

# Cardiac sodium channelopathies

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**Abstract** Cardiac sodium channels are protein complexes that are expressed in the sarcolemma of cardiomyocytes to carry a large inward depolarizing current ( $I_{Na}$ ) during phase 0 of the cardiac action potential. The importance of  $I_{Na}$  for normal cardiac electrical activity is reflected by the high incidence of arrhythmias in cardiac sodium channelopathies, i.e., arrhythmogenic diseases in patients with mutations in *SCN5A*, the gene responsible for the pore-forming ion-conducting  $\alpha$ -subunit, or in genes that encode the ancillary  $\beta$ -subunits or regulatory proteins of the cardiac sodium channel. While clinical and genetic studies have laid the foundation for our understanding of cardiac sodium channelopathies by establishing links between arrhythmogenic diseases and mutations in genes that encode various subunits of the cardiac sodium channel, biophysical studies (particularly in heterologous expression systems and transgenic mouse models) have provided insights into the mechanisms by which  $I_{Na}$  dysfunction causes disease in such channelopathies. It is now recognized that mutations that increase  $I_{Na}$  delay cardiac repolarization, prolong action potential duration, and cause long QT syndrome, while mutations that reduce  $I_{Na}$  decrease cardiac excitability, reduce electrical conduction velocity, and

induce Brugada syndrome, progressive cardiac conduction disease, sick sinus syndrome, or combinations thereof. Recently, mutation-induced  $I_{Na}$  dysfunction was also linked to dilated cardiomyopathy, atrial fibrillation, and sudden infant death syndrome. This review describes the structure and function of the cardiac sodium channel and its various subunits, summarizes major cardiac sodium channelopathies and the current knowledge concerning their genetic background and underlying molecular mechanisms, and discusses recent advances in the discovery of mutation-specific therapies in the management of these channelopathies.

**Keywords** Arrhythmia · Action potential · Cardiac electrophysiology · Cardiomyocyte · Ion channels

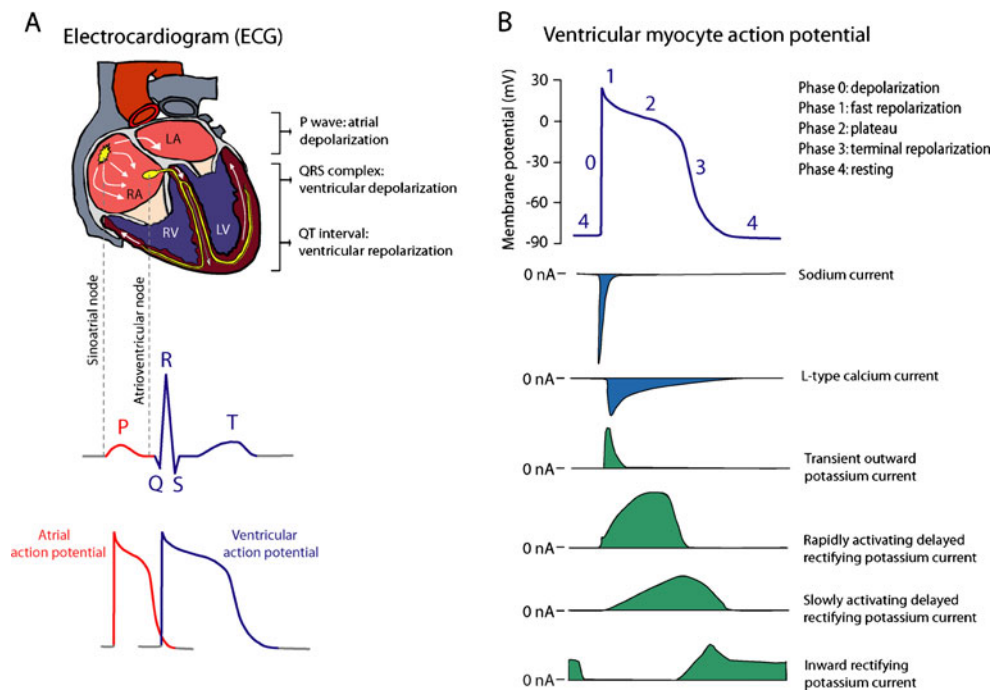
## Introduction

Cardiac sodium channels are transmembrane proteins that are located in the sarcolemma of atrial and ventricular myocytes and the Purkinje fibers. They enable a large and rapid influx of sodium ions (sodium current;  $I_{Na}$ ) during the rapid upstroke (phase 0) of the cardiac action potential.  $I_{Na}$  underlies the initiation and propagation of action potentials, and, by doing so, it determines cardiac excitability and conduction velocity of electrical stimuli through the heart (Fig. 1a, b) [9]. To a lesser extent, cardiac sodium channels are also present in the sinoatrial node and the atrioventricular node, where they contribute to pacemaker activity [45]. The importance of sodium channels for normal electrical activity of the heart is emphasized in arrhythmogenic diseases caused by inherited or de novo (mostly heterozygous) mutations in *SCN5A*, the gene that encodes the cardiac sodium channel (“cardiac sodium channelopathies”). These diseases include long QT syndrome type 3 (LQT-3), Brugada

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**Fig. 1** The cardiac electrical activity and cardiac ion currents. **a** The electrical activity of the heart is represented on the surface electrocardiogram (ECG), and results from coordinated action potential generation in individual cardiomyocytes. The electrical activity starts by the spontaneous generation of action potentials in pacemaker cells in the sinoatrial node. Propagation of these action potentials creates an excitation wave through the atria, leading to atrial

depolarization. After traveling through the atrioventricular node, the excitation wave reaches the ventricles, and leads to ventricular depolarization. **b** The cardiac action potential is generated by transmembrane inwardly and outwardly directed ion currents. The inward (depolarizing) sodium and calcium currents are pointed downwards and colored *blue*. The outward (repolarizing) potassium currents are pointed upwards and colored *green*

syndrome (BrS), progressive cardiac conduction disease (PCCD), dilated cardiomyopathy (DCM), sick sinus syndrome (SSS), atrial fibrillation (AFib), sudden infant death syndrome (SIDS), and overlap syndromes [5]. In overlap syndromes, patients display overlapping clinical symptoms or arrhythmia forms of more than one disease [42]. Although less prevalent, mutations in genes encoding proteins that regulate the expression or function of the sodium channel may also cause these diseases [5].

The effects of *SCN5A* mutations on the normal structure, expression, and function of cardiac sodium channels are routinely investigated by cloning and expression of channel proteins in heterologous systems (e.g., human embryonic kidney cells, Chinese hamster ovary cells, *Xenopus* oocytes). The effects of mutations in genes encoding the regulatory proteins are studied by co-expression of the mutant protein with the normal sodium channel protein. Additionally, genetically engineered mice carrying mutations in a specific gene of interest are increasingly used to study the effect of mutations in the native environment of the myocyte [17]. These expression models, in conjunction with various experimental techniques (e.g., electron crystallography to study channel structure, polymerase chain reaction to study mRNA expression, Western blot and immunohistochemistry to study protein expression, co-immunoprecipitation to study

protein–protein associations, and patch-clamp technique to measure currents) have greatly enhanced our understanding of the role of sodium channel dysfunction in the pathophysiology of cardiac sodium channelopathies. Furthermore, they have provided basic rationale for gene-specific and even mutation-specific approaches in the clinical management of subject with such channelopathies. This review briefly describes the structure and function of the cardiac sodium channel and its modulation by regulatory proteins. Moreover, it discusses the well-recognized cardiac sodium channelopathies and the nature and role of sodium channel dysfunction in the underlying mechanisms of these diseases.

