Sodium channel dysfunction in inherited and acquired cardiac diseases
Casini, S.

Citation for published version (APA):
Casini, S. (2008). Sodium channel dysfunction in inherited and acquired cardiac diseases Masso delle Fate Edizioni

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

General Introduction

Simona Casini, Arthur A.M. Wilde, and Hanno L. Tan

Published in part as a book chapter in: “Electrical diseases of the heart: genetics, mechanisms, treatment, prevention”, edited by Ihor Gussak, Charles Antzelevitch, Arthur Wilde, Paul Friedman, Michael Ackerman, Win-Kuang Shen, Springer Verlag, 2008
List of abbreviations

AMP: adenosine monophosphate
ARVC: arrhythmogenic right ventricular cardiomyopathy
BrS: Brugada syndrome
CAMs: cell adhesion molecules
cAMP: cyclic adenosine monophosphate
CaMKII: calcium/calmodulin dependent protein kinase II
Cyto-D: cytochalasin-D
DADs: delayed afterdepolarizations
DGC: dystrophin glycoprotein complex
DMD: Duchenne muscular dystrophy
dV/dt_max: maximal action potential upstroke velocity
EADs: early afterdepolarizations
G-protein: guanine nucleotide-binding protein
Gαs: α-subunit of the guanine nucleotide-binding stimulatory (G) protein
HF: heart failure
ICD: implantable cardioverter defibrillator
I_Kr: rapid component of delayed rectifier potassium current
I_Na: sodium current
I_NaL: late sodium current
GTP: guanosine triphosphate
LQTS: long QT syndrome
Na_1.5: α-subunit of the cardiac sodium channel
MBD: membrane binding domain
PCCD: progressive cardiac conduction disease
PKA: protein kinase A
PKC: protein kinase C
SIDS: sudden infant death syndrome
SSS: sick sinus syndrome
SUDS: sudden unexplained death syndrome
RV: right ventricle
RVOT: right ventricle outflow tract
TdP: Torsade de Pointes
V_{1/2}: half-point potential
VF: ventricular fibrillation
Introduction

Sodium ion (Na\(^+\)) influx through cardiac Na\(^+\) channels is essential for cardiac excitation as it generates the action potential upstroke in cells of the working myocardium and the specialized conduction system. In consequence, Na\(^+\) channel function plays a major role in initiation, propagation and maintenance of the normal cardiac rhythm. Mutations in SCN5A, the gene encoding the \(\alpha\)-subunit of the cardiac Na\(^+\) channel, the so-called “inherited sodium channelopathies”, are known to evoke various life-threatening disorders of cardiac rhythm that can vary from tachyarrhythmias (fast heart rhythms) to bradyarrhythmias (slow heart rhythms) and may require implantation of pacemakers or implantable cardioverter/defibrillators (ICDs) to prevent sudden cardiac death \(^1\) (Table 1). At the same time, common sequence variants (polymorphisms) in the Na\(^+\) channel have also been implicated as risk factors in cardiac diseases and determinants of drug sensitivity \(^2\). Finally, recent studies have linked Na\(^+\) current dysfunction to structural cardiac defects, notably cardiac fibrosis, dilated cardiomyopathy and, possibly, arrhythmogenic right ventricular cardiomyopathy (ARVC). These structural changes may be conducive to life-threatening arrhythmias based on reentrant excitation \(^1\).

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 as well as our understanding of how Na\(^+\) channel dysfunction may act as a major pathophysiologic mechanism in various diseases, including common acquired diseases (Table 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 both SCN5A mutations and common variants, and concepts regarding arrhythmia susceptibility derived from studies of these conditions.
<table>
<thead>
<tr>
<th>Cardiac diseases</th>
<th>Reported changes in $I_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inherited Primary Electric Disease</strong></td>
<td></td>
</tr>
<tr>
<td>Atrial standstill</td>
<td>↓</td>
</tr>
<tr>
<td>Brugada syndrome</td>
<td>↓</td>
</tr>
<tr>
<td>LQT3 syndrome</td>
<td>↑</td>
</tr>
<tr>
<td>Progressive cardiac conduction disease</td>
<td>↓</td>
</tr>
<tr>
<td>SIDS</td>
<td>↑↓</td>
</tr>
<tr>
<td>Sick sinus syndrome</td>
<td>↓</td>
</tr>
<tr>
<td>SUDS</td>
<td>↓</td>
</tr>
<tr>
<td><strong>Structural Disease</strong></td>
<td></td>
</tr>
<tr>
<td>ARVC*</td>
<td>↓</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>↓</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>↓</td>
</tr>
<tr>
<td><strong>Acquired Disease</strong></td>
<td></td>
</tr>
<tr>
<td>Acquired Brugada syndrome</td>
<td>↓</td>
</tr>
<tr>
<td>Acquired LQTS</td>
<td>↑</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>↑↓</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>↑↓</td>
</tr>
</tbody>
</table>

*Table 1: ARVC, Arrhythmogenic Right Ventricular Cardiomyopathy; LQTS, Long QT syndrome, SIDS, Sudden Infant Death Syndrome; SUDS, Sudden Unexplained Death Syndrome; * not fully resolved, ↑ increased net $I_{Na}$, ↓ decreased net $I_{Na}$*
1. The cardiac sodium channel structure and function

The α-subunit pore-forming region

The pore-forming α-subunit of the cardiac Na⁺ channel (Naᵥ1.5), encoded by SCN5A, is the main component of the cardiac Na⁺ channel complex. Expression of SCN5A alone in heterologous expression systems is sufficient to generate Na⁺ current (I₉Na). The α-subunit consists of 4 homologous domains (D1–DIV), each composed of 6 membrane-spanning segments (S1-S6) linked by intracellular and extracellular loops (Fig. 1).

The S6 segments line the inner pore ³, ⁴, while their extracellular linkers (P-loops) fold back into the membrane and define the outer mouth of the pore ³,⁵. Four amino acids, aspartic acid (D), glutamic acid (E), lysine (K), and alanine (A), one in each P-loop, form the so-called DEKA ring. This ring is considered the selectivity filter of the Na⁺ channel and allows the distinction between different ions, favouring the passage of Na⁺ ions ⁶,⁷. Finally, the S4 segments, which are positively charged due to the presence of the amino acids arginine and lysine, act as a voltage sensor ⁸,⁹.

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 concert in the outward direction to allow for opening of the channel, a process called activation ⁸,⁹. This increase in Na⁺ permeability causes the sudden membrane depolarization that characterizes the rapid upstroke of the action potential. Activation of the channel lasts a few milliseconds and is followed by fast inactivation, a non-conducting state from which the channel cannot re-open. Membrane repolarization is necessary to allow Na⁺ channels to recover from inactivation to the resting state (closed state), from which they can reopen during the next cardiac cycle.
Fast inactivation is mediated by the intracellular linker between domains III and IV. The hydrophobic IFM motif (isoleucine-phenylalanine-methionine) is the amino acid sequence that binds to the inner vestibule of the pore via hydrophobic interactions, and occludes it (Fig. 1) \(^{10, 11}\). The DIIIS4-S5 and DIVS4-S5 linkers are believed to form the inactivation gate receptor for the IFM motif \(^{12-16}\). It was recently demonstrated that interactions between the C-terminus and the intracellular DIII-DIV linker are required to stabilize channel inactivation \(^{17, 18}\). These interactions play a critical role in the heart by preventing the occurrence of a small persistent (non-inactivating) inward \(\text{Na}^+\) current, also called late \(\text{Na}^+\) current (\(I_{\text{NaL}}\)). This current would prolong the action potential and render the heart susceptible to arrhythmias that are initiated by secondary depolarizations occurring before the cell has fully repolarized, so-called early afterdepolarizations (EADs) \(^{17, 18}\).

Beside this fast inactivation process (time frame: a few milliseconds), \(\text{Na}^+\) channels can undergo a slower inactivation process (slow inactivation) when the membrane remains depolarized for a longer time. That more stable, non-conducting, conformational state develops in cardiac \(\text{Na}^+\) channels in about 50-100 ms and requires a prolonged period of hyperpolarization to recover from \(^{19, 20}\). However, unlike activation and fast inactivation, the molecular basis of slow inactivation is still poorly understood. Serial mutagenesis and chimeric analysis suggest a key role of the P-loops \(^{21-23}\). A second hypothesis involves a rearrangement of amino acid residues within the putative pore–lining S6 segments that would constrict the inner pore region \(^{24, 25}\).

