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Review

Defective interactions of protein partner with ion channels and transporters as alternative mechanisms of membrane channelopathies☆

Crystal F. Kline, Peter J. Mohler *

The Dorothy M. Davis Heart and Lung Research Institute, Department of Internal Medicine, Division of Cardiovascular Medicine, Department of Physiology & Cell Biology, The Ohio State University Wexner Medical Center, USA

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ABSTRACT

The past twenty years have revealed the existence of numerous ion channel mutations resulting in human pathology. Ion channels provide the basis of diverse cellular functions, ranging from hormone secretion, excitation–contraction coupling, cell signaling, immune response, and trans-epithelial transport. Therefore, the regulation of biophysical properties of channels is vital in human physiology. Only within the last decade has the role of non-ion channel components come to light in regard to ion channel spatial, temporal, and biophysical regulation in physiology. A growing number of auxiliary components have been determined to play elemental roles in excitable cell physiology, with dysfunction resulting in disorders and related manifestations. This review focuses on the broad implications of such dysfunction, focusing on disease-causing mutations that alter interactions between ion channels and auxiliary ion channel components in a diverse set of human excitable cell disease. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé

Contents

1. Introduction

Most ion channels are recognized as central players of larger multi-subunit complexes. Ion channels associate with a number of scaffolding, anchoring, regulatory, and signaling proteins. Co-targeting of these auxiliary subunits to the channel complex has evolved to allow for rapid and localized regulation of ion channels in response to specific stimuli. Electrophysiological properties of cells, once thought to be

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* Corresponding author at: Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical Center, 473 W. 12th Ave, Columbus, OH 43210, USA. Tel.: +1 614 292 7766; fax: +1614 247 7799.
E-mail address: peter.mohler@osumc.edu (P.J. Mohler).

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exclusively attributed to ion channels, have expanded to include these auxiliary subunits. While mutations in ion channel genes can cause dysfunction, a growing number of ion channel-associated proteins have come to the forefront in the study of human excitable cell pathology. This new paradigm has emerged based on growing evidence demonstrating variants in genes involved in the expression, localization, and regulation of ion channels also result in excitable cell disease. These findings have expanded the view of excitable cell disease, uncovered new mechanisms for ion channel regulation, and provided novel targets for pharmacological treatment of aberrant ion channel activity in excitable cells.

2. Liddle syndrome: dysregulation of Nedd4-2-mediated regulation of ENaC and hypertension

The last, and rate-limiting, step in Na^+ reabsorption by the kidney occurs in the distal convoluted tubules, connecting tubules, and collecting ducts where the epithelial sodium channel (ENaC) regulates Na^+ absorption [1,2]. ENaC selectively transports Na^+ down an electrochemical gradient established by the Na^+/K^+ ATPase at the basolateral membrane of polarized epithelial cells [3]. Consequently, the directional movement of Na^+ produces an osmotic gradient that drives the movement of water in the same direction [4–6].

ENaC is selectively expressed at the apical membrane in a variety of epithelia, such as the kidney, lung, and colon [7]. Since entry of Na^+ through ENaC is the last and rate-limiting step for Na^+ absorption, [3,7,8] regulation of this channel is vital to the regulation of extracellular fluid volume, blood volume, and blood pressure. The activity of ENaC is tightly controlled by a number of hormones (aldosterone, vasopressin, and insulin) and intracellular mediators (Na^+, Ca^{2+}, pH, cAMP, protein kinases A and C, and extracellular proteases) [3,9]. The necessity of this tight regulation is illustrated by the number of human diseases that have been linked to dysfunction in ENaC, including Liddle syndrome, [10,11] cystic fibrosis, [12] pseudohypoaldosteronism type I (PHA-I), [13,14] and pulmonary edema [15,16].

Liddle syndrome, first described in 1963, was originally determined to be an autosomal dominant form of endocrine hypertension [17]. Activating mutations of the epithelial sodium channel (ENaC) were initially described, resulting in increased sodium reabsorption/potassium wasting in the distal nephron. As a result, affected patients experience hypertension, hypokalemia, low aldosterone and renin levels, salt sensitivity volume expansion, and metabolic alkalosis [17]. ENaC is composed of three similar subunits, α, β, and γ. While the stoichiometric ratio of these subunits in a channel complex is debated, the current view is a ratio of 1:1:1 [18]. Each subunit consists of intracellular N- and C-termini, two transmembrane domains, and an extracellular loop [19]. The C-terminal region of each subunit contains a conserved proline-rich sequence, called the PY motif [19]. The original gene variants (frameshifts and premature stop codons) identified in the etiology of Liddle syndrome were found to result in the truncation of the C-terminus, with specific deletions of the PY motifs (PPxY) of the β (*SCNN1B*) and γ (*SCNN1G*) subunits [10,11].

