyndt *et al.*[93] described a large French family with a missense mutation (G1406R) in which mutation carriers exhibited either BS or PCCD phenotypes, suggesting that modifier gene(s) may influence the phenotypic consequence of this SCN5A mutation. Similarly, the deletion of a lysine (ΔK1500) was associated with BS, LQTS, and PCCD.[94] Later studies have also reported combinations of others phenotypes, e.g., SSS in conjunction with conduction disease and BS.[95] Probably the most consistent link is that between BS and PCCD. In a study of 78 individuals carrying a SCN5A mutation associated with BS, the spontaneous (i.e., in the absence of pharmacological challenge) BS phenotypes were present only in 36%, while 76% exhibited conduction defects. Moreover, the conduction defects worsened with age in mutation carriers.[38]

How a single mutation can result in a different and sometimes opposing phenotype, is not fully resolved. In addition to specific gating changes (as described for the 1795insD mutation[7]), other explanations have been proposed. For instance, the intrinsic heterogeneity of the myocardial substrate with which the mutant Na^+ channel interacts may be relevant. Epicardial myocytes have a characteristic spike-and-dome AP morphology, due to a large I_{to}, while endocardial cells do not. Mutations that act to reduce I_{Na}, in the presence of a large repolarizing current, may result in premature AP repolarization, loss of AP dome, and BS-type ST elevation on the ECG (repolarization disorder hypothesis). Conversely, in endocardial cells, persistent I_{Na}, in the presence of smaller repolarizing currents, may prolong AP duration (LQT3 phenotype).[96] Still, this theory does not explain why a single mutation may cause different phenotypes in various members of the same family. Other studies have revealed the role of gender. In a single family, the G1406R mutant caused BS only in male carriers (four of six), while PCCD was found in six of six female and two of six male carriers.[93] This is consistent with the observation that while SCN5A variants are equally transmitted between both sexes, the BS phenotype is more prevalent in males. The male predominance might be partially due to the intrinsic differences in ventricular AP between males and females.[97] I_{to} density is higher in males, rendering them more susceptible to the effect of I_{Na} reduction. In men, this may result more readily in excessive repolarization as proposed in the repolarization disorder hypothesis.[98] Conversely, females may be protected, because $I_{Ca\text{-}L}$ is more strongly expressed in their epicardium.[99] Moreover, other studies suggested that the male hormone testosterone may be accountable for the gender differences in BS.[100]

Of note, the modulating role of genetic factors in determining the clinical phenotype is now emerging from recent studies. For instance, the D1275N mutation caused atrial standstill in the presence of homozygous loss-of-function Cx40 promoter polymorphisms,[69] but DCM and conduction defects in their absence.[68,70] Similarly, the common polymorphism H558R in SCN5A produced no appreciable I_{Na} gating changes in isolation, but it attenuated the gating defects caused by a SCN5A mutation identified in a child with conduction disease.[14] Another study also reported a modulatory role of this polymorphism on a LQT3-linked SCN5A mutant.[101] The presence of such polymorphisms and differences in their prevalence among various ethnic groups may underlie clinically relevant differences among ethnic groups. For instance, the S1102Y polymorphism in SCN5A is mainly present in subjects of African descent[15] (it was also found in a single white family, but there, it was deemed a disease-causing mutation responsible for LQT3[102]). S1102Y was found in a patient of African descent who sustained QT prolongation and TdP on amiodarone (with associated hypokalemia and dilated cardiomyopathy as confounders for QT prolongation).[15] Similarly, Bezzina *et al.*[13] recently identified a haplotype variant in the promoter region of SCN5A that occurred with an allele frequency of 22% in Asian subjects, but was absent in whites and blacks. This haplotype resulted in reduced SCN5A transcription and was associated with slower cardiac conduction. It was suggested that this variability in SCN5A transcription may contribute to differences in phenotype as a function of ethnicity.[13]

Acquired Cardiac Arrhythmias

Whereas the inherited arrhythmias described above are explained by disturbed properties of mutant Na^+ channels, "acquired arrhythmias" are precipitated by environmental factors that act on the electrical activity of the heart.