Finally, upon depolarization, closed-state inactivation (inactivation from the closed state without prior activation) may also occur \(^{26}\) and can be clinically relevant \(^{27}\). The regions involved in closed-state inactivation await identification.
Fig. 1. Schematic drawing representing the structure and modulation sites for the \( \alpha \)-subunit of the cardiac sodium channel (Na\(_1\).1.5) and its associated \( \beta_1 \)-subunit. The \( \alpha \)-subunit is composed of 4 homologous domains (DI–DIV), each composed of 6 membrane-spanning segments (S1-S6) linked by intracellular and extracellular loops. The positively charged segments S4 act as voltage sensor, while the P-loops form the selectivity filter. The IFM motif (isoleucine-phenylalanine-methionine), located in the DIII-DIV linker, is involved in fast inactivation, together with the DIIS4-S5 and DIVS4-S5 linkers, which act as fast inactivation docking sites. Syntrophin proteins regulate the Na\(^+\) channel by binding to the last three amino acids (serine-isoleucine-valine, SIV) of the C-terminus. The \( \beta_1 \)-subunit is formed by a single membrane-spanning segment, with a large extracellular portion and a smaller intracellular segment. The \( \beta_1 \) extracellular domain is critical for \( \alpha \)-\( \beta_1 \) interactions and cell adhesion, while the intracellular portion is involved in \( \alpha \)-\( \beta_1 \) interactions and ankyrin recruitment. Of note, phosphorylation of the tyrosine residue 181 (Y181), modulates the ankyrin-\( \beta_1 \) association. PKA, protein kinase A; PKC, protein kinase C.
1.2 The cardiac sodium channel as macromolecular complex

It is now clear that the $\alpha$-subunit of the cardiac $\text{Na}^+$ channel does not exist in isolation \textit{in vivo}, but in association with $\beta$-subunits, cytoskeleton proteins, cell adhesion molecules, extracellular matrix proteins, regulatory kinases and phosphatases. Proper functioning of these proteins and of their interactions appears to be crucial for $\text{Na}^+$ channel function and/or localization. Indeed, mutations in genes that encode for accessory proteins of the $\text{Na}^+$ channel macromolecular complex, for instance the $\beta_4$-subunit, the membrane protein caveolin-3, or the cytoskeleton protein ankyrin-B, can also cause cardiac rhythm diseases, such as sudden infant death syndrome (SIDS) and Long QT syndrome (LQTS). Finally, $\text{SCN5A}$ mutations that abolish the interactions between the $\text{Na}^+$ channel $\alpha$-subunit and ankyrin-G or those that alter the $\alpha$-$\beta_1$ subunit interactions may cause Brugada syndrome (BrS) and Long QT syndrome type 3 (LQT3), respectively. Thus, understanding how different proteins interact with each other and how alterations of a specific part of the macromolecular complex can lead to a certain disease, will help future development of drugs that are effective in the treatment of cardiac arrhythmias.

The regulatory role of $\beta$-subunits

The $\text{Na}^+$ channel $\alpha$-subunit interacts with smaller accessory proteins known as $\beta$-subunits. $\beta$-subunits are glycosylated proteins with a single sarcolemma-spanning segment, a large extracellular immunoglobulin (Ig)-like N-terminal domain, and a small intracellular C-terminal portion (Fig. 1). The Ig-like domain of $\beta$-subunits is structurally homologous to the Ig superfamily that also includes cell adhesion molecules (CAMs). So far, four $\beta$-subunit isoforms ($\beta_1$-$\beta_4$) have been identified, which are all present in the human heart. Moreover, alternative splice
variants have also been described for the β1-subunit, β1A and β1B. β1 and β3 share significant homology and are both non-covalently associated with the α-subunit. On the other hand, β2 and β4 are similar and linked to the α-subunit via disulfide bonds.

The role of β-subunits in modulating the cardiac Na+ channel is still under investigation. Co-expression of β1 with Na1.5 in heterologous expression systems (Xenopus oocytes and mammalian cells) has produced contrasting results. Reported effects of β1 on Na1.5 function range from no effect or increased peak I\textsubscript{Na} with no changes in gating, to significant shifts in voltage dependence of (in)activation, changes in the rate of recovery from inactivation, reduced slow inactivation, and reduced I\textsubscript{NaL}. Co-expression of β3 with Na1.5 has also been shown to increase I\textsubscript{Na} and change the kinetic properties of Na1.5, while co-expression of β2 resulted in no obvious effect on Na1.5 in vitro. The lack of any electrophysiological effect of the β2-subunit on Na1.5 contrasts with co-immunoprecipitation experiments which showed that β1 and β2 associate with Na1.5 in cardiac myocytes. A possible explanation could be that, differently from the in vivo situation, upon heterologous expression, Na1.5 does not associate with β2-subunit, or simply that the effects of β2 in vivo do not include regulation of Na1.5 expression and gating. Finally, co-expression of the β1-subunit with Na1.5 resulted in no changes in kinetic properties of Na1.5, while a negative shift of voltage dependence of inactivation and a slowed recovery from inactivation were recently described.

The availability of transgenic mice has allowed the study of the α-β subunits interactions in vivo. β2\textsuperscript{-/-} knockout mice showed severely disturbed neuronal Na\textsuperscript{+} channel expression and gating, but no cardiac phenotype was described, confirming that β2 might not affect cardiac Na\textsuperscript{+} channel function. In contrast, studies performed in β1\textsuperscript{-/-} mice showed that loss of β1 had both neurological and cardiac effects. β1\textsuperscript{-/-} mice exhibited longer

---

General Introduction
QT and RR intervals than wild-type mice\textsuperscript{51}. Electrophysiological investigation in ventricular myocytes isolated from $\beta_1^{-/-}$ mice revealed increased densities of both $I_{\text{Na}}$ and $I_{\text{NaL}}$ with no other changes in channel gating. A longer action potential duration and slower rate of action potential repolarization were also found, consistent with the QT prolongation phenotype and the increase in $I_{\text{NaL}}$\textsuperscript{51}. The finding that the absence of $\beta_1$ increased $I_{\text{Na}}$ density contrasts with most in vitro reports which show an increase of $I_{\text{Na}}$ in the presence of $\beta_1$. These discrepancies may be explained by the fact that the physiological effects of $\beta_1$ are cell-type specific and/or that heterologous expression systems do not fully recapitulate the situation in cardiac myocytes.

Confirming the key role of $\beta$-subunits in cardiac $\text{Na}^+$ channel regulation, a novel missense mutation (L179F) in $SCN4B$, the gene encoding the $\beta_4$ subunit, was recently identified in a 21-months-old girl affected by Long QT syndrome (LQT10)\textsuperscript{28}. Patch-clamp studies in HEK293 cells stably expressing Na\textsubscript{v}1.5 and transfected with this mutant $\beta_4$ subunit revealed increased $I_{\text{NaL}}$ compared to Na\textsubscript{v}1.5 expressed alone or together with the wild-type $\beta_4$ subunit\textsuperscript{28}.

In addition to modulation of $\text{Na}^+$ channel expression and gating, $\beta$-subunits appear also to play a role in cell adhesion, thereby functioning as a contact point between the intracellular and extracellular environments\textsuperscript{52}. In particular, $\beta_1$ and $\beta_2$ interact with the extracellular matrix proteins tenascin C and R, both expressed in brain and heart\textsuperscript{53, 54}, and with other CAMs, such as contactin and neurofascin 186, resulting in an enhanced expression of the neuronal $\text{Na}^+$ channel isoform Na\textsubscript{v}1.2\textsuperscript{55, 56}.

Finally, $\beta_1$ and $\beta_2$ associate with the cytoskeleton anchoring proteins ankyrin-G and ankyrin-B\textsuperscript{57-59}. $\beta_1$-ankyrin interactions appear to be modulated by phosphorylation of the intracellular tyrosine residue 181 ($\beta_1\text{Y181}$, Fig. 1). Mutation of this tyrosine residue to glutamate ($\beta_1\text{Y181E}$), mimicking phosphorylation by introducing negative charges, resulted in the
abolishment of β₁-mediated ankyrin recruitment in heterologous expression systems\textsuperscript{58}.

**The regulatory role of the cytoskeletal proteins actin and tubulin**

The cytoskeleton with its network of fibrous proteins determines cell morphology, and is essential for cellular motility, intracellular transport, and cell division. The cardiac cytoskeleton is mainly composed of microfilaments, intermediate filaments, and microtubules (Fig. 2). Globular actin subunits assemble into long filamentous polymers called F-actin. Two F-actin chains twist around each other and form the microfilaments. Microtubules are cylindrical polymers made of heterodimers of α- and β-tubulin. The intermediate filaments, so called because of their intermediate diameter between microfilaments and microtubules, consist of homodimers and/or heterodimers of several proteins, of which desmin is the most important, the protein that connects the sarcomeres and keeps them in register during contraction\textsuperscript{60,61}.

**Fig. 2. Schematic drawing representing the main components of the cellular cytoskeleton.** Microtubules, microfilaments and intermediate filaments form a network that keeps in place the different cellular organelles, participate in cell motility, cell division and intracellular signaling. Figure adapted from “Pathophysiology: Concepts of Altered Health States” 7\textsuperscript{th} Edition, by Carol Mattson Porth published by Lippincott Williams and Wilkins, 2004.
In pathological conditions, such as heart failure (HF) and ischemia, where Na\(^+\) channel function is often compromised (see the paragraphs entitled “congestive heart failure” and “ischemic heart disease”, below), derangement of the cytoskeleton is also present. In particular, increases in tubulin content and/or the degree of tubulin polymerization, along with desmin disorganization and lack of contractile proteins, are frequently observed in HF animal models and in explanted failing human hearts \(^{60,61}\). Moreover, disruption in contractile proteins and microtubules have been reported in ischemia \(^{62}\).

Experimental data on Na\(^+\) channel modulation by cytoskeleton proteins are still scarce, and this subject requires further investigation. Cytoskeleton integrity seems to be crucial for cardiac Na\(^+\) channel gating and expression \(^{63-65}\). Inhibition of F-actin polymerization with the use of cytochalasin-D (Cyto-D) mainly resulted in a decreased peak \(I_{Na}\) in rat ventricular myocytes \(^{63}\). Of note, \(I_{Na}\) reduction was also observed in ischemia \(^{66}\), a condition where disruption in contractile proteins has been observed \(^{62}\). Opposite effects on \(I_{Na}\) density were found with the use of colchicine, a drug that inhibits tubulin polymerization and increases the free tubulin concentration. Incubation of neonatal rat cardiomyocytes with colchicine caused an increase in \(I_{Na}\) density. The effect was guanine nucleotide-binding protein (G-protein)-dependent, since removal of guanosine triphosphate (GTP) from the patch pipette prevented \(I_{Na}\) enhancement by colchicine \(^{65}\).