### The cardiac sodium channel

Voltage-gated sodium channels are dynamic transmembrane proteins in excitable cells (e.g., cardiomyocytes, skeletal muscle cells, neurons) that open and close to conduct ions. They are large molecular complexes containing a pore-forming ion-conducting  $\alpha$ -subunit and ancillary  $\beta$ -subunits, and several regulatory proteins [1, 9, 29, 33, 39, 48, 77, 89]. The  $\alpha$ -subunit is called  $\text{Na}_v1.5$ , and is encoded by *SCN5A*. It is organized into a cytoplasmic N terminus, four homologous domains (DI–DIV) that are connected to each other by

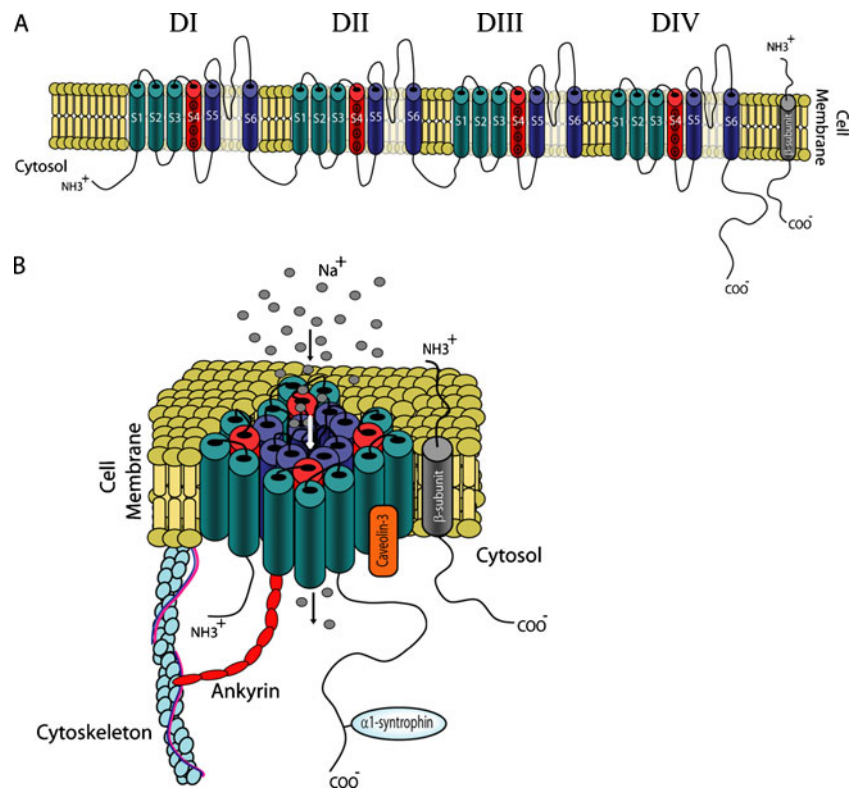
cytoplasmic linkers, and a cytoplasmic C terminus. Each domain consists of six transmembrane  $\alpha$ -helical segments (S1–S6), connected to each other by alternating extracellular and cytoplasmic loops. The four domains fold around an ion-conducting pore, which is lined by the extracellular loops (P-loops) between S5 and S6 segments (Fig. 2a, b). Some amino acids in the P-loops confer the selectivity for sodium ions. Gating represents time-dependent transitions between distinct conformational states of the channel protein due to molecular movements in response to membrane potential changes (“voltage-dependent gating”) [9]. When cardiomyocytes are excited by electrical stimuli from adjacent cells or by artificially applied stimuli during patch-clamp experiments, their resting membrane potential (approximately  $-85$  mV) depolarizes. This triggers an outward movement of the positively charged S4 segments (voltage sensors), which leads to channel activation (i.e., opening of the pore). Inactivation (closing of the pore) starts simultaneously with activation, but since inactivation is slower than activation, channels remain transiently open to conduct  $I_{Na}$  during phase 0 of the action potential (Fig. 3a, b). Inactivation comprises different conformational states, including fast, intermediate, and slow inactivation. Fast inactivation is coupled to activation and initiated by the outward movement of the S4 segment of DIV. This triggers the amino acids isoleucine, phenylalanine, and methionine (IFM motif; “the lid”) and the neighboring glycine and proline (“the hinges”) in the DIII–DIV linker to occlude the pore by binding to multiple amino acids in the

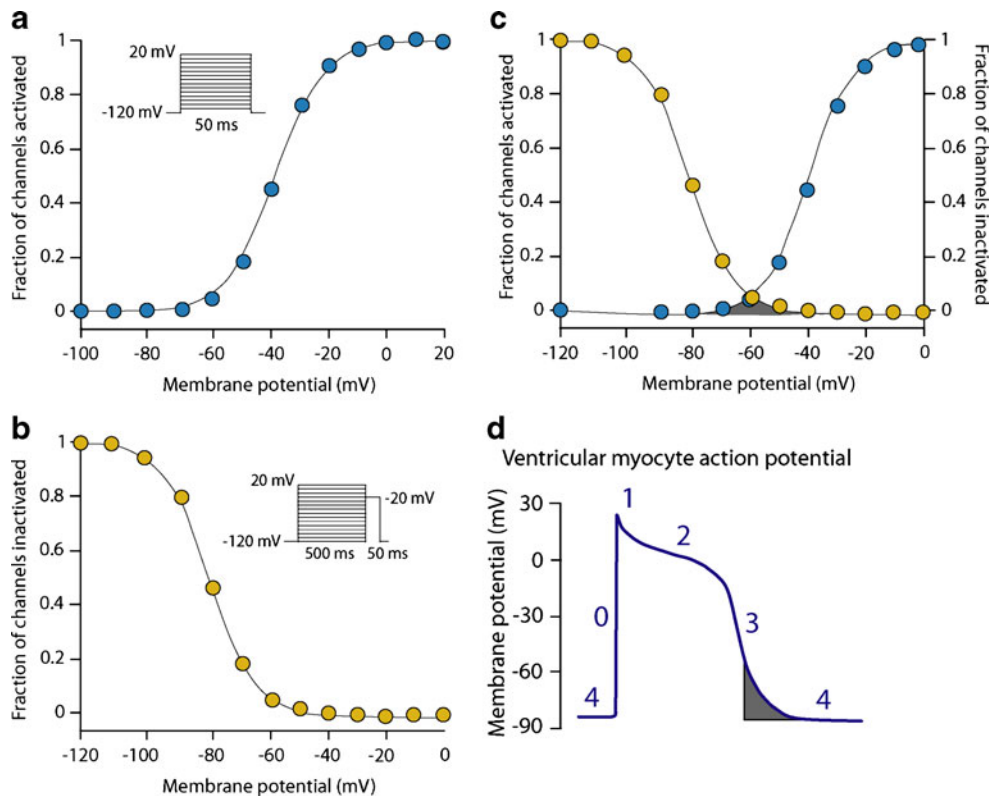
cytoplasmic loops between the S4 and S5 segments of DIII and DIV (“the dock”) [9]. The molecular movements leading to slow inactivation are less well understood. However, mutations in the P-loops, the S6 segments and the C terminus have been reported to affect this state. In any case, more than 99% of sodium channels are inactivated at the end of action potential phase 1, and can be reactivated only after recovery from inactivation during action potential phase 4. Slow inactivation requires much longer recovery times than fast inactivation. Finally, a very small fraction of sodium channels may reactivate during action potential phase 3. The current through these channels ( $<1\%$  of the peak  $I_{Na}$ ) is called the window current, since it arises when the sarcolemma reaches a potential that is depolarized sufficiently to reactivate some channels, but not enough to cause complete inactivation (Fig. 3c, d). The voltage range for the window current is very restricted and narrow in healthy hearts, granting it a small role during the cardiac action potential [41].