Sodium absorption through ENaC is altered in two non-mutually exclusive ways: altering the open probability (P_o) of the channel [20] or varying the membrane density of the channel [21–24]. There is overwhelming evidence to demonstrate that the most potent down-regulation of ENaC is via ubiquitination of the membrane channel and subsequent endocytosis. Liddle syndrome is linked to a defect in the ubiquitin-mediated down-regulation of β and γ ENaC. Specifically, it has been demonstrated that it is the Nedd4-2-mediated ubiquitination of ENaC that leads to endocytosis of the channel [25] and that dysfunction in the ability of Nedd4-2 to ubiquitinate ENaC results in enhanced Na^+ absorption and hypertension in Liddle syndrome (Fig. 1) [26]. Specifically, the E3 ubiquitin ligase Nedd4-2 binds to the PY motif in the C-terminal regions of ENaC subunits and facilitates ubiquitination of lysine residues located in the N-terminal domains of the β and γ ENaC subunits. Nedd4-2 has been shown to effectively suppress ENaC activity by enhancing the endocytosis of the channel from the apical membrane [27–30]. Liddle syndrome mutations effectively remove the PY motifs, resulting in an inability of Nedd4-2 to mediate the ubiquitination and endocytosis of ENaC. The result is an accumulation of active channels at the cell surface, sustained Na^+ (and fluid) absorption in the distal nephron, and hypertension. In summary, the dynamic relationship between ENaC and Nedd4-2 illustrates the complex mechanisms underlying human disease as well as potential new molecular targets to tune membrane excitability.

3. Yotiao and LQT1: PKA-mediated phosphorylation of KCNQ1 and the development of long QT syndrome

Late phase repolarization of the cardiac action potential is largely determined by the slow delayed rectifier current, I_{Ks} [31]. This cardiac membrane current is controlled by potassium channel comprised of

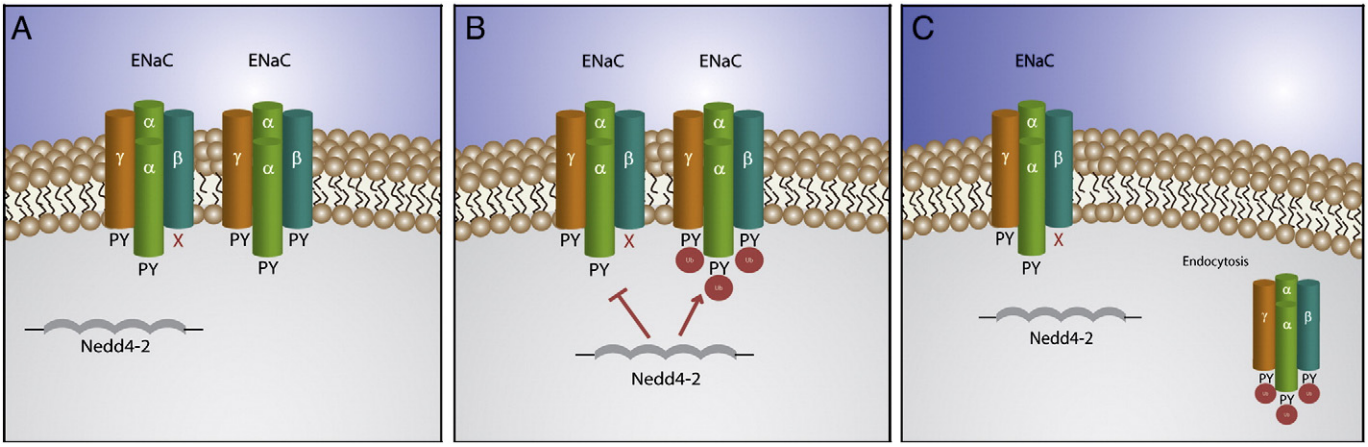

Fig. 1. Regulation of ENaC by Nedd4-2. A–B. The ubiquitin ligase Nedd4-2 recognizes PY motifs in wild type ENaC but not mutated forms of the β or γ subunits, which lack the PY motif. C. Ubiquitinated ENaC is endocytosed, leading to a reduction in ENaC at the plasma membrane, reducing activity. On the other hand, mutated forms of ENaC, that are resistant to ubiquitination, remain at the plasma membrane, and increase channel activity.

four pore-forming KCNQ1 α-subunits and auxiliary KCNE1 β-subunits [32,33]. Individuals with variants in *KCNQ1* or *KCNE1* may develop type 1 or type 5 long QT syndromes, respectively. Specifically, these patients demonstrate arrhythmias and sudden cardiac death in response to stimulation of the sympathetic nervous system [34].