It was proposed that the increase in \(I_{Na}\) occurred via GTP activation of the \(\alpha\)-subunit of the G-stimulatory protein (G\(\alpha\)s), in line with previous findings showing that, in rat ventricular myocytes, G\(\alpha\)s increases the number of functional Na\(^+\) channels \(^{67}\). Direct effects of GTP-bound \(\alpha,\beta\) tubulin dimers on Na\(^+\) channels were also considered possible, while the protein kinase A (PKA) pathway was excluded \(^{65}\) (see the paragraph entitled “Protein kinase A”, below) since, in neonatal myocytes, colchicine did not increase the amount of cyclic adenosine monophosphate (cAMP) \(^{68}\).
Finally, modulation of voltage-dependent gating properties by actin- and tubulin-based elements of the cytoskeleton was also reported. In rat ventricular myocytes exposed to Cyto-D, the activation/inactivation coupling was shifted towards more positive $V_{1/2}$ of activation $^{64}$. This result suggests a reduction of cell excitability in conditions that affect cytoskeleton integrity, e.g., ischemia $^{62}$. Finally, taxol, a microtubule stabilizer, shifted the activation/inactivation coupling towards more negative values of $V_{1/2}$ of activation $^{64}$. The latter finding may decrease the threshold of $I_{Na}$ activation and produce premature excitation. Moreover, it can result in a larger overlap between activation and inactivation (increased window current), which can cause action potential prolongation $^{69}$. The latter is one of the hallmark signs of HF. Accordingly, tubulin polymerization was found to be increased in this condition $^{60, 61}$. Of interest, administration of taxol in the treatment of cancer is associated with a variety of cardiac disturbances in patients, such as ventricular tachycardia, bradycardia, atrioventricular conduction block, and bundle branch block $^{70, 71}$. These cardiac disorders might be related to Na$^+$ channel dysfunction.

The regulatory role of ankyrins

Ankyrins are a family of intracellular proteins with the function of anchoring membrane protein complexes to the actin/spectrin cytoskeleton network. Therefore, ankyrins are responsible for targeting proteins to specialized cell compartments $^{72, 73}$.

So far, three different genes that encode ankyrin proteins have been identified. Ankyrin-B and ankyrin-G, encoded by $ANK2$ and $ANK3$ respectively, are ubiquitously expressed, while ankyrin-R, encoded by $ANK1$, is prevalently expressed in erythrocytes, and in a subset of neurons and skeletal muscle. Alternative splice variants of all three ankyrin proteins have been identified $^{72, 73}$.

Canonical ankyrins consist of four different domains: an N-terminal membrane-binding domain (MBD), a spectrin-binding domain, a death
domain, and a C-terminal domain (Fig. 3). MBD mediates interactions mainly with ion channels, transporters, and cells adhesion molecules, but also with cytoplasmic proteins such as clathrin and tubulin. The central region of ankyrins (spectrin-binding domain), which interacts with the actin-binding protein \( \beta \)-spectrin, mediates the physical linkage to the cytoskeleton. Adjacent to the spectrin-binding domain there is a death domain of 95 amino acids. Death domains were first reported in proteins which mediate the apoptosis pathway. The role of the death domain for ankyrins function is still unknown. However, the death domain of ankyrin-G has been shown to interact with the pro-apoptotic molecule Fas to promote cell death in renal tubules. The death domain, together with the C-terminus domain, forms the so-called “regulatory” domain (Fig. 3). Unlike other shared regions, the C-terminus domain is the most divergent amongst the ankyrin proteins, suggesting that it may be critical for the specific activities of ankyrin proteins in vivo.

Fig. 3. Schematic drawing representing the four domains which form the structure of the cytoskeleton anchoring protein ankyrin-B. To date, 18 mutations have been identified in ANK2, the gene encoding ankyrin-B, almost all of them are concentrated in the regulatory domain. In bold are indicated the mutations already characterized, in italic the one that await electrophysiological characterization.

Ankyrin-G and -B are expressed in the heart and both are involved in different types of cardiac arrhythmias. In vitro binding assays showed that ankyrin-G associates with a nine amino acid sequence located in the DII-
DIII linker of Na\textsubscript{v}1,5\textsuperscript{32} (Fig. 1). The missense mutation E1053K, recently identified in the ankyrin binding motif of Na\textsubscript{v}1,5 and linked to BrS, abolishes the interaction between Na\textsubscript{v}1,5 and ankyrin-G\textsuperscript{32}. Mutant Na\textsubscript{v}1,5 transfected in rat myocytes, exhibited a reduced cell membrane expression as compared to wild-type\textsuperscript{32}. Of note, this effect appeared to be cell-specific, since both mutant and wild-type Na\textsubscript{v}1,5 transfected in HEK293 cells displayed similar $I_{Na}$ densities, indicating a normal membrane Na\textsubscript{v}1,5 expression\textsuperscript{32}.

The role of ankyrin-B in regulating Na\textsuperscript{+} channel function is less clear. Patients with mutations in ANK2 (Fig. 3) have clinical features of Long QT syndrome (LQT4)\textsuperscript{31}, but also bradycardia, sinus arrhythmias, idiopathic ventricular fibrillation, delayed conduction/conduction block, and catecholaminergic polymorphic ventricular tachycardia. This diversity has resulted in the name “ankyrin-B syndrome”\textsuperscript{76, 77}. Although some of these arrhythmia types suggest impairment of Na\textsuperscript{+} channel function, a direct interaction between Na\textsuperscript{+} channel and ankyrin-B could not be established. Cardiomyocytes isolated from ankyrin-B\textsuperscript{+/-} mice, which share several clinical features with patients who carry an ankyrin-B mutation, exhibited altered calcium homeostasis that can lead to EADs and delayed afterdepolarizations (DADs), and, consequently, to cardiac arrhythmias, despite normal localization and cell surface expression levels of Na\textsuperscript{+} channels\textsuperscript{31, 76}. On the other hand, ventricular myocytes isolated from ankyrin-B\textsuperscript{-/-} mice did show several Na\textsuperscript{+} channel abnormalities compared to wild-type myocytes. These include reduced $I_{Na}$ density, due to fewer functional Na\textsuperscript{+} channels, and increased $I_{NaL}$, resulting from longer channel openings and re-openings, similar to what is observed in LQT3-associated mutant Na\textsuperscript{+} channels\textsuperscript{78}. These findings suggest that complete loss of ankyrin-B expression might be necessary to compromise cardiac Na\textsuperscript{+} channel functioning.
The regulatory role of syntrophin and dystrophin

The dystrophin glycoprotein complex (DGC) acts as a link between the extracellular matrix and the intracellular cytoskeleton, since the N-terminal portion of dystrophin binds to the intracellular actin and its C-terminus binds to β-dystroglycan. The latter is connected to α-dystroglycan, in turn bound to the extracellular protein laminin (Fig. 4)\(^79\). Na\(^+\) channels bind dystrophin via syntrophins, which are a family of adaptor proteins contained in the DGC. All syntrophins contain an amino acid sequence (PDZ motif), allowing the binding to the C-termini of several proteins. In particular, the last three amino acids (serine-isoleucine-valine) of the Na\(_{v}1.5\) C-terminus interact with the syntrophin PDZ motif (Fig. 1)\(^80-82\).

Up to now, five syntrophin isoforms have been isolated (α1, β1, β2, γ1, and γ2) with different localizations\(^83, 84\). Recently, it has been suggested that α\(_1\), β\(_1\), and β\(_2\) are the binding partners of the Na\(^+\) channel in the cardiac muscle, while γ syntrophins were not detected in the heart\(^82, 85\).
Besides the role of anchoring the cardiac Na\(^+\) channel to the actin cytoskeleton, the syntrophin/DGC complex may also modulate its function. Alteration of the Na\(^+\) channel-syntrophin dystrophin network could, indeed, be the cause of cardiac abnormalities, in particular, conduction defects, observed in patients with muscular dystrophies such as Duchenne muscular dystrophy (DMD)\(^\text{86}\). DMD is an inherited syndrome caused by mutations in the dystrophin gene on chromosome X, which encodes different dystrophin isoforms, such as the m-dystrophin, expressed both in the heart and in the skeletal muscle. Mutations in the gene encoding dystrophin produce either a marked decrease or a complete absence of the protein\(^\text{87}\).

The \textit{mdx}^\text{5cv} mouse represents an animal model for DMD that well recapitulates the muscle phenotype and some of the cardiac defects observed in DMD patients\(^\text{88}\). The \textit{mdx}^\text{5cv} mouse carries a nucleotide substitution in exon 10 of the dystrophin gene, which generates an aberrant splice site resulting in a premature stop-codon\(^\text{89}\). Ventricular myocytes isolated from these dystrophin-deficient mice revealed reduced Na\(^+\) channel protein content and \(I_{\text{Na}}\) compared to wild-type mice. No changes in Na\(^+\) channel gating were reported. Moreover, \textit{mdx}^\text{5cv} mice showed electrocardiogram (ECG) signs of conduction defects (prolonged QRS and P wave durations). Histological analysis of ventricular sections showed no signs of interstitial fibrosis. In addition, no differences in nuclear and/or cytoplasmic sizes that might have indicated the presence of cardiac hypertrophy were reported\(^\text{82}\). In conclusion, whether cardiac Na\(^+\) channel abnormalities due to the destruction of the Na\(^+\) channel-syntrophin-dystrophin interactions might be the cause of cardiac dysfunction observed in DMD patients is still under investigation.

**The regulatory role of caveolin-3**
Caveolins are important components of caveolae, small invaginations of the plasma membrane in which several signaling molecules and ion channels are
present, including Na\(^+\) channels \(^{90}\). Up to now, three different genes encoding for caveolins have been identified. \(CAV1\) and \(CAV2\) encode caveolin-1 and caveolin-2, two isoforms which have a relatively restricted tissue distribution \(^{91, 92}\), while \(CAV3\) encodes for caveolin-3, exclusively expressed in smooth, skeletal, and cardiac muscle \(^{93, 94}\). In particular, Na\(^+\) channels and caveolin-3 co-localize in rat ventricular myocytes \(^{95, 96}\) and in human myocardium \(^{29}\).