Although  $Na_v1.5$  is sufficient to generate sodium current in heterologous expression systems, the obtained current is quite different from  $I_{Na}$  present in isolated cardiomyocytes. This may be due to the absence of ancillary  $\beta$ -subunits and regulatory proteins in heterologous systems (Fig. 2b). So far, four  $\beta$ -subunits are known in the heart ( $\beta1$  to  $\beta4$ ), which are encoded by four genes (*SCN1B* to *SCN4B*) [1, 29, 33, 48, 89]. The  $\beta$ -subunits are proteins with an extracellular N terminus, one transmembrane segment, and a cytoplasmic C terminus. They increase the expression of

**Fig. 2** Molecular structure of the cardiac sodium channel.

**a** Cartoon of the  $\alpha$ -subunit ( $Na_v1.5$ ) and the  $\beta$ -subunit of the cardiac sodium channel.  $Na_v1.5$  consists of four domains (DI–DIV), each containing six transmembrane segments (S1–S6); S4 segments are positively charged and act as voltage sensors. The  $\beta$ -subunit consists of one single transmembrane segment. **b** The four domains of  $Na_v1.5$  fold around an ion-conducting pore, which is lined by the loops between the S5 and S6 segments. The expression and function of  $Na_v1.5$  is regulated by  $\beta$ -subunits and several directly or indirectly interacting regulatory proteins





**Fig. 3** Voltage-dependent activation and inactivation of the cardiac sodium channel. **a** Using the patch-clamp technique, the membrane potential dependence of activation is studied by applying 50 ms depolarizing voltage steps from a holding potential of  $-120$  mV (*inset*). The activation curve is obtained by dividing the amplitude of the resulting sodium current at each voltage step by the maximum peak sodium current amplitude, and plotting versus the corresponding voltage. **b** The membrane potential dependence of inactivation is studied by applying 500 ms depolarizing voltage steps from a holding potential of  $-120$  mV to inactivate the channels (prepulse). Next, the

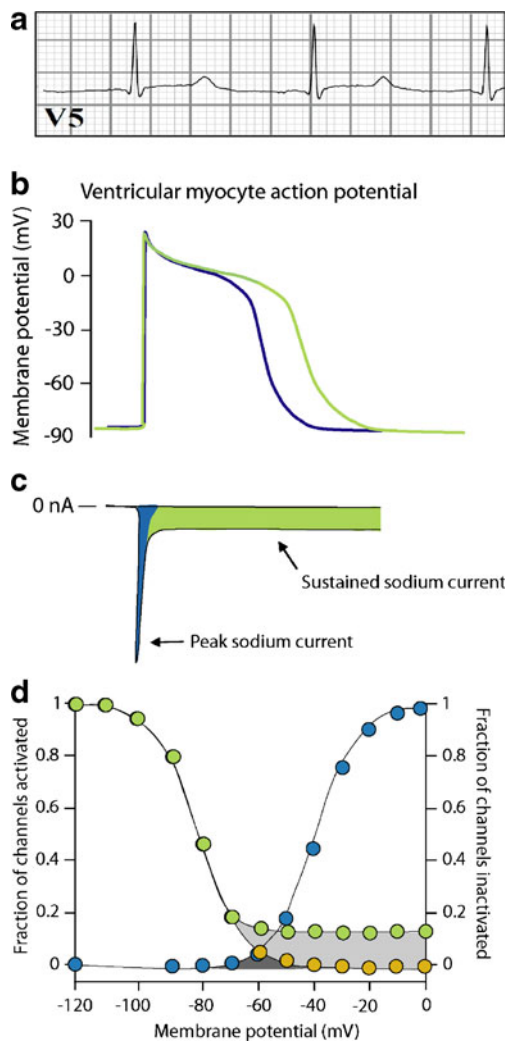
fraction of channels that is not inactivated by the prepulse is measured by applying a voltage step to 20 mV (test pulse). The inactivation curve is obtained by dividing the amplitude of sodium current at each test pulse by the maximum peak sodium current amplitude, and plotting versus the corresponding prepulse voltage. **c** The window current (*gray area*) arises when the sarcolemma reaches a potential that is depolarized sufficiently to reactivate some channels, but not enough to cause complete inactivation. **d** The voltage range for the window current is normally narrow and achieved during phase 3 of the ventricular action potential

$\text{Na}_v1.5$  in the sarcolemma, augment the amplitude of  $I_{\text{Na}}$ , modulate its gating properties, and play crucial roles in the interaction of  $\text{Na}_v1.5$  proteins to extracellular matrix molecules, cytoplasmic cytoskeleton apparatus, and components of cardiac intercellular junctions (e.g., cadherins, connexins). The  $\beta$ -subunits may also contribute to the preferential localization of  $\text{Na}_v1.5$  proteins in the intercalated disks.  $\text{Na}_v1.5$  proteins may also directly interact with several regulatory proteins, including enzymes involved in their glycosylation and phosphorylation (e.g.,  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II) [1, 77], adaptor proteins that connect them to the cytoskeleton (e.g., ankyrins) [77], and proteins that mediate their trafficking from the endoplasmic reticulum (ER) to the sarcolemma (e.g., glycerol-3-phosphate dehydrogenase 1-like protein) [39]. Of note, mutations in genes encoding  $\beta$ -subunits and regulatory proteins are occasionally found in patients with clinical phenotypes similar to arrhythmogenic diseases caused by *SCN5A* mutations. This reflects the importance of these

proteins for the normal functioning of the cardiac sodium channel [5].

### Long QT syndrome type 3

Long QT syndrome (LQTS) is a disease characterized by prolonged QT intervals on the surface electrocardiogram (ECG), and increased risk for sudden death due to ventricular tachyarrhythmias, in particular torsades de pointes. Prolonged QT intervals reflect increased action potential durations in ventricular myocytes, and correspond with delayed ventricular repolarization (Fig. 4a, b). Heritable LQTS is classified into different types, and each type is linked to mutations in a gene encoding a protein that is directly (ion channel) or indirectly ( $\beta$ -subunit or regulatory protein) involved in repolarization [50]. LQT-3 is linked to mutations in *SCN5A*, and covers approximately 13% of all genotyped individuals with LQTS [31, 87]. So far, more than



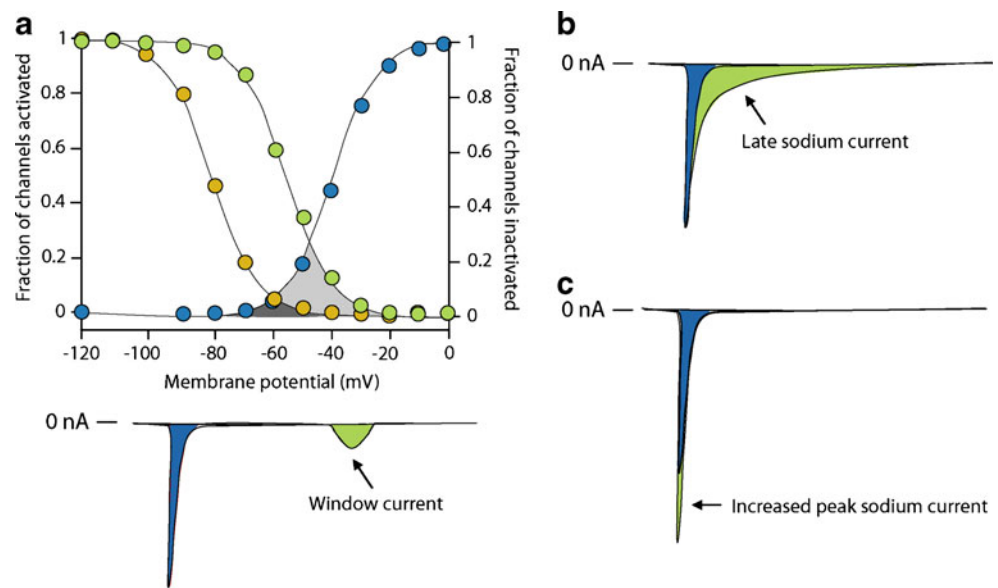
**Fig. 4** Long QT syndrome type 3. **a** Prolonged QT intervals on the surface ECG of an individual with LQT-3. **b** QT interval prolongation results from delayed repolarization of ventricular action potentials. **c** Delayed repolarization in LQT-3 is often due to the presence of abnormal sustained non-inactivating sodium current (green area). **d** Sustained current results from incomplete inactivation of the sodium channels (green circles)