I_{Ks} is regulated by stimulation of the sympathetic nervous system, contributing to shortening of the cardiac action potential despite concurrent increases in heart rate. β-adrenergic receptors (β-ARs) modulate excitation–contraction coupling through signaling cascades that lead to PKA-dependent phosphorylation of several proteins. β-AR agonists, such as norepinephrine, activate stimulatory G-proteins, resulting in the activation of adenylate cyclase and an increase in cAMP production. The increase in cAMP activates PKA [35]. Sympathetic regulation of the cardiac action potential is mediated via PKA-mediated phosphorylation of KCNQ1 [36,37]. Facilitation of PKA-mediated phosphorylation of KCNQ1 requires assembly with the A-kinase anchoring protein (AKAP) Yotiao (Fig. 2) [38]. A decade ago, Marx and colleagues identified Yotiao as a necessary targeting protein for the KCNQ1 channel complex in the cardiomyocyte [38]. Studies in brain had previously identified Yotiao as an AKAP [39,40].

Mutations in KCNQ1 and Yotiao which disrupt the complex result in type 1 and type 11 long QT syndromes, respectively [41]. For example, a mutation in KCNQ1 (G589D), originally identified by Piippo and colleagues [42], was determined to disrupt the interaction between Yotiao and I_{KS} complex, rendering the I_{KS} complex insensitive to β-adrenergic stimulation [38]. As a result, the channel had a significantly increased threshold of activation [38]. Modeling studies suggested that this mutation was likely to produce QT_c prolongation and extrasystoles in conjunction with sympathetic stimulation [43]. Counterpoint to mutations in *KCNQ1*, the inherited S1570L mutation in Yotiao also results in a reduced interaction between Yotiao and the I_{KS} complex [44]. Specifically, this mutation resides in a region of Yotiao which coordinates its interaction with KCNQ1 [44]. While S1570L does not eliminate the interaction with the I_{KS} complex, it produces a significant reduction in its association with KCNQ1, reduces cAMP-induced KCNQ1 phosphorylation, and eliminates the functional response of the I_{KS} complex to cAMP [44]. Overall, this results in delayed repolarization of the action potential [44]. Together, these data demonstrate the necessity of macromolecular complexes for normal cardiac physiology.

4. The ryanodine receptor and CPVT: defects in a macromolecular regulatory complex

Ryanodine receptors are critical players in cardiac excitation–contraction coupling, where opening of the plasma membrane L-type Ca^{2+} channel triggers an intensified release of Ca^{2+} from the sarcoplasmic reticulum, i.e. Ca^{2+}-induced Ca^{2+} release. Cardiac RyRs are primarily tetramers of RyR_2 monomers with large regulatory domains located in the cytosol [45]. RyR_2 channels exist as extensive macromolecular complexes, comprised of over a dozen regulatory subunits, including calmodulin (CaM), protein kinase A (PKA), Ca^{2+}/calmodulin-dependent kinase II (CaMKII), protein phosphatases I and 2A (PP1, PP2A), calstabin2 (also known as FK506-binding protein FKBP12.6), phosphodiesterase 4D3 (PDE4D3), triadin, and calsequestrin [45,46].

Human variants in the RyR_2 gene have been linked to human catecholaminergic polymorphic ventricular tachycardia (CPVT), [42,47] a disease directly linked with sympathetic-dependent polymorphic arrhythmia with risk of sudden cardiac death. Specifically, CPVT-associated gain-of-function variants sensitize RyR_2 to activation by cytosolic Ca^{2+} [48,49] or delay Ca^{2+}-dependent inactivation [50]. Multiple studies have supported this, demonstrating that cardiomyocytes isolated from transgenic knock-in mice (heterozygous for CPVT-associated mutations) show gain-of-function activity, where catecholamine-induced spontaneous Ca^{2+} release events were observed [46,51].