The nature of the interactions between the cardiac Na\(^+\) channel and caveolin-3 is not completely resolved yet. It has been shown that, in rat ventricular myocytes, increased I\(_{\text{Na}}\) secondary to \(\beta\)-adrenergic stimulation through a G\(\alpha\)s (PKA independent) \(^{67}\) pathway is due to the opening of caveolae that act as a local storage, which promotes the insertion of cardiac Na\(^+\) channels at the cell surface \(^{95, 96}\). Moreover, mutations in \(CAV3\) were identified in some patients with congenital Long QT syndrome (LQT9) \(^{29}\) and sudden infant death syndrome (SIDS) \(^{30}\). Co-expression of Na\(_{\text{1.5}}\) and these caveolin-3 mutants significantly increased I\(_{\text{NaL}}\) current in HEK293 cells \(^{29,30}\), as previously shown in LQT3- and SIDS-associated \(SCN5A\) mutations.

Of note, DGC is also a component of caveolae \(^{97,98}\), therefore, the interaction between cardiac Na\(^+\) channel and caveolin-3 might also occur through proteins of the DGC. The observation that transgenic mice overexpressing caveolin-3 have a phenotype similar to that observed in DMD, is suggestive for such an interaction. In these mice, dystrophin and its associated glycoproteins are downregulated not only in the skeletal muscle, but also in the heart. Caveolin-3 over-expression resulted in cardiac muscle degeneration, with the presence of inflammation and interstitial fibrosis \(^{99}\). Moreover, QRS duration of the ECG was significantly prolonged in these caveolin-3 transgenic mice \(^{99}\).

Taken all together, these findings show the importance of the interactions between Na\(^+\) channels and the caveolin-3/dystrophin protein complex, although the underlying mechanisms are not fully understood yet.
The regulatory role of protein kinases and phosphatases

Neurotransmitters and hormones regulate the cardiac Na\(^+\) channel thorough enzyme cascades that have as downstream targets kinases and phosphatases. Here, we will discuss the modulatory role on the cardiac Na\(^+\) channel of some of them.

**Protein kinase A**

\(\beta\)-adrenergic receptor stimulation regulates cardiac Na\(^+\) channel activity by activation of the stimulatory G protein which can follow two different parallel mechanisms: the “direct pathway”, and the “indirect pathway”.

As described previously (see the paragraph entitled “The regulatory role of caveolin-3”), the direct pathway (PKA independent) involves the action of the G\(\alpha\)s protein which increases \(I_{Na}\) presumably through the promotion of membrane channel insertion from caveolae \(^{95,96}\).

The indirect pathway (PKA dependent) occurs through G\(\alpha\)s stimulation of the intracellular enzyme adenylyl cyclase which transforms adenosine monophosphate (AMP) into cAMP. This second messenger is then able to activate the protein kinase A (PKA). PKA phosphorylation of two conserved serines (Ser\(^{525}\) and Ser\(^{528}\)) located in the DI-DII linker of Nav1.5 \(^{100}\) (Fig. 1) results in a consistent enhancement of \(I_{Na}\) \(^{100-102}\). A recent study showed that, in the presence of PKA activators, HEK293 cells expressing Nav1.5 revealed an increased channel membrane expression \(^{101}\). The authors suggested that Nav1.5 phosphorylation may either facilitate channel release from the endoplasmic reticulum or the recruitment of a population of Na\(^+\) channels located in the caveolae \(^{100,101}\).

**Protein kinase C**

Protein kinases C (PKC) constitute a family of serine/threonine kinases which comprises at least 11 different isoforms, almost all expressed in human ventricular myocytes \(^{103}\). PKC activation in response to \(\alpha_1\)-adrenergic receptor stimulation drives phosphorylation of several substrates. PKC
activation caused a severe reduction in $I_{Na}$ density at a diastolic holding potential (56% at -94 mV) and a negative shift of the voltage-dependence of steady-state inactivation in rat cardiac Na$^+$ channels heterologously expressed in a mammalian cell line$^{104}$. Moreover, single channel recordings revealed a reduced probability of Na$^+$ channel opening in the presence of a PKC activator, while single channel conductance was unaffected$^{104}$. Similar results were reported in oocytes transfected with the human cardiac Na$^+$ channel isoform, i.e., reduction in $I_{Na}$ magnitude, but no gating changes$^{105}$. Phosphorylation of a serine at position 1503 in the DIII-DIV linker of Na$_v$1.5 (Fig. 1) was partly responsible for the effect of PKC activation on $I_{Na}$$^{105}$. In a follow-up study, it was proposed that PKC activation reduced $I_{Na}$ amplitude by promoting channel internalization, rather than a direct effect on Na$^+$ channel function$^{106}$. Of note, PKC and PKA regulate the cardiac Na$^+$ channel differently.

**Calcium/calmodulin dependent protein kinase II**

Calcium/calmodulin dependent protein kinase II (CaMKII) is a serine/threonine kinase which is widely expressed and transduces intracellular calcium increases into phosphorylation of many different proteins$^{107}$. Recently, a role for CaMKII in cardiac Na$^+$ channel modulation has been reported$^{108}$. It was shown that CaMKII co-immunoprecipitates with and phosphorylates cardiac Na$^+$ channels, although neither the mode of this interaction nor the phosphorylation site were investigated. Besides, over-expression of CaMKII$\delta$c in rabbit myocytes (CaMKII$\delta$ is the predominant isoform in the heart and CaMKII$\delta$c is its cytosolic splice variant) induced a negative shift of voltage-dependence of inactivation, increased slow inactivation, and slowed the recovery from inactivation. All these effects were calcium dependent. Finally, increased $I_{NaL}$ and slowed Na$^+$ current decay were also observed. Overall, increased slow inactivation and slowed recovery from inactivation decrease $I_{Na}$ availability, especially at fast heart rates,$^{19, 20}$ while, at slower heart rates, the slowed current decay and the
increased $I_{\text{Na}}$ can cause action potential prolongation, consistent with the LQT3 syndrome. Interestingly, the altered phenotype caused by the over-expression of CaMKIIδ was similar to the one observed in patients and in mice carrying the 1795insD mutation\(^{19,109}\), and included both LQT3 and BrS characteristics (see the paragraph entitled “Overlap syndromes and modulating factors”, below). Since the expression and the activity of CaMKII are up-regulated in human HF\(^{110,111}\) and in HF animal models\(^{112}\), these gating changes might cause an acquired form of arrhythmia. This idea is supported by the fact that, in transgenic mice that over-expressed CaMKIIδ and showed signs of HF, it was possible to induce ventricular tachycardia\(^{108}\).

**Calcineurin**

Calcineurin is a calcium-activated serine/threonine phosphatase that is closely involved in the generation of cardiac hypertrophy\(^{113}\). Transgenic mice over-expressing a constitutively active form of calcineurin showed massive cardiac hypertrophy, followed by fibrosis, congestive HF and premature death\(^{114}\). A subsequent study revealed that those premature sudden deaths were caused by progressive conduction defects leading to conduction block\(^{115}\). Patch-clamp experiments in isolated cardiomyocytes showed an age-dependent reduction of both $I_{\text{Na}}$ density and maximal action potential upstroke velocity ($dV/dt_{\text{max}}$), a measure of $I_{\text{Na}}$, which paralleled the *in vivo* QRS prolongation and the decrease in QRS amplitude that immediately preceded the episodes of heart block\(^{116}\). Chronic *in vitro* treatments of myocytes with ryanodine, BAPTA-AM and thapsigargin, all substances which disturb calcium handling, could rescue the effects of calcineurin over-expression. Finally, also the PKC inhibitor bisindolylmaleimide rescued $dV/dt_{\text{max}}$ and $I_{\text{Na}}$ density to values indistinguishable from those seen in wild-type cells, in line with the previous findings that PKC activation decreases $I_{\text{Na}}$ density\(^{104-106}\). Western blot and immunohistochemistry assays did not show changes in $Na^+$ channel
expression. Thus, the authors concluded that over-expression of calcineurin altered calcium homeostasis leading to PKC activation, which resulted in a decreased activity of Na⁺ channels ¹¹⁶.

1.3 Sodium channel dysfunction in inherited and acquired cardiac disease

1.3.1 Inherited primary electrical disease

Brugada syndrome
BrS 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 ECG of BrS patients is characterized by ST segment elevation in the right precordial leads (V1-V3), often in conjunction with signs of conduction slowing ¹¹⁷, ¹¹⁸ (Fig. 5). The ECG signs of the syndrome are dynamic and often concealed, but can be unmasked by Na⁺ channel blockers, or during a febrile state ¹¹⁹-¹²¹. 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 ¹²², ¹²³. In Southeast Asia, the disease is the leading cause of death in males under the age of 40, second only to car accidents.

Fig. 5. Representative electrocardiogram of a Brugada syndrome patient. Note ST segment elevation with high take-off (J point) and negative T waves, typically seen in right precordial leads V1-V2.
BrS exhibits an autosomal dominant pattern of inheritance, with incomplete penetrance and male predominance. Most drugs do not provide fully effective prevention of arrhythmias and ICDs are the only recommended form of therapy to prevent sudden death\textsuperscript{124}.