80 *SCN5A* mutations have been identified in patients with LQT-3, and nearly 50% of them have been studied heterologously [93]. Most of these mutations are missense mutations, and are found to cause sodium channel gain-of-function, by disrupting fast inactivation and thereby causing an abnormal sustained (or persistent) non-inactivating sodium current ( $I_{\text{sus}}$  or  $I_{\text{pst}}$ ; Fig. 4c, d) [13]. Mutations causing  $I_{\text{sus}}$  are mainly clustered in  $\text{Na}_v1.5$  regions that are involved in fast inactivation (i.e., S4 segment of DIV, the DIII–DIV linker, and the cytoplasmic loops between the S4 and S5 segments of DIII and DIV), or in regions that stabilize fast inactivation (e.g., the C terminus) [31, 52, 76, 93]. The C terminus probably interacts with the DIII–DIV linker to stabilize the occlusion of the pore during inactivation [60].

Other less common mechanisms of *SCN5A* mutations to cause LQT-3 include increased window current [86, 90], slower inactivation [66, 90], faster recovery from inactivation [2, 19], and larger peak  $I_{\text{Na}}$  density [64]. The window current increases when inactivation of mutant sodium channels occurs at more positive (depolarized) potentials (i.e., delayed inactivation), while activation is not changed. This widens the voltage range during which the sodium channel may reactivate without inactivation (Fig. 5a) [86, 90]. Slower inactivation allows longer channel openings, and causes a slowly inactivating sodium current (Fig. 5b) [66, 90]. To distinguish it from  $I_{\text{sus}}$  (which does not inactivate), this current is called the late sodium current ( $I_{\text{NaL}}$ ) in this review. However, in the literature, the terms  $I_{\text{sus}}$ ,  $I_{\text{pst}}$ , and  $I_{\text{NaL}}$  are often used interchangeably. Comparable to  $I_{\text{sus}}$ , both the window current and  $I_{\text{NaL}}$  exert their effects during phases 2 and 3 of the action potential, where normally no or very small sodium current is present. However, the other two mechanisms (i.e., faster recovery from inactivation and larger peak  $I_{\text{Na}}$  density) involve increased influx of sodium ions during phase 0 of the action potential (Fig. 5c). Faster recovery from inactivation leads to larger peak  $I_{\text{Na}}$  by increasing the fraction of channels available for activation during subsequent depolarizations. Finally, larger  $I_{\text{Na}}$  density may result from increased expression of mutant  $\text{Na}_v1.5$  through enhanced mRNA translation or protein trafficking to the sarcolemma, decreased protein degradation, or altered modulation by  $\beta$ -subunits and regulatory proteins. Importantly, one single *SCN5A* mutation may cause several changes in the expression and/or gating properties of the resulting sodium channels. Regardless of the mechanism, increased sodium current ( $I_{\text{sus}}$ , window current,  $I_{\text{NaL}}$ , or peak  $I_{\text{Na}}$ ) upsets the balance between depolarizing and repolarizing currents in favor of depolarization. The resulting delay in the repolarization process triggers early afterdepolarizations (i.e., re-activation of L-type calcium channel during phases 2 or 3 of the action potential), especially in Purkinje fiber myocytes where action potential durations are intrinsically longer [22, 27]. Early afterdepolarizations are believed to induce torsades de pointes [92].

Arrhythmic events in LQT-3 usually occur at rest or during sleep when the heart rate is slow. Accordingly,  $I_{\text{sus}}$  is larger at slower stimulus frequencies, suggesting that the degree of  $I_{\text{sus}}$  may be a strong determinant for arrhythmias to occur [70]. This is confirmed by a report of homozygous carriers of an *SCN5A* mutation who displayed longer QT intervals and more frequent arrhythmias than heterozygous carriers of the same mutation. Consistently, homozygous expression of the mutation in heterologous system caused larger  $I_{\text{sus}}$  densities than heterozygous expression of the mutation [40]. The definite role of  $I_{\text{sus}}$  in LQT-3 is further reflected by the effectiveness of drugs that inhibit  $I_{\text{sus}}$  in the treatment of patients with LQT-3. Such drugs (e.g., ranola-

**Fig. 5** Alternative mechanisms of sodium channel gain-of-function in long QT syndrome type 3. **a** Increased window current due to delayed inactivation of cardiac sodium channels (green circles). Increased window current is carried at potentials corresponding to phases 2 and 3 of the ventricular action potential (green area), remote from the peak sodium current during phase 0 (blue area). **b** Slower inactivation creates a late sodium current (green area). **c** Increased peak sodium current



zine, mexiletine, flecainide) shorten QT intervals in patients with LQT-3 by preferentially blocking  $I_{\text{sus}}$  over peak  $I_{\text{Na}}$  [11, 51, 69]. However, this effect may be mutation specific. Mexiletine has been shown to be especially effective in patients with *SCN5A* mutations that shift inactivation toward more negative potentials (i.e., earlier inactivation) [66]. Moreover, in high concentrations these drugs may also block peak  $I_{\text{Na}}$ , and exert pro-arrhythmic effects by decreasing cardiac excitability and slowing electrical conduction velocity. Finally, beta-blockers (the cornerstone therapy in most long QT syndrome patients, particularly those with LQTS type 1 and, somewhat less so, in LQTS type 2) seem to be less effective in LQT-3 [70]. This may be due to their pro-arrhythmic effect by slowing the heart rate and consequently increasing  $I_{\text{sus}}$ , which opposes their anti-arrhythmic effect (i.e., preferential block of  $I_{\text{sus}}$  by direct binding to  $\text{Na}_v1.5$ ). The anti-arrhythmic effect of beta-blockers in LQT-3 may also be mutation-specific (e.g., when inactivation occurs at more negative potentials), and limited to some agents (propranolol, carvedilol, but not metoprolol) [10].