It is increasingly recognized that acquired arrhythmias may involve subclinical genetic variations (polymorphisms) that can alter the structure or the electrophysiological properties of the myocardial substrate, thereby contributing to unique drug responses in carriers of these gene variants. Similarly, some of the knowledge obtained in the study of rare inherited SCN5A-related disorders can be now extended to common acquired diseases. Na^+ channel gating derangements caused by these conditions may have relevance both in the presence of Na^+ channel polymorphisms and in their absence.

Drug-Induced Brugada Syndrome

Given the role of I_{Na} reduction in BS, various conditions and drugs that reduce I_{Na} may cause/mimic BS. Class IC Na^+ channel blockers are the most frequent causes of acquired BS, but many other drugs (not necessarily drugs aimed primarily at cardiac disease) are able to induce ST-segment elevations. Moreover, a BS-like phenotype may be evoked by environmental factors, including electrolyte abnormalities, hyperthermia, hypothermia, elevated insulin level, acute ischemia, and mechanical compression of the right ventricular outflow tract (RVOT).[103] It is likely that polymorphisms can enhance individual susceptibility to the BS phenotype in the presence of drugs or other triggers, although up to now no cases were reported.

Drug-Induced Long QT Syndrome

A SCN5A polymorphism (S1102Y) was found in a patient of African descent who sustained QT prolongation and TdP on amiodarone (with associated hypokalemia and dilated cardiomyopathy as confounders for QT prolongation).[15] This polymorphism appeared to be ethnicity related, being commonly present in subjects of African descent. Subsequent biophysical analysis revealed mild Na^+ channel gain-of-function, primarily caused by enhanced persistent I_{Na} and increased I_{Na} density. The biophysical effects of S1102Y are so subtle that it is anticipated that most individuals who carry it do not develop arrhythmias, unless additional acquired risk factors are present, such as drug use, hypokalemia, or structural heart disease.

A SCN5A mutation (L1825P) was isolated in a woman who showed drug-induced LQTS after cisapride treatment.[104] In isolation, this mutation was subclinical, and exerted clinical effects only when uncovered by the use of cisapride. The

mutant channel exhibited both gain-of-function Na^+ channel features (persistent I_{Na}, slowed decay of inactivation), characteristic of LQT3, and loss-of-function features (decreased peak current, negative shift of inactivation, positive shift of activation, enhanced closed-state inactivation) typical of BS. It was suggested that the superimposition of the subclinical features of L1825P channels at baseline, and the proarrhythmic effects of cisapride, may have been the cause of the LQTS phenotype in the patient.

Congestive Heart Failure

AP and QT prolongation are well-known features of congestive heart failure (HF) that contribute to enhanced risk of arrhythmias and sudden death in this disease. An increased persistent I_{Na}, which can contribute to AP prolongation, was shown in two different dog models of HF[105,106] and in failing human ventricular myocytes.[106] Accordingly, experimental block of persistent I_{Na} by saxitoxin or lidocaine shortened AP duration and abolished EADs in myocytes of failing hearts.[105] In contrast, down-regulation of I_{Na} was found in two different canine HF models,[106–108] and in human ventricular cells.[106] A decrease in I_{Na} density may reduce excitability, thereby slowing myocardial conduction and contributing to reentrant arrhythmias. However, Wiegerinck *et al.*[109] did not observe any differences in peak current between HF and CTR in a volume/pressure overload HF rabbit model, in line with another study that did not reveal changes in I_{Na} in dogs with pacing-induced HF.[110] The contrasting results of these studies may be due to species/model differences.

While these studies did not address the possible presence of Na^+ channel polymorphisms, these observations may point to a novel target for a reduction in risk of arrhythmia in congestive heart failure.