To date, a significant number of $SCN5A$ mutations have been reported to contribute to BrS. Functional analysis employing expression systems showed that all produce $\text{Na}^+$ channel loss-of-function. However, various mechanisms of $I_{\text{Na}}$ reduction have been described. These include decreases in $I_{\text{Na}}$ density and gating changes (Table 2). Of note, $SCN5A$ mutations account only for 18%-30% of BrS cases, suggesting that other genes might be involved\textsuperscript{124}. A second locus on chromosome 3, close to but apart from the $SCN5A$ locus, was linked to BrS\textsuperscript{125}. Here, an A280V mutation in the glycerol-3-phosphate dehydrogenase 1-like gene ($GPD1-L$)

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reported Biophysical Mechanisms of Increase in Net Sodium Current (Gain-of-Function) and Reduction in Net Sodium Current (Loss-of-Function)</strong></td>
</tr>
</tbody>
</table>

**Gain-of-function**

- persistent current (disruption of fast inactivation)
- changes in voltage dependence of activation (hyperpolarizing shift) and/or 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/or inactivation (hyperpolarizing shift)
- slower recovery from inactivation
- enhanced slow inactivation
- enhanced closed-state inactivation

was identified. This mutant, when co-transfected with Na\textsubscript{1.5} into a HEK293 cell line, resulted in reduction in $I_{\text{Na}}$ magnitude, due to a decreased
Nav1.5 surface expression, when compared to wild-type. Thus, GPD1-L is a novel Na\textsuperscript{+} channel modulator in the heart\textsuperscript{126}. Another study showed a modulatory effect on the BrS phenotype of the rapid delayed rectifier potassium current (I\textsubscript{Kr}) encoded by KCNH2\textsuperscript{127}. More recently, loss-of-function mutations in the \( \alpha \)-subunit (CACNA1C) and the \( \beta \)-subunit (CACNB2b) of the cardiac calcium channel have been shown to cause an overlap syndrome characterized by a shorter than normal QT interval and a BrS phenotype\textsuperscript{128}.

How I\textsubscript{Na} reduction causes the characteristic features of BrS, is unresolved. One hypothesis (repolarization disorder hypothesis), proposes that reduced I\textsubscript{Na} may exacerbate the effects of the intrinsic difference in density of the transient outward potassium current, I\textsubscript{to}, between epicardial layers (high I\textsubscript{to} density) and endocardial layers (low I\textsubscript{to} density)\textsuperscript{129}. According to this hypothesis, this strong repolarizing current renders epicardial cells more sensitive than endocardial cells to the effects of reductions in I\textsubscript{Na}. An alternative hypothesis revolves around slowing of impulse propagation in the right ventricle (RV), particularly, the RV outflow tract (depolarization disorder hypothesis)\textsuperscript{130-132}. Both hypotheses are supported by clinical and experimental data, rendering it likely that BrS is not fully explained by one single mechanism. Yet, evidence of conduction abnormalities in patients with BrS is accumulating. Widening of P wave and QRS duration, and prolongation of PQ and HV intervals, all of which represent conduction abnormalities, are often observed in BrS patients. Greater prolongation of PQ and HV intervals at baseline, and excessive QRS interval prolongation after the use of Na\textsuperscript{+} channel blockers are more likely to be found in BrS patients who carry a SCN5A mutation than in those who do not\textsuperscript{133}. Moreover, in a study of 78 individuals carrying a SCN5A mutation associated with BrS, resting ECGs showed a spontaneous BrS ECG pattern in 28 of 78 (36\%) mutation carriers, while 59 of 78 (76\%) exhibited conduction defects. The conduction defects worsened with age in mutation carriers, leading in five cases to pacemaker implantation\textsuperscript{134}. This study showed that the most common phenotype of gene carriers of a BrS-type
SCN5A mutation are progressive cardiac conduction defects similar to the Lenègre disease phenotype (see the paragraph entitled “Progressive cardiac conduction disease”, below).

Finally, recent studies have highlighted the role of other pathophysiological derangements, e.g., fibrosis \(^{130, 135}\) (see the paragraph entitled “Cardiac fibrosis”, below). These studies all contribute to the emerging notion that BrS might have multiple etiologies.

**SUDS**

Sudden Unexplained Death Syndrome (SUDS) is a disorder 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 \(^{136}\). The patients, almost exclusively men, have structurally normal hearts and an ECG patterns similar to BrS. SCN5A mutations, which resulted in “loss-of-function" alterations such as in BrS, were identified in SUDS patients. These data suggest that SUDS and BrS are phenotypically, genetically and functionally the same disorder \(^{137}\).

**Long QT syndrome**

The congenital Long QT syndrome (LQTS) is an inherited disorder estimated to affect 1/3000 individuals, with the onset of symptoms typically occurring within the first 2 decades of life \(^{138}\). 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 of the ECG (Fig. 6), which is coupled at the cellular level to prolonged action potential duration and delay in myocardial repolarization \(^{138}\). Action potential prolongation can lead to EADs which may initiate a life-threatening form of polymorphic ventricular tachycardia called Torsade de Pointes (TdP) \(^{139}\). LQTS is a heterogeneous disorder with
different genotypes and corresponding phenotypes. So far, mutations in 10 different genes were associated with LQTS. Five genes encode pore-forming ion channel α-subunits, three of which are potassium channel α-subunits (KCNQ1 [LQT1], KCNH2 [LQT2], SCN5A [LQT3], KCNJ2 [LQT7 or Andersen syndrome], CACNA1C [LQT8 or Timothy syndrome]), the others encode ion channel regulatory proteins (KCNE1 [LQT5], KCNE2 [LQT6], ANKB [LQT4], CAV3 [LQT9], SCN4B [LQT10]) 140. SCN5A mutations (LQT3) account for ≈10% of genotyped LQTS patients 141.

In general, Na⁺ channel mutations linked to LQT3 are associated with disrupted inactivation, as was originally identified in the ΔKPQ mutation 142, 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 $I_{NaL}$ (gain-of-function) during the action potential plateau that prolongs action potential duration and may account for the development of arrhythmogenic triggered activity, such as EADs. Although most of the mutations associated with LQT3 produce $I_{NaL}$, some mutations exhibited other gating disorders that also lead to prolongation of the QT interval. These include shifts in the voltage dependence of activation and/or inactivation that can result in a larger overlap between the two curves (increased window current) 69, 143, 144, faster
recovery from inactivation \(^{145}\) and decreased slow inactivation \(^{146}\) (Table 2). Because a very delicate balance of inward and outward currents maintains the action potential plateau, even subtle current alterations during repolarization may provoke QT prolongation and TdP.

The identification of LQTS patients with mutations in genes that encode regulatory proteins of the cardiac Na\(^+\) channel macromolecular complex (ankyrin-B \([\text{ANK2}]\), caveolin-3 \([\text{CAV3}]\), and the \(\beta_1\)-subunit \([\text{SCN4B}]\)) \(^{28, 29, 31}\) suggests that changes in any single component of the protein complex that constitutes the cardiac Na\(^+\) channel might affect its correct functioning. Of note, \(\approx\)25\% of patients with LQTS do not present genetic defects in the known LQTS-susceptibility genes \(^{147}\). Thus, Na\(_v\)1.5 associated proteins, especially \(\beta\)-subunits, seem very promising candidates for being yet unidentified LQTS genes. Accordingly, patch-clamp experiments in myocytes isolated from \(\beta_1^{+/}\) mice showed both increased I\(_{\text{Na}}\) and I\(_{\text{NaL}}\) \(^{51}\). Down-regulation of the \(\beta_1\)-subunit or destructions of Na\(_v\)1.5-\(\beta_1\) interactions might contribute to a prolonged QT phenotype. Indeed, in D1790G mutant Na\(^+\) channels associated with LQT3, the effect of the mutation is due to the loss of the ability of Na\(_v\)1.5 to be modulated by \(\beta_1\) \(^{33}\).

Genotype-phenotype studies have shown that the risks of cardiac events and the circumstances under which they occur differ amongst 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. Of note, the genotype-phenotype relationships for LQTS that involve ion channel interacting proteins have not been determined yet.

\(\beta\)-Adrenergic receptor blockers remain the cornerstone of therapy in LQTS, although this treatment may be less efficacious in \(\text{SCN5A}\) mutation carriers \(^{148}\). Clinical and \textit{in vitro} evidence suggest that Na\(^+\) channel blockers such as mexiletine may reduce I\(_{\text{NaL}}\) and shorten the QT interval in \(\text{SCN5A}\) mutation carriers, but there are no data to indicate a reduction in
mortality 149, 150. Flecainide has also been observed to shorten QT intervals151, but some have cast doubts on the safety of this therapy 152.

SIDS
Sudden infant death syndrome (SIDS) continues to be the most common cause of post-neonatal infant death, accounting for about 25% of all deaths between 1 month and 1 year of age. SIDS is a complex, multifactorial disorder, the cause of which is still not fully understood. However, much is known now 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, drugs, and infant-related risk factors, such as non-supine sleeping position and soft bedding. Emerging evidence also shows an increased number of genetic risk factors responsible for SIDS 153.

The first molecular proof that LQTS may be involved in SIDS was provided by a study of an infant who was resuscitated from ventricular fibrillation (VF). The infant showed a severely prolonged QTc interval (648 ms). Genetic analysis demonstrated a de novo SCN5A mutation (S941N) 154. In the following years, many other LQT3-associated SCN5A mutations143,155, 156 and mutations in KCNQ1 (LQT1) and KCNH2 (LQT2) genes encoding for potassium channel α-subunits 156-159 were reported to cause SIDS. Mutations in CAV3 were also found responsible for some cases of SIDS 30. Yet, while a study which was performed in a large cohort of SIDS victims revealed the presence of LQTS mutations in ≈10% of all patients, about half of the genetic variants were located in SCN5A 156.

Other studies demonstrated that BrS can also be considered as a cause of sudden death in children. Indeed, mutations in SC5NA and GPD1-L, both linked to BrS, were found in cases of SIDS 160-162.