#### LQTS and mutations in sodium channel regulatory proteins

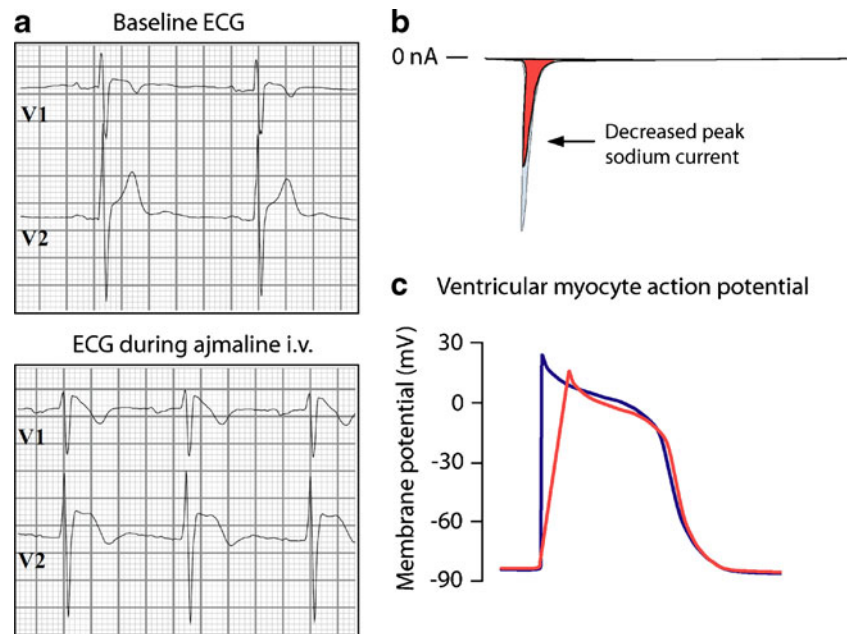
Three less common types of LQTS are caused by mutations in genes encoding proteins that regulate the expression or function of  $\text{Na}_v1.5$  proteins. A mutation in *SCN4B*, encoding the  $\beta 4$ -subunit, has been linked to LQTS type 10. When co-expressed heterologously with *SCN5A*, the mutation shifted the inactivation toward more positive potentials, but did not change the activation. This resulted in increased window currents at membrane potentials corresponding to the phase 3 of the action potential [48]. Mutations in *CAV3*, encoding caveolin-3, are linked to LQTS type 9 (LQT-9). Caveolin-3 co-localizes and interacts

with  $\text{Na}_v1.5$  proteins at the sarcolemma of cardiomyocytes (Fig. 2b). When co-expressed with *SCN5A*, the mutant caveolin-3 proteins induce  $I_{\text{sus}}$  through a yet unknown mechanism [81]. Finally, a mutation in *SNTA1* has been linked to LQTS type 12. *SNTA1* encodes  $\alpha 1$ -syntrophin, a cytoplasmic adaptor protein that enables the interaction between  $\text{Na}_v1.5$ , nitric oxide synthase (NOS), and sarcolemmal calcium ATPase (PMCA). PMCA inhibits nitric oxide synthesis by NOS. By inducing nitrosylation of  $\text{Na}_v1.5$  proteins, nitric oxide decreases channel inactivation and increases  $I_{\text{sus}}$  densities. The mutation in *SNTA1* disrupted the interaction between  $\text{Na}_v1.5$  and PMCA, and consequently caused increased  $\text{Na}_v1.5$  nitrosylation and  $I_{\text{sus}}$  densities [77].

#### Brugada syndrome

BrS is diagnosed in subject with high risk for sudden death due to ventricular tachyarrhythmias (polymorphic ventricular tachycardia and ventricular fibrillation), accompanied by typical coved-type ST segment elevation in the right-precordial ECG leads  $V_1$  to  $V_3$  [16]. The ECG changes are often concealed, but can be unmasked by a drug challenge test using Class 1A or 1C anti-arrhythmic drugs (e.g., ajmaline, flecainide, pilsicainide), which block the cardiac sodium channel (Fig. 6a) [91]. This indicates the crucial role of  $I_{\text{Na}}$  inhibition in the pathophysiology of BrS. Accordingly, 10–30% of subject with BrS carry a mutation in *SCN5A*, including missense mutations, nonsense mutations, and nucleotide deletions or insertions [91, 93]. The latter may alter mRNA splicing or create a premature stop codon by shifting the open reading frame, and result in truncated  $\text{Na}_v1.5$  proteins. More than 100 *SCN5A* mutations are linked

**Fig. 6** Brugada syndrome. **a** Coved-type ST segment elevation in the right-precordial ECG leads V<sub>1</sub> and V<sub>2</sub> after intravenous (i.v.) administration of sodium channel blocking drug ajmaline in an individual with BrS. **b** BrS-linked *SCN5A* mutations often lead to peak sodium current reduction. **c** Reduced peak sodium current decreases the upstroke velocity of action potential phase 0, which slows cardiac electrical conduction

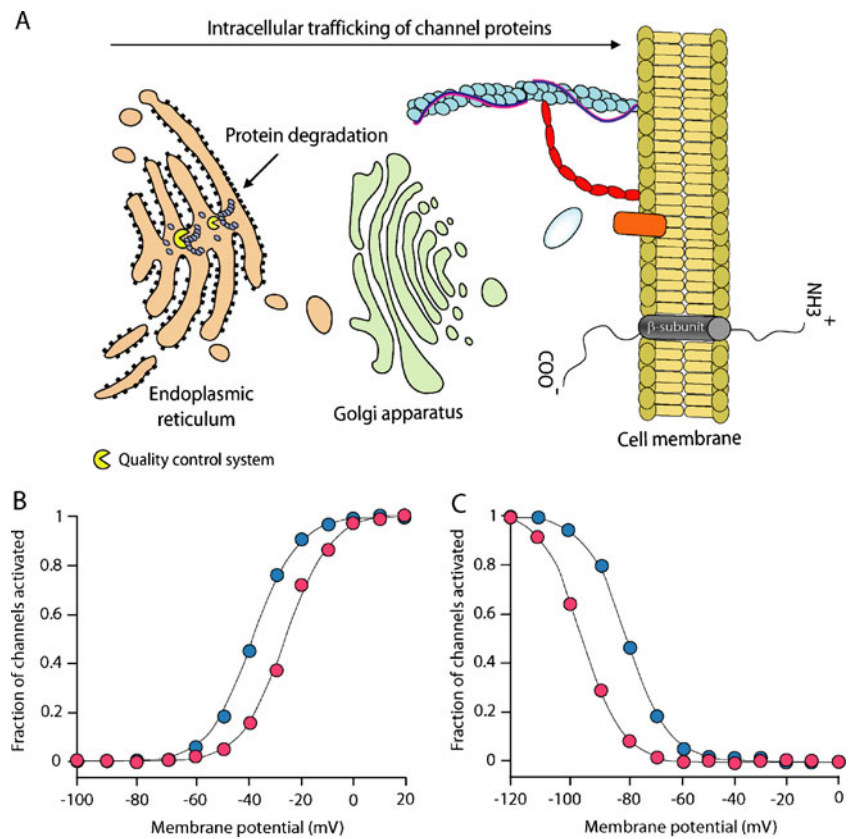


to BrS. Virtually all mutations that are heterologously expressed (<50%) lead to sodium channel loss-of-function (i.e., reduced peak  $I_{Na}$ ; Fig. 6b). The underlying mechanisms include decreased expression of  $Na_v1.5$  proteins [78], expression of non-functional channels [35], and altered gating properties [6, 15, 21, 73]. Decreased sarcolemmal expression of  $Na_v1.5$  proteins results from premature degradation of the mutant proteins by the quality control system in the ER (Fig. 7a) [78]. Expression may also be decreased because mutant  $Na_v1.5$  proteins fail to interact with  $\beta$ -subunits or regulatory proteins, which mediate their normal localization on the sarcolemma. Some mutant  $Na_v1.5$  proteins pass the ER quality control system and traffic normally to the sarcolemma, but form channels that conduct no or very small  $I_{Na}$  (non-functional) [35]. The third mechanism (altered gating properties) comprises delayed activation (i.e., activation at more positive potentials) [73], earlier inactivation (i.e., inactivation at more negative potentials) [15], faster inactivation [21], and enhanced slow inactivation [6]. Delayed activation, earlier inactivation, and faster inactivation reduce  $I_{Na}$  by decreasing the probability of the channels to reside in the activated state (Fig. 7b, c). Enhanced slow inactivation means that mutant channels preferentially enter into the slow inactivation state. As mentioned earlier, the slow inactivation state requires relatively long recovery times during the action potential phase 4. At fast heart rates, phase 4 becomes too short for such channels to recover completely from slow inactivation. This leads to an accumulation of the channels in the slow inactivation state, and  $I_{Na}$  reduction. Although BrS-linked *SCN5A* mutations are in general randomly located in various regions of  $Na_v1.5$ , mutations that cause enhanced slow

inactivation are mainly located in the P-loops, the S6 segments, and the C terminus [93].