Ischemic Heart Disease

Although several studies have revealed ischemia-related functional changes in various cardiac ion channels and transporters,[111] it is likely that Na^+ channel dysfunction plays an important role in reentrant arrhythmias under ischemic conditions.[112] Ischemia-induced arrhythmias can evolve from a site of slowed conduction near the ischemic border zone,[113] consistent with a loss of Na^+ channel function.

Supporting these findings, in an experimental dog model of chronic ischemia, myocytes obtained from the ischemic zone exhibited reduced I_{Na} and altered inactivation gating properties (negative shift of inactivation, slowed recovery from inactivation, enhanced closed-state inactivation) when compared to myocytes of nonischemic regions.[114] These results are consistent with previous findings in which maximal AP upstroke velocity (V_{max}), a measure of I_{Na}, was found to be significantly reduced in myocytes of infarction border zones.[115,116] The reduced I_{Na} can contribute to conduction slowing, thereby facilitating reentrant arrhythmias.

Consistent with a higher affinity for the inactivated channel conformation(s), lidocaine is able to produce an enhanced use-dependent I_{Na} block in border zone cells, suggesting that changes in Na^+ channel inactivation gating in ischemic border zones may provoke a proarrhythmic pharmacological effect.[117] This could be a molecular mechanism for the enhanced risk of life-threatening proarrhythmia conferred by potent Na^+ channel blockers in patients who experienced cardiac ischemia in the CAST trial.[118]

These data suggest that gating changes may contribute to electrophysiological heterogeneity during ischemia, which predispose to reentrant arrhythmias. Accordingly, in rats with 3- to 4-week-old experimentally induced myocardial infarctions, an increased persistent Na^+ was found when compared to controls.[119]

Other experiments revealed that Na^+ influx carried by the persistent Na^+ current appeared to be a major contributor to the rise of intracellular Na^+ observed during ischemia[120] and hypoxia.[121] Moreover, exposure of the heart to ischemia is known to increase lysophosphatidylcholine, palmitoyl-L-carnitine, and reactive oxygen species (e.g., hydrogen peroxide), and these substances are themselves reported to increase persistent I_{Na}.[122–124] Persistent I_{Na} can trigger arrhythmias either by prolonging AP duration with subsequent EADs or by increasing intracellular Na^+ loading, leading to calcium overload, and, consequently, to delayed afterdepolarizations (DADs).[125] In both mechanisms, persistent I_{Na} represents an attractive target for therapeutic intervention. A selec-

tive blocker of this current without significant action on peak Na^+ current may be a promising development.[126,127]

Summary

While the spectrum of diseases associated with SCN5A mutations is still expanding, it is becoming increasingly clear that even subtle changes in Na^+ channel gating can dramatically affect cardiac rhythm, especially if exacerbated by environmental factors that act on the electrical activity of the heart. Biophysical studies have revealed Na^+ channel gating defects that, in many cases, can explain the phenotype associated with the rhythm disorder. However, recent studies have shown significant overlap between aberrant rhythm phenotypes, and single mutations have been found to evoke different rhythm disorders. It is now evident that the correlation between mutation and the clinical phenotype is not always straightforward. Many factors, such as the presence of polymorphisms, humoral regulation, auxiliary subunits, and transcriptional regulation may play a role. Moreover, it is becoming clear that Na^+ channel-related diseases involve not only derangements in cardiac excitability, but also structural cardiac derangements, that may not be detectable using current clinical imaging modalities, but that nevertheless may act in concert with reductions in cardiac excitability to facilitate reentrant arrhythmias. With the discovery of novel disease entities associated with SCN5A variants, and the elucidation of the biophysical ways in which these variants impact on I_{Na}, we are now beginning to obtain the insights needed to apply the concepts derived from studies of rare inherited SCN5A-associated diseases to common acquired disease.

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