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.
Progressive cardiac conduction disease

Progressive cardiac conduction disease (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 (Fig. 7). Complete 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 \textit{SCN5A} and to another chromosomal locus (19q13.2-q13.3) \cite{163,164} where the involved gene is yet unknown. The causal relationship between \textit{SCN5A} mutations and PCCD was first reported in a large French family and in a Dutch family \cite{165}. In the French family, P wave width, PR intervals, and QRS intervals prolonged with age, however, in the Dutch family, the proband was a child\cite{165}. This suggests that the resulting phenotype may be progressive or immediate.

The first study on the biophysical properties of a PCCD associated \textit{SCN5A} mutation (G514C) showed reduced I_{Na} \cite{27}. Patch-clamp studies revealed opposing gating changes that resulted in attenuated I_{Na} reduction. As confirmed by modelling studies, this decrease in I_{Na} was sufficient to cause conduction disease, but insufficient to cause BrS. Although through

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig7.png}
\caption{Representative electrocardiogram of a patient with progressive cardiac conduction disease. Note marked QRS widening, PQ interval prolongation, and leftward QRS axis deviation.}
\end{figure}
different mechanisms, all subsequent PCCD-associated \( SCN5A \) mutants studied were characterized by a loss of \( \text{Na}^+ \) channel function \( 166-169, 170, 171-173 \). The decrease in \( I_{\text{Na}} \) would result in a reduction of action potential upstroke velocity, thereby slowing 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 \(^{174}\). Of note, the same mutation was previously found both in a family with atrial standstill \(^{146}\) (see the paragraph entitled “Atrial standstill”, below) and in one with dilated cardiomyopathy \(^{175, 176}\) (see the paragraph entitled “Dilated cardiomyopathy”, below).

**Atrial standstill**

Atrial standstill is a rare arrhythmia that consists of loss in electrical and mechanical activity of the atria. Atrial standstill is characterized by bradycardia, absence of P waves, and junctional escape rhythm \(^{177}\). Whereas, in most reports, atrial standstill is secondary to other diseases, familial atrial standstill without underlying cardiac disorder is extremely rare and identification of genetic factors for atrial standstill is hampered by the small number of affected individuals in each family.

The first mutation associated with atrial standstill was found in \( SCN5A \) (D1275N) and resulted in a loss-of-function \( \text{Na}^+ \) channel. Of interest, the affected individuals carried both the mutation and were homozygous for two linked polymorphisms in \( \text{Cx} 40 \), which encode the atrium-specific gap junction protein connexin 40\(^{146}\). These \( \text{Cx} 40 \) polymorphisms were localized in the promoter region and resulted in reduced connexin 40 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 \( \text{Cx} 40 \) variant was used to explain why the phenotype in this family was restricted to the atrium. Of note, the Finnish PCCD family, mentioned above, carried only the D1275N
mutation\textsuperscript{174}. This supports the idea that D1275N is responsible for conduction slowing/reduced excitability, while the \textit{Cx40} polymorphisms are required to evoke an atrial phenotype. Interestingly, the ventricular conduction in the atrial standstill family was also slightly impaired. Recently, another \textit{SCN5A} mutation (L212P) was linked to atrial standstill \textsuperscript{178}. 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 \textit{Cx40} polymorphisms as described previously \textsuperscript{146}, but was heterozygous for it.

Taken together, these studies indicate that genetic defects in \textit{SCN5A} most likely underlie atrial standstill, but that additional genetic factors which modulate atrial electrical coupling seem to be necessary to the clinical manifestation of this inherited arrhythmia. The chance of the simultaneous occurrence of the rare \textit{Cx40} genotype and a \textit{SCN5A} mutation is low. This could explain the extremely rare prevalence of this arrhythmia.

**Sick sinus syndrome**

Idiopathic sick sinus syndrome (SSS) is characterized by sinus bradycardia and sinus arrest in the absence of structural heart disease. In three independent studies, mutations in \textit{HCN4}, the gene encoding the $\alpha$-subunit of the pacemaker current, $I_p$, were identified \textsuperscript{179,181}. These \textit{HCN4} mutations resulted in loss-of-function of $I_p$. A role of $I_{Na}$ in sinoatrial node depolarization is also starting to be recognized \textsuperscript{182,183}, and \textit{SCN5A} mutations were identified in 5 of 10 children (7 families) with sinus node disease \textsuperscript{184}. Compound heterozygosity was, however, necessary, as members of the studied families who carried mutations in only one allele were clinically unaffected. The \textit{SCN5A} mutations resulted in loss-of-function. The phenotype in these individuals included bradycardia that progressed in some cases 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.

1.3.2 Sodium channel dysfunction in structural defects

Cardiac fibrosis

The first association between reduced $I_{\text{Na}}$ and structural defects was derived from a study of two infants of asymptomatic parents who exhibited prolonged ECG conduction intervals (PR, QRS) associated with episodes of wide complex tachycardia \(^{166}\). Genetic analysis revealed compound heterozygosity for two $SCN5A$ mutations which both caused $I_{\text{Na}}$ reduction (due to a truncated protein resulting from a premature stop codon in W156X, in conjunction with severely reduced $I_{\text{Na}}$ density of R225W channels). The postmortem examination of the heart of one child of this family, who died from severe reduction in cardiac excitability and ventricular tachyarrhythmias, showed fibrosis and necrosis in the left and right ventricle (RV), and fibrosis of the atrioventricular node and specialized conduction system, providing the first evidence to suggest that morphological changes within the heart may occur secondary to Na$^+$ channel defects. Similarly, studies of the explanted heart of a BrS patient with a loss-of-function $SCN5A$ mutation (G1935S) revealed severe fibrosis in the RV outflow tract (RVOT) \(^{130}\). 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 BrS 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 \(^{135}\). All mutations resulted in loss-of-function $I_{\text{Na}}$, characteristic of BrS. One mutation carrier showed fibrofatty myocardial replacement, suggestive of arrhythmogenic right ventricular cardiomyopathy (ARVC). Of note, in the BrS patients with $SCN5A$ mutations, myocyte apoptosis in both the left ventricle and the RV was significantly higher than in control.
The association between reduced $I_{\text{Na}}$ and structural defects is supported by experimental studies. In a $SCN5A$ knock-out 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 $I_{\text{Na}}$, as found in patch-clamp studies. 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, heterogeneous expression of connexin 43 and decreased expression of connexin 40, was also identified. A subsequent study from the same group revealed that only the synergism between reduced $I_{\text{Na}}$, increased fibrosis, and impaired intracellular coupling could lead to severe decrease in conduction velocity. Furthermore, conduction slowing was particularly evident in the RV, in agreement with the PCCD and BrS phenotypes. In conclusion, these studies suggest that a monogenic ion channels defect can lead to myocardial structural defects.

**Arrhythmogenic right ventricular cardiomyopathy**

ARVC is an inherited heart muscle disease characterized by specific derangements of the RV (fibrosis, fibrofatty replacement of myocardium) and ventricular tachycardia which may culminate in VF and sudden death. Of interest, the clinical features of ARVC may exhibit significant overlap with those of BrS. Most notably, the hallmark ST elevations of BrS have also been reported in ARVC, and may be provoked by $\text{Na}^+$ channel blockers, similar to BrS. Conversely, autopsy studies of BrS patients revealed fatty tissue deposition in the RV. In particular, in biopsies from the RV, fatty tissue infiltration mimicking ARVC was observed in 8 of 22 BrS cases (36%). Most recently, in a study of 18 unrelated patients with the clinical phenotype of BrS, endomyocardial biopsies revealed structural derangements in all, with histopathological
findings suggestive of ARVC in a patient who carried a \textit{SCN5A} mutation$^{135}$. Although overlap may exist between ARVC and BrS, at present, ARVC has been linked to genes which are different from those responsible for BrS. Only the \textit{ARVC5} locus has been mapped to a region on the same chromosome which contains \textit{SCN5A} and \textit{GPD1-L}, two genes associated with BrS, but the \textit{ARVC5} gene has not been identified yet$^{195}$. These data do not only substantiate a possible overlap between some cases of ARVC and BrS, but they may also exemplify the potential of \textit{SCN5A} mutations to be causally involved in structural cardiac defects.

**Dilated cardiomyopathy**

Dilated cardiomyopathy (DCM) is an idiopathic, genetically heterogeneous, disorder characterized by HF and an enhanced incidence of cardiac arrhythmias. The majority of the identified genes encode structural proteins of the cytoskeleton and contractile apparatus. Linkage analysis of a family with DCM identified a locus for DCM on chromosome 3 (3p22-p25), which contains \textit{SCN5A}$^{196}$. Subsequent \textit{SCN5A} screening of the family members revealed the presence of the missense mutation D1275N, as reported in studies by McNair et al.$^{175}$ and Olson et al.$^{176}$. In these studies, affected members had DCM and signs of conduction disease, along with atrial fibrillation. Of interest, this phenotype is distinct from a previous study by Groenewegen et al.$^{146}$, in which the D1275N mutation caused familial atrial standstill in individuals who carried this mutation and were homozygous for two linked polymorphisms in \textit{Cx40} (in the study of McNair et al., no DCM affected individual was homozygous for the \textit{Cx40} polymorphisms). 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$^{174}$. Also in this case, no individuals homozygous for the \textit{Cx40} polymorphisms were detected. 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. Initially, it was proposed that contractile dysfunction and DCM observed by McNair et al.\textsuperscript{175} and Olson et al.\textsuperscript{176} may be secondary to chronically increased heart rates during atrial fibrillation\textsuperscript{197}, but this idea is not supported by the study of Laitinen-Forsblom et al.\textsuperscript{174} in which patients had no DCM, although they did have 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 a two base pair insertion 2550-2551insTG, leading to a premature stop codon and truncation of the encoded protein). Cardiac biopsies revealed interstitial fibrosis in 2 of 4 mutation carriers (in the T220I and R814W families)\textsuperscript{176}. Of note, the T220I mutation was already reported as a recessive allele in SSS\textsuperscript{184}.