$I_{Na}$  reduction decreases the upstroke velocity of action potential phase 0, and, as a result, slows atrial and ventricular electrical conduction (Fig. 6c). This is often reflected by prolonged atrioventricular and ventricular conduction intervals (PR and QRS intervals, respectively) on the ECGs of BrS patients with an *SCN5A* mutation (Fig. 6a) [91]. During electrophysiological studies in such patients, electrical conduction is particularly delayed between the His bundle and the ventricles (HV interval prolongation), indicating the importance of  $I_{Na}$  for the initiation and propagation of action potentials in Purkinje fiber myocytes and the ventricular conduction system [72]. The right-precordial ST segment elevation is less well understood, and explained by two hypotheses. The first hypothesis focuses on the presence of transmural voltage gradients due to heterogeneity in action potential duration between the right ventricular epicardium and endocardium. Indeed, action potential durations are shorter in the epicardium, where the repolarizing transient outward potassium current ( $I_{to}$ ) is more prominently expressed.  $I_{Na}$  reduction would further shorten epicardial action potential durations, and facilitate reentrant excitation waves between depolarized endocardium and prematurely repolarized epicardium [7]. The second hypothesis involves preferential conduction slowing in the right ventricular outflow tract. Regional differences in conduction velocity in the right ventricular epicardium would be aggravated by  $I_{Na}$  reduction, and trigger the occurrence of epicardial reentrant excitation waves [49]. This hypothesis is supported by the increased prevalence of mild (subclinical) structural abnormalities in the right ventricles of BrS patients

**Fig. 7** Mechanisms of sodium channel loss-of-function in Brugada syndrome, progressive cardiac conduction disease, and sick sinus syndrome.  
**a** Decreased sarcolemmal expression of  $\text{Na}_v1.5$  due to premature degradation of mutant channel proteins by the quality control system in the endoplasmic reticulum.  
**b** Delayed activation of cardiac sodium channels (red circles).  
**c** Earlier inactivation of cardiac sodium channels (red circles)



(e.g., inflammation, fatty tissue replacement, and fibrosis) [24]. Regardless of the mechanism, since ST segment elevation is equally seen in *SCN5A* mutation carriers and non-carriers, other genetic variants (e.g., in genes encoding ion channels or regulatory proteins) and exogenous factors (e.g., electrolyte imbalances, hormones, body temperature) may contribute to the pathophysiology of BrS [4, 30, 46, 74].

Arrhythmic events in BrS occur more frequently in men, and at rest or during sleep. Experimental studies suggest that the effects of gender on the BrS phenotype may be due to intrinsic differences in ion channel expression between men and women (e.g., higher  $I_{to}$  densities in men) or due to differences in hormone levels (e.g., higher testosterone levels in men) [7, 74]. The latter is confirmed by the disappearance of typical BrS ECG changes after castration [46]. The occurrence of arrhythmias during sleep may be due to an increased vagal activity and/or decreased sympathetic activity. This is confirmed in BrS patients by right-precordial ST segment elevation following intracoronary injection of acetylcholine, and decreased levels of norepinephrine in the synaptic cleft on positron emission tomography [32, 54]. Moreover, the typical ECG changes and arrhythmias in BrS may also be triggered by fever [4]. Although the mechanism is not fully understood, some *SCN5A* mutations have been shown to alter the gating properties of cardiac sodium channels in a temperature-dependent manner, e.g., more slow inactivation at higher temperatures [6, 21]. Finally,

the BrS ECG changes are reported to worsen during exercise. This may be partially attributed to an enhanced slow inactivation in mutant channels, leading to an accumulation of the mutant channels in the slow inactivation state at fast heart rates. However, other factors (e.g., autonomic nervous system, ion current imbalances) may also play a role [3].

So far, no mutation-specific therapy is available for BrS. Decreased sarcolemmal expression of mutant  $\text{Na}_v1.5$  proteins can be restored with cardiac sodium channel blocking drugs (e.g., mexiletine). Such drugs bind to mutant proteins, and act as molecular chaperones to rescue their trafficking from the ER to the sarcolemma [78]. However, it is questionable whether these drugs can be used as therapy, because (once expressed on the sarcolemma) the mutant proteins display arrhythmia-causing gating defects (e.g.,  $I_{sus}$ ) [58]. Moreover, since sodium channel blocking drugs reduce  $I_{Na}$ , they may aggravate ECG changes or trigger arrhythmias in BrS, and should therefore be avoided [30, 59]. An implantable cardioverter defibrillator and adequate treatment of fever are currently the only effective therapies to prevent sudden death in BrS.

#### BrS and mutations in sodium channel regulatory proteins

$I_{Na}$  reduction in BrS may also be due to mutations in genes encoding  $\beta$ -subunits or regulatory proteins of the cardiac sodium channel. A mutation in *SCN1B* was found in one



family with BrS. When heterologously expressed, the mutation resulted in formation of truncated  $\beta 1$ -subunits, which failed to interact with  $\text{Na}_v 1.5$  proteins and to increase  $I_{\text{Na}}$  densities as normal  $\beta 1$ -subunits did [89]. A missense mutation in *SCN3B* was found in one subject with BrS. The mutation reduced  $I_{\text{Na}}$  by disrupting the trafficking of  $\text{Na}_v 1.5$  proteins from the ER to the sarcolemma, and by altering the gating properties (e.g., earlier and faster inactivation) [29]. Finally, a mutation in *GPD1-L*, encoding glycerol-3-phosphate dehydrogenase 1-like protein (GPD1-L), was found in a family with BrS [39]. Recently, GPD1-L was shown to inhibit the phosphorylation of  $\text{Na}_v 1.5$  proteins at residues where phosphorylation would lead to  $I_{\text{Na}}$  reduction (probably by decreasing the sarcolemmal expression of  $\text{Na}_v 1.5$  proteins). The mutant GPD1-L failed to inhibit the phosphorylation of  $\text{Na}_v 1.5$  proteins, and resulted in reduced  $I_{\text{Na}}$  densities [79].

### Progressive cardiac conduction disease

PCCD (also called Lev or Lenègre disease) manifests as progressive prolongation of the conduction parameters (P wave, PR, and QRS intervals), and right or left bundle branch block, without ST segment elevation or QT interval prolongation (Fig. 8). A complete atrioventricular block may develop and cause syncope or sudden death. The ECG changes indicate slowing of electrical conduction through the atria, atrioventricular node, His bundle, Purkinje fibers, and ventricles, accompanied by an age-related degenerative process, in which fibrosis affects only the cardiac conduction system. In heritable PCCD, conduction slowing may be attributed to loss-of-function mutations in *SCN5A*. The mutations are reported to reduce  $I_{\text{Na}}$  by decreasing the expression of channel proteins on the sarcolemma, expressing non-functional channels, or altering the gating properties (e.g.,



**Fig. 8** Prolonged conduction parameters (P wave, PR and QRS intervals), and right bundle branch block in an individual with progressive cardiac conduction disease

delayed activation, earlier inactivation, enhanced slow inactivation, or slower recovery from inactivation) [63, 67, 75, 93]. These loss-of-function mechanisms are also described for BrS-linked *SCN5A* mutations (Fig. 7). Moreover, a single *SCN5A* mutation may cause PCCD alone (isolated) or PCCD combined with BrS (overlap syndrome) [35, 73]. This suggests that, in addition to  $I_{\text{Na}}$  reduction, which is enough to cause conduction slowing in PCCD or BrS, other factors are required for the development of the typical right-precordial ST segment elevation in BrS.