In conclusion, while the study of Olson et al.\textsuperscript{176} 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.

1.3.3 Overlap syndromes and modulating factors
Several $SCN5A$ mutations which resulted in overlapping phenotypes characterized by a combination of various features of the above described disorders have been identified. The 1795insD mutant was the first to be reported. Carriers of this mutation exhibited features of LQT3 and BrS\textsuperscript{19}. Patch-clamp studies revealed that these opposing phenotypes (gain-of-function in LQT3, and loss-of-function in BrS) may be explained by distinct gating changes. Thus, enhanced $I_{NaL}$ accounted for action potential prolongation and LQT3, while, concurrently, enhanced slow inactivation resulted in reduced $I_{Na}$ availability and ST elevation (BrS) at fast heart rates. A subsequent study revealed that this $SCN5A$ mutation 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 action potential by $I_{\text{NaL}}$ and decreased sinus node diastolic depolarization rate by a -10 mV shift of Na\(^+\) channel steady-state inactivation \(^{198}\). Missense mutations of the same residue to a histidine (Y1795H) or cysteine (Y1795C) resulted in BrS and LQTS, respectively \(^{199}\).

A large French family with a missense mutation in $\text{SCN5A}$ (G1406R) was reported in which mutation carriers exhibited either a BrS or PCCD phenotype \(^{171}\), suggesting that modifier gene(s) may influence the phenotypic consequence of this mutation. Similarly, the deletion of a lysine (ΔK1500) was associated with BrS, LQTS and PCCD \(^{200}\). Later studies have also reported combinations of other phenotypes, e.g., SSS in conjunction with conduction disease and BrS \(^{201,202}\), and BrS with short QT syndrome \(^{128}\).

Probably the most consistent link is that between BrS and PCCD. In a study of 78 individuals who carried a $\text{SCN5A}$ mutation associated with BrS, the spontaneous (i.e., in the absence of pharmacological challenge) BrS phenotypes were present only in 36%, while 76% exhibited conduction defects. Moreover, the conduction defects worsened with age in mutation carriers \(^{134}\). Evidence that genetic modifiers play a role in the variability of cardiac conduction parameters and the clinical expression of the cardiac Na\(^+\) channel diseases, was provided by a study in which two different strains of mice (129P2 and FVB/N) that both carried the 1798insD mutation, equivalent to 1795insD in humans \(^{109}\), presented different phenotypes \(^{203}\). In particular, the 129P2 mutant mice showed more severe PQ prolongation compared to the FVB/N mutant mice, and QRS prolongation following flecainide administration was also more pronounced in the 129P2 mice. Total RV activation time was longer in 129P2 mice than in FVB/N mice. In 91% of old 129P2 mice, it was possible to induce arrhythmias as opposed to 43% in old FVB/N mice. Finally, old 129P2 mice developed more severe fibrosis than FVB/N mice \(^{203}\).

How a single mutation can result in different and sometimes opposing phenotypes is not fully resolved. In addition to specific gating changes (as described for the 1795insD mutation \(^{19}\)), 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 action potential morphology, due to a large \(I_{\text{to}}\), while endocardial cells do not. Mutations that act to reduce \(I_{\text{Na}}\), in the presence of a large repolarizing current, may result in premature action potential repolarization, loss of action potential dome, and BrS-type ST elevation in the ECG (repolarization disorder hypothesis). Conversely, in endocardial cells, \(I_{\text{NaL}}\) in the presence of smaller repolarizing currents, may prolong action potential duration (LQT3 phenotype)\(^{204}\).

Other studies have revealed the role of gender. In a single family, the G1406R mutant caused BrS only in male carriers (4 of 6), while PCCD was found in 6 of 6 female, and 2 of 6 male carriers\(^{171}\). This is consistent with the observation that, while \(SCN5A\) mutations are equally transmitted between both sexes, the BrS phenotype is more prevalent in males. The male predominance might be partially due to the intrinsic differences in ventricular action potential between males and females. \(I_{\text{to}}\) density is higher in males, rendering them more susceptible to the effect of \(I_{\text{Na}}\) reduction\(^{205}\). In men, this may result more readily in excessive repolarization as proposed in the repolarization disorder hypothesis\(^{206}\). Conversely, females may be protected, because \(I_{\text{Ca-L}}\) is more strongly expressed in their epicardium\(^{207}\). Moreover, other studies suggested that the male hormone testosterone may be accountable for the gender differences in BrS\(^{208}\).

Importantly, 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\(^{146}\), but DCM and conduction defects in their absence\(^{174}\). Similarly, the common polymorphism H558R in \(SCN5A\) produced no appreciable \(I_{\text{Na}}\) gating changes in isolation, but it attenuated the gating defects caused by a \(SCN5A\) mutation identified in a child with conduction disease\(^{169}\). Other studies also reported a modulatory role of this polymorphism on a LQT3-linked
SCN5A mutant 209 and a BrS-linked SCN5A mutant 210, respectively. In both cases, the H558R polymorphism restored normal Na\textsuperscript{+} channel trafficking. On the other hand, H558R further accentuated the decrease in peak I\textsubscript{Na} caused by a SCN5A mutation identified in a patient with acute myocardial infarction 211 (see the paragraph entitled “Ischemic heart disease”, below).

The presence of such polymorphisms and differences in their prevalence among various ethnic groups may underlie clinically relevant differences. For instance, the S1102Y polymorphism in SCN5A is mainly present in subjects of African descent 212 (it was also found in a single white family, but there, it was deemed a disease-causing mutation responsible for LQT3 213), and was associated with increased arrhythmia susceptibility in an African-American who sustained QT prolongation and TdP on amiodarone (see the paragraph entitled “Drug-induced LQTS”, below) 212. Finally, homozygosity for the same polymorphism has been associated with increased risk of SIDS in African-American infants 214.

Similarly, a haplotype variant consisting of six polymorphisms in the promoter region of SCN5A was found to occur 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 in both normal Japanese individuals and Japanese BrS patients. It was suggested that this variability in SCN5A transcription may contribute to differences in phenotype between various ethnic groups 215.

1.3.4 Acquired cardiac arrhythmias

Whereas inherited SCN5A linked arrhythmias are explained by disturbed properties of mutant Na\textsuperscript{+} channels, ‘acquired arrhythmias’ are precipitated by environmental factors that act on the electrical activity of the heart. However, it is increasingly being recognized that acquired arrhythmias may involve subclinical genetic variations (polymorphisms) that can alter the structure or the electrophysiological properties of the myocardial substrate,
and may thus interact with environmental triggers to form the pathophysiological basis for cardiac arrhythmias. For instance, such mechanisms may contribute to unique drug responses in carriers of these gene variants. Similarly, some of the knowledge obtained in studies of rare inherited \textit{SCN5A} related disorders can be now extended to common acquired diseases.

**Drug-induced BrS**

Given the role of $I_{Na}$ reduction in BrS, various conditions and drugs that reduce $I_{Na}$ may cause/mimic BrS. Class IC antiarrhythmic drugs (potent Na$^+$ channel blockers) are the most frequent causes of acquired BrS, but many other drugs (not necessarily drugs aimed primarily at cardiac disease) are also able to induce ST segment elevations. Moreover, a BrS-like phenotype is more likely to occur in susceptible individuals in the presence of environmental factors, including electrolyte abnormalities, elevated insulin level, acute myocardial infarction, ischemia, mechanical compression of RVOT, or hyperthermia. It is likely that polymorphisms can enhance individual susceptibility to the BrS phenotype in the presence of drugs or other triggers, although up to now no cases were reported.

**Drug-induced LQTS**

Many drugs have been found to provoke “acquired LQTS”, most of which reduce $I_{Kr}$. Moreover, a genetic predisposition to drug-provoked LQTS has been reported. A \textit{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). This polymorphism appeared to be ethnicity-related, being commonly present in subjects of African descent. Subsequent expression of S1102Y in HEK293 cells revealed mild gain-of-function compared to wild-type channels, primarily caused by enhanced $I_{NaL}$ and increased peak $I_{Na}$. Simulation studies showed that these subtle
biophysical changes *per se* did not alter the action potential. However, under simulated block of $I_{Kr}$, a common effect of many drugs (including amiodarone), and hypokalemia, action potential prolongation was observed, which would enhance the risk of arrhythmias caused by excessive QT prolongation, e.g., TdP $^{212}$. In conclusion, 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.

Similarly, a $SCN5A$ mutation (L1825P) was isolated in a woman who showed drug-induced LQTS after cisapride treatment $^{218}$. In isolation, this mutation was subclinical, and exerted clinical effects only when uncovered by the use of cisapride. The mutant channel, when heterologously expressed, exhibited both gain-of-function Na$_v$1.5 channel features (increased $I_{NaL}$, slowed $I_{Na}$ decay), characteristic of LQT3, and loss-of-function features (decreased peak $I_{Na}$, negative shift of inactivation, positive shift of activation, enhanced closed-state inactivation), consistent with BrS. The authors suggested that the superimposition of the biophysical features of L1825P channels, while subclinical at baseline, and the proarrhythmic effects of cisapride may have been the cause of the LQTS phenotype in the patient.