Whether the age-dependent fibrosis of the conduction system is a primary degenerative process in PCCD, or a physiologic process that is accelerated by  $I_{\text{Na}}$  reduction remains to be investigated. However, the latter is suggested by histological studies, in which fibrosis was found in cardiac biopsies of BrS patients [24], and animal studies in heterozygous *SCN5A* knockout mice (50%  $I_{\text{Na}}$  reduction), in which conduction defects and myocardial fibrosis that deteriorated progressively with age were observed [65]. So far, no molecular therapy for PCCD exists and the implantation of a pacemaker is the most effective treatment.

### PCCD and mutations in sodium channel regulatory proteins

Two loss-of-function mutations in *SCN1B* have been identified in patients with PCCD who carried no mutation in *SCN5A*. One mutation resulted in truncated  $\beta 1$ -subunits that failed to interact with  $\text{Na}_v 1.5$  proteins, to increase  $I_{\text{Na}}$  densities and to alter the gating properties as normal  $\beta 1$ -subunits did. The other mutation decreased the ability of normal  $\beta 1$ -subunits to increase  $I_{\text{Na}}$  densities and to facilitate the activation of the co-expressed sodium channels [89]. Interestingly, normal and mutant  $\beta 1$ -subunits were expressed to a higher degree in the Purkinje fibers than in the ventricles. This indicates that  $I_{\text{Na}}$  reduction in Purkinje fiber myocytes may underlie prolongation of PR and QRS intervals, and right or left bundle branch block in PCCD.

### Dilated cardiomyopathy

DCM is characterized by ventricular dilatation and impaired systolic function, which may proceed into congestive heart failure. Although DCM is a final common pathway of various acquired diseases, up to 50% of cases are reported to be idiopathic (i.e., without any obvious aetiological trigger). Approximately 20% of idiopathic DCM cases display familial prevalence, and have been linked to mutations in various genes that encode proteins involved in the contractile apparatus and the cytoskeleton. Initially, linkage analysis in a large family with DCM mapped the disease locus to a region on the short arm of chromosome 3 (3p22-p25), which harbors the *SCN5A* gene [55]. Afterwards, a missense mutation in

*SCN5A* was associated with the disease phenotype in this family [47]. Since then, several other missense and truncation mutations in *SCN5A* have been linked to DCM [25, 53, 56]. Remarkably, most of these mutations are found in patients who display multiple phenotypes, including DCM, sinoatrial node dysfunction, atrial flutter, atrial fibrillation, atrioventricular block, bundle branch block, ventricular tachycardia, and/or ventricular fibrillation. When expressed heterologously, DCM-linked *SCN5A* mutations usually do not disrupt the sarcolemmal expression of channel proteins, but cause diverse loss-of-function and gain-of-function changes in their gating properties (e.g., earlier or delayed activation, earlier inactivation, disrupted fast inactivation, and increased  $I_{NaL}$  or window current) [25, 53]. Although it remains unclear how such divergent gating changes lead to DCM, it is speculated that *SCN5A* mutations in DCM disrupt the interactions between cardiac sodium channels and intracellular (or extracellular) proteins that are essential for normal cardiomyocyte structure and architecture. However, it must be noted that mutations linked to DCM cases with conduction defects often cause  $I_{Na}$  reduction. This is supported by reduced *SCN5A* gene transcription and  $I_{Na}$  densities in a mouse model with DCM that also displayed prolonged conduction parameters [28]. No molecular therapy for DCM exists, and the standard therapy for congestive heart failure is applied.

### Sick sinus syndrome

SSS encompasses various forms of arrhythmia that result from sinoatrial node dysfunction. Arrhythmias may be transient, are diagnosed electrocardiographically, and include sinus bradycardia, sinus arrest, and atrial tachycardia–bradycardia syndrome (i.e., atrial tachycardia alternating with episodes of sinus bradycardia; Fig. 9). Patients may suffer from syncope and require lifelong pacemaker therapy. Heritable SSS is associated with loss-of-function mutations in *SCN5A*, and often linked to compound heterozygous mutations in patients with severe symptoms at relatively young age [14].  $I_{Na}$  reduction results from decreased sarcolemmal expression of mutant channel proteins, expression of non-functional channels, and altered gating properties (e.g., delayed activation, earlier inactivation;

Fig. 7) [14, 37]. Not surprisingly, SSS may manifest concomitantly with other phenotypes that are linked to *SCN5A* loss-of-function mutations (i.e., BrS, PCCD) [37, 44, 73].

Pacemaker cells in the sinoatrial node reach the voltage range for the sodium window current during action potential phase 4. The small inward window current contributes to the gradual depolarization of the sarcolemma of these cells, and allows spontaneous action potential generation, which underlies cardiac automaticity. In addition to their contribution to cardiac pacemaker activity, sodium channels also play an essential role in the propagation of action potentials from the central area of the sinoatrial node through its peripheral regions to the surrounding atrial muscle [37, 84]. Experimental studies in heterozygous *SCN5A* knockout mice suggest that the main mechanism of  $I_{Na}$  reduction to cause SSS indeed involves reduced automaticity, and conduction slowing or blocking of action potentials from the sinoatrial node to the surrounding atrial muscle [36]. Interestingly, LQT-3 patients with *SCN5A* gain-of-function mutations may also suffer from sinus bradycardia and sinus arrest. Computer simulation models showed that  $I_{susc}$  in pacemaker cells may decrease sinus rate and induce sinus arrest by delaying the repolarization and prolonging action potential durations [83].

### Atrial fibrillation

AFib is the most prevalent clinically relevant cardiac arrhythmia, and is characterized by a disorganized electrical activation of the atria. It usually affects elderly patients with structural heart disease. However, AFib may also occur in young patients with structurally normal hearts (i.e., lone AFib), and display a familial occurrence. *SCN5A* mutations were first linked to lone AFib in patients with DCM [56]. Next, a common polymorphism (H558R) in *SCN5A* was found more often in patients with lone AF than matched controls. Of note, H558R is known to reduce  $I_{Na}$  in heterologous systems [18]. Subsequently, an *SCN5A* loss-of-function mutation was identified in a family with lone AFib [23]. It was speculated that  $I_{Na}$  reduction may predispose to AFib by slowing the electrical conduction velocity. Conduction slowing is an essential determinant for

**Fig. 9** Sinus arrest in an individual with sick sinus syndrome. The patient also suffered from sinus bradycardia



maintaining reentrant excitation waves in the atria. This mechanism is supported by an increased prevalence of lone AFib, accompanied with prolonged atrial conduction intervals (e.g., P wave duration), in BrS patients [34]. Nevertheless, two *SCN5A* gain-of-function mutations have also been linked to lone AFib. Interestingly, both mutations were found to induce gain-of-function by delaying channel inactivation [38, 43]. In one mutation, larger  $I_{Na}$  densities corresponded with increased atrial excitability in mutation carriers [43]. The other mutation induced spontaneous action potential formation when expressed in atrial cardiomyocytes [38]. Thus, loss-of-function mutations in *SCN5A* predispose to AFib by facilitating the maintenance of reentrant excitation waves, while gain-of-function mutations may initiate AFib by increasing atrial excitability.

Although both gain-of-function mutations discussed above did not prolong QT intervals, another *SCN5A* gain-of-function mutation has recently been described in a family with LQT-3 and lone AFib. The mutation was shown to induce gain-of-function by causing  $I_{sus}$ , suggesting that  $I_{sus}$  may promote AFib by prolonging action potential duration and triggering EADs. Importantly, through inhibition of  $I_{sus}$ , flecainide not only shortened the QT intervals in the affected family members, but also effectively restored the normal sinus rhythm [12]. In contrast, flecainide therapy in patients with AFib due to *SCN5A* loss-of-function mutations may be contraindicated, because flecainide also blocks the peak  $I_{Na}$ , and therefore may aggravate  $I_{Na}$  reduction in these patients.

#### AFib and mutations in sodium channel regulatory proteins

Recently, mutations in *SCN1B* and *SCN2B* (encoding the  $\beta$ 1- and  $\beta$ 2-subunits of the cardiac sodium channel, respectively) have been identified in patients with lone AFib. Interestingly, the reported patients with AFib and mutations in *SCN1B* or *SCN2B* often displayed an ECG pattern suspect for BrS. When heterologously co-expressed with *SCN5A*, the mutant  $\beta$ 1-subunits failed to increase  $I_{Na}$  as normal  $\beta$ 1-subunits did, and caused delayed channel activation. Mutant  $\beta$ 2-subunits also induced loss-of-function, but only by delaying channel activation [88].

#### Sudden infant death syndrome

SIDS is diagnosed when an infant under the age of 1 year suddenly and unexpectedly dies, and when a detailed review of the clinical history, extensive examination of the death scene, and a complete medical autopsy fail to provide an explanation for the death. Although various exogenous factors are recognized to increase the risk for SIDS (e.g., tobacco and alcohol use by the mother, low socioeconomic

status, prone sleeping), higher SIDS rates in some ethnicities suggest a role for genetic factors in the development of SIDS. Initially, this suggestion was reinforced when SIDS was linked to prolonged repolarization intervals as seen in LQTS [71]. This was followed by anecdotal reports linking SIDS to gain-of-function mutations in *SCN5A* and to mutations that were previously associated with LQT-3 [68, 90]. Subsequently, postmortem genetic testing in a population-based cohort indicated that gain-of-function mutations in *SCN5A* may be the most prevalent genetic cause of SIDS [8]. *SCN5A* mutations in SIDS commonly increase  $I_{sus}$ , mostly in combination with altered gating properties that result in  $I_{Na}$  gain-of-function (e.g., earlier activation, delayed inactivation, or increased window current). Interestingly, some SIDS-linked *SCN5A* mutations display only  $I_{sus}$  under acidic conditions, supporting the role of exogenous factors (here acidosis) in the development of SIDS [85]. Less frequently, *SCN5A* loss-of-function mutations have also been found in infants with SIDS [57]. However, it may be possible that in these patients SIDS represents a severe form of BrS that manifests during early childhood [62].

#### SIDS and mutations in sodium channel regulatory proteins

Recently, postmortem genetic testing in population-based cohorts identified mutations in *CAV3* and *GPD1-L* as possible causative genetic factors in the development of SIDS [20, 80]. As mentioned earlier, mutations in *CAV3* are linked to LQT-9, and mutations in *GDPL-1* are found in patients with BrS [39, 81]. When co-expressed heterologously with *SCN5A*, SIDS-linked mutations in *CAV3* or *GDPL-1* exerted similar effects on the cardiac sodium channel as their equivalents in LQT-9 or BrS, respectively (i.e., increased  $I_{sus}$  in the presence of mutant *CAV3* proteins, and decreased peak  $I_{Na}$  in the presence of mutant *GDPL-1* proteins). These data support the hypothesis that SIDS may be a severe form of LQTS or BrS during infancy.

#### Overlap syndromes

Overlap syndromes involve overlapping clinical symptoms or arrhythmias of various arrhythmogenic diseases (i.e., sodium channelopathies). The designation is also used when a mutation causes various arrhythmogenic phenotypes in different families or members of one family. Not surprisingly, *SCN5A* loss-of-function mutations have often been associated with overlapping phenotypes of BrS and PCCD or BrS and SSS [15, 35, 42, 44, 73, 83]. Since the underlying molecular mechanisms of these diseases implicate  $I_{Na}$  reduction, it is plausible that the clinical phenotype is determined by the degree of  $I_{Na}$  reduction, the

nature of altered gating properties, the presence of exogenous factors (e.g., electrolyte imbalance, drugs, hormones, body temperature, subclinical cardiac structural changes) and co-inherited genetic variants (e.g., polymorphisms in *SCN5A* or genes encoding regulatory proteins).

More surprisingly, some *SCN5A* mutations cause symptoms of both LQT-3 ( $I_{Na}$  gain-of-function) and BrS ( $I_{Na}$  loss-of-function) in members of one family, or LQT-3 in one family and BrS in another [15, 26, 42]. Although exogenous and genetic factors discussed before may play a role, such mutations are believed to alter gating properties in a manner that results in both  $I_{Na}$  gain-of-function and loss-of-function. For example, the insertion of an aspartic acid residue at position 1795 of the  $Na_v1.5$  protein (1795insD) was the first mutation found in a multigenerational family with ECG signs of both LQT-3 and BrS [15]. While QT interval prolongation was found to be caused by an increased  $I_{sus}$ , BrS was shown to be the result of delayed activation, earlier inactivation, and slower recovery from inactivation of the mutant channels [82]. Interestingly, while *SCN5A* mutations that are linked to LQT-3 often lead to increased  $I_{sus}$  levels, mutations linked to overlap syndromes (LQT-3 and BrS with or without SSS) usually also induce earlier inactivation (i.e., inactivation at more negative membrane potentials) and enhanced tonic block by flecainide [42]. Finally, administration of sodium channel blocking drugs may induce typical ECG signs of BrS in patients with LQT-3, limiting the use of such drugs to inhibit  $I_{sus}$  and restore delayed repolarization in these patients [61].

## Conclusion

Cardiac sodium channelopathies that are described in this review emphasize the importance of  $I_{Na}$  for normal cardiac electrical activity. The association of most channelopathies to mutations not only in *SCN5A* but also in genes encoding the  $\beta$ -subunits or regulatory proteins indicates that, for normal functioning of the cardiac sodium channel, the contribution of various channel subunits is required. Most of our understanding of the molecular mechanisms of cardiac sodium channelopathies originates from experimental studies in heterologous expression system and transgenic mouse models. Although these models have greatly increased our knowledge of the structure and function of the cardiac sodium channel in normal hearts, and the nature and role of  $I_{Na}$  dysfunction in diseased hearts, they have by and large failed to explain the mechanism of different phenotypes or diseases caused by one single mutation in *SCN5A*. Since the native environment of cardiomyocytes is absent, the effects of intracellular and extracellular molecules on the function of cardiac sodium channels are greatly lost in heterologous expression models. Although this shortcoming is remedied in

transgenic mouse models, the absence of environmental factors and co-inherited genetic variants (e.g., polymorphisms, mutations in non-coding genetic regions) in mouse models remains a limitation. These limitations compel careful interpretation of the experimental data and their translation into the patient phenotypes, and make it clear that future research is needed to design more appropriate expression systems (e.g., cardiomyocytes derived from induced pluripotent stem cells). Future research is also needed to discover novel gene-specific and mutation-specific pharmacological therapies in the management of cardiac sodium channelopathies. Currently, molecular therapy for cardiac sodium channelopathies due to  $I_{Na}$  loss-of-function is lacking, and the efficiency of sodium channel blocking drugs to restore QT intervals in patients with LQT-3 seems to be limited to mutations that induce earlier inactivation of the sodium channels.

**Conflict of interest** The authors have declared that no conflict of interest exists.

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