**Congestive heart failure**

Action potential and QT prolongation are well-known features of congestive HF, and contribute to enhanced risk of arrhythmias and sudden death in this disease. Augmented $I_{NaL}$ was shown in two different dog models of HF $^{219,220}$ and in human ventricular myocytes isolated from HF patients $^{220}$. $I_{NaL}$ can trigger arrhythmias either by prolonging action potential duration with subsequent EADs, or by increasing intracellular Na$^+$ loading, thereby eliciting an increase in intracellular calcium levels via the Na$^+$/Ca$^{2+}$ exchanger. Intracellular calcium overload may evoke cardiac arrhythmias based on delayed afterdepolarizations (DADs) $^{221}$. In both mechanisms, $I_{NaL}$
represents an attractive target for therapeutic intervention. Accordingly, experimental block of $I_{\text{Na}}$ by saxitoxin or lidocaine shortened action potential duration and abolished EADs in myocytes isolated from failing hearts.\textsuperscript{219}

Down-regulation of $I_{\text{Na}}$ was also found in two different canine HF models\textsuperscript{220, 222, 223} and in human ventricular myocytes\textsuperscript{220}. A decrease in $I_{\text{Na}}$ density may reduce excitability, thereby slowing myocardial conduction and contributing to re-entrant arrhythmias. However, no differences in peak $I_{\text{Na}}$ between HF and control were found in a volume/pressure overload HF rabbit model\textsuperscript{224}, in line with a dog model of pacing-induced HF\textsuperscript{225}. The contrasting results of these studies may be due to the species/model differences.

In the failing animal models in which reductions in $I_{\text{Na}}$ density were reported, there were no changes in Na\textsuperscript{+} channel mRNA levels\textsuperscript{220, 223}, in line with a study in which no changes in mRNA levels were found in patients with end-stage HF\textsuperscript{226}. However, a recent study reported three new $SCN5A$ mRNA splice variants, previously undetected in the human heart, which resulted in prematurely truncated proteins. When transfected into a HEK293 cell line stably expressing the full-length Na\textsuperscript{+} channel, all variants showed a significant reduction in $I_{\text{Na}}$ as compared to the native channel alone\textsuperscript{227}. The authors then examined the levels of these truncated transcripts in explanted heart from HF patients and in controls. The total percentage of short variants went from 12.5\% of the total $SCN5A$ mRNA in controls, to 54.9\% in HF patients and at the same time the full-length Na\textsuperscript{+} channel transcript was reduced from 87.5\% in controls to 45.1\% in HF. This led to a 63\% reduction in cardiac Na\textsuperscript{+} channel protein level in HF ventricular tissue compared to controls, as detected by Western blot analysis\textsuperscript{227}. Therefore, this study showed for the first time that abnormal $SCN5A$ splicing may contribute to the $I_{\text{Na}}$ reduction observed in HF.

Finally, post-transcriptional mechanisms might also be involved. For instance, while activation of PKA or G\alpha\textsubscript{s} proteins via $\beta$-adrenergic receptor activation increases Na\textsuperscript{+} channel numbers by promoting channel
insertion into the sarcolemma, this process may be impaired in HF, because β-adrenoceptors are downregulated. On the other hand, abnormalities in calcium homeostasis, as reported in HF, can activate multiple downstream targets, e.g., PKC. Activation of Ca$^{2+}$-dependent PKC reduces $I_{Na}$ density. Finally, cytoskeleton derangements, observed in HF, can also contribute to reduced $I_{Na}$.

While these studies did not address the possible presence of SCN5A mutations or polymorphisms, these observations may point to a novel target for reduction of arrhythmia risk in congestive HF.

**Ischemic heart disease**

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

Supporting these findings, myocytes obtained from the ischemic zone of a 5-day-old infarct created by coronary ligation in a canine heart, exhibited reduced $I_{Na}$ density and altered inactivation gating properties (negative shift of inactivation, slowed recovery from inactivation, enhanced closed-state inactivation) when compared to myocytes of non-ischemic regions. These results are consistent with previous findings in which $dV/dt_{\text{max}}$, a measure of $I_{Na}$, was found to be significantly reduced in myocytes of infarction border zones. Moreover, augmented $I_{Na,L}$, likely due to hypoxic conditions, seems to contribute to the increased intracellular Na$^+$ loading observed in ischemia. Accordingly, ranolazine, a drug that potently inhibits $I_{Na,L}$, in addition to blocking other ion currents such as $I_{Kr}$ and $I_{Ca,L}$ (L-type calcium current), was recently approved in the treatment of angina pectoris. These data suggest that Na$^+$ channel derangements may
contribute to electrophysiological heterogeneity during ischemia, which predisposes to re-entrant arrhythmias.

Although the identification of patients at risk of VF during acute myocardial infarction remains difficult, a recent report has highlighted the importance of a family history of sudden death, pointing to the possibility of a genetic predisposition. In line with this hypothesis, a recent study reported on a cohort of 19 individuals admitted to the hospital with acute myocardial infarction. All patients presented episodes of VF. One patient, in particular, had an arrhythmic storm, displaying 6 episodes of polymorphic ventricular tachycardia and VF within the first 12 hours. Subsequent SCN5A analysis revealed the presence of the common H558R polymorphism in 5 of the 19 patients and of the R34C polymorphism in 1 patient. The patient with the arrhythmic storm was the only one to have a SCN5A mutation. He carried the G400A missense mutation and the common H558R polymorphism on the same allele. Heterologous expression of G400A, G400A+H558R, and wild-type Na+ channels in mammalian cells revealed decreased peak I_{Na} for G400A mutant channels compared to wild-type. The presence of the H558R polymorphism resulted in a further reduction of peak I_{Na}. The fact that the patient developed his first VF episode at the age of 70 years and only in the setting of myocardial infarction supports the notion that SCN5A mutations can be a predisposing factor for acquired arrhythmic syndromes.
1.4 Aim of the thesis

The cardiac Na\(^+\) channel is responsible for the initiation of the rapid upstroke of the action potential in the conduction system and working myocardial cells. Given its central role, it is not surprising that mutations in the pore-forming region (\(\alpha\)-subunit) and, as recently shown, in the accessory proteins of the cardiac Na\(^+\) channel can cause primary electrical diseases, the so-called “inherited sodium channelopathies” (LQT3, BrS, SIDS, PCCD, atrial standstill and SSS), that are associated with various life-threatening arrhythmias. On the other hand, a link between Na\(^+\) channel dysfunction and structural cardiac defects, notably cardiac fibrosis, dilated cardiomyopathy and, possibly, arrhythmogenic right ventricular cardiomyopathy, is also emerging.

The focus of the research presented in this thesis is on the alterations in cardiac Na\(^+\) channel function that occur in cardiac inherited and acquired diseases. Using a combination of several techniques, including sequence analysis, patch-clamp experiments, computer simulations, histopathologic studies, and immunohistochemical studies, alterations in Na\(^+\) channel gating properties and expression are characterized, and related to the cardiac rhythm disturbances observed.

In Chapter 2, we report the functional consequences of a novel SCN5A mutation, G1319V, identified in a BrS patient. We conducted patch-clamp experiments in HEK293 cells, transfected either with the mutant or the wild-type Na\(^+\) channel, to study how the mutation affected the electrophysiological properties of the protein and how these derangements could explain the corresponding BrS phenotype. Subsequently, using a three-dimensional model of the DIIIS4-S5 linker, we discussed how a glycine to valine substitution at the 1319 position could impact on the structure-function relation of the cardiac Na\(^+\) channel.

In Chapter 3, we performed a combined electrophysiological, genetic, histopathologic and computational study of the heart of a BrS patient who underwent cardiac transplantation because of an excessively
high incidence of VF. The study was aimed to further elucidate the pathophysiological mechanisms underlying BrS.

In **Chapter 4**, we studied how cardiac Na\(^+\) channel function may be regulated by the cytoskeletal component tubulin. This study was provoked by the clinical observation that the use of the anti-tumor drug taxol, which causes tubulin polymerization, may cause various cardiac arrhythmias in patients. These arrhythmias possess electrophysiological features which suggest derangement of Na\(^+\) channel function. We studied the physiological consequence of exposure to taxol of HEK293 cells transfected both with the \(\alpha\)-subunit of the cardiac Na\(^+\) channel alone or together with the \(\beta_1\)-subunit, using a combination of the patch-clamp technique and immunohistochemistry.

In **Chapter 5**, we aimed to study how Na\(^+\) channel function may be regulated by intracellular calcium levels. Such regulation may be relevant in diseases associated with derangements in intracellular calcium levels, e.g., HF. Thus, we studied Na\(^+\) channel function in ventricular myocytes isolated from control rabbits and rabbits in which HF was induced by combined volume-pressure overload. We used various patch-clamp techniques (whole-cell configuration, single channel recording, alternate voltage/current-clamp mode) to study Na\(^+\) channel function at calcium concentrations that are present during the cardiac action potential (0-500 nM).
References


13. Miyamoto K, Nakagawa T, Kuroda Y. Solution structures of the cytoplasmic linkers between segments S4 and S5 (S4-S5) in domains III


38. Yu FH, Westenbroek RE, Silos-Santiago I, et al. Sodium channel \( \beta_4 \), a new disulfide-linked auxiliary subunit with similarity to \( \beta_2 \). *J Neurosci* 2003; 23:7577-7585.


43. Nuss HB, Chiamvimonvat N, Perez-Garcia MT, et al. Functional association of the \( \beta_1 \) subunit with human cardiac (hH1) and rat skeletal muscle (mu 1) sodium channel alpha subunits expressed in Xenopus oocytes. *J Gen Physiol* 1995; 106:1171-1191.


46. Valdivia CR, Nagatomo T, Makielski JC. Late Na currents affected by \(\alpha\) subunit isoform and \(\beta_1\) subunit co-expression in HEK293 cells. *J Mol Cell Cardiol* 2002; 34:1029-1039.