In 2003, Marks and colleagues proposed a model to explain the decreased stability of the closed conformation state of mutant RyR_2 channels. While another hypothesis suggested that dysfunctional regulation of RyR2 was due to abnormal intramolecular associations within the RyR2 molecule [52], the Marks' model suggested a role for the channel-stabilizing subunit FKBP12.6 [49]. Specifically, their work proposed that binding to FKBP12.6 was decreased in CPVT-linked mutations in *RyR2*. Further work from the group supported this model, demonstrating that the binding affinity of FKBP12.6 is reduced for CPVT-associated *RyR2* mutants [49]. Moreover, several studies have demonstrated that the dissociation of FKBP12.6 destabilizes the closed state of the channel, leading to a gain-of-function phenotype [53]. Studies using FKBP12.6 knockout mice further strengthened the link between RyR_2-FKBP12.6 interactions and ventricular arrhythmias [49,54]. Following β-adrenergic stimulation, FKBP12.6$^{-/-}$ mice experience ventricular tachycardia,

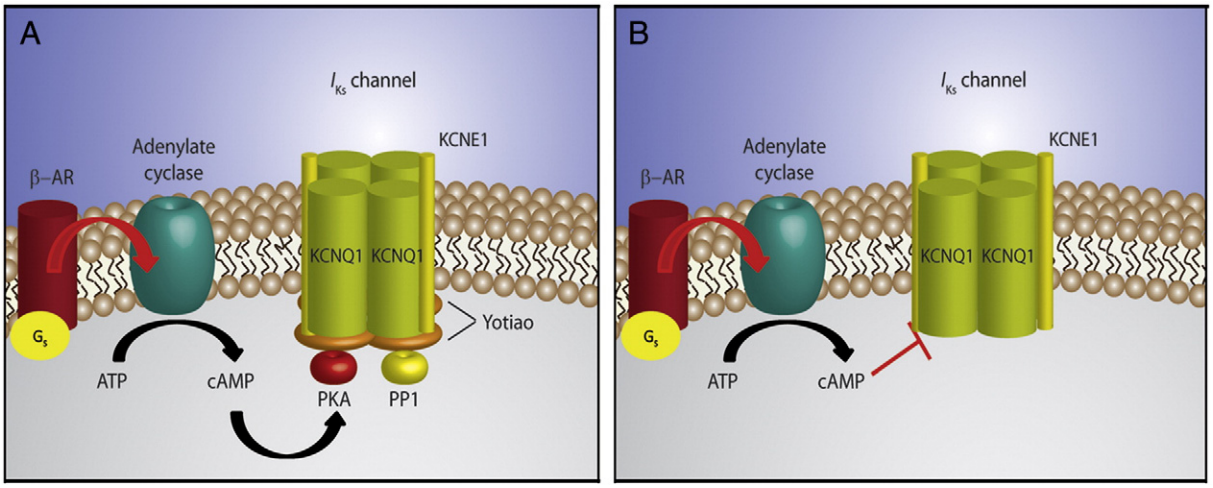

Fig. 2. Regulation of I_{Ks} by the AKAP Yotiao. A. The I_{Ks} channel consists of KCNQ1 α-subunits and KCNE1 β-subunits. The AKAP Yotiao complexes protein kinase A (PKA) and protein phosphatase 1 (PP1) to the I_{Ks} channel. Stimulation of the β-adrenergic receptor (β-AR) leads to activation of adenylate cyclase, the production of cAMP, and activation of protein kinase A. PKA and PP1 regulate I_{Ks} via phosphorylation/dephosphorylation activities. B. Mutations which disrupt the association between Yotiao and the I_{Ks} channel complex become insensitive to β-adrenergic stimulation.

which is very similar to what is observed in transgenic mice harboring CPVT-associated mutations in *RyR2* [46,51]. Moreover, an experimental drug used to enhance FKBP12.6 binding to RyR2 (JTV519) inhibits arrhythmias in FKBP12.6$^{+/-}$ mice [55]. Despite these findings, there is still controversy involving the role of FKBP12.6 in the pathogenesis of CPVT [56]. Future studies are needed to fully elucidate the molecular mechanisms associated with RyR_2-associated CPVT in humans.

5. Ankyrins: critical components of the excitable cell membrane

Ankyrins have been established as integral components in the targeting, retention, and regulation of a number of ion channels, transporters, pumps, and cytosolic proteins in excitable cells. Three ankyrin gene products, ankyrin-R (*ANK1*), ankyrin-B (*ANK2*), and ankyrin-G (*ANK3*) are expressed in vertebrates, each with unique expression profiles and disease associations. In the past decade, studies in humans and mice have illustrated a critical role for ankyrin function in the heart and pancreatic beta cell. Dysfunction in ankyrin-B has been associated with defective intracellular Ca^{2+} handling (LQT4 or Ankyrin-B syndrome), as well as permanent neonatal diabetes mellitus, while dysfunction in ankyrin-G binding with $Na_v1.5$ has been linked with Brugada syndrome.

5.1. Ankyrin-B syndrome: lost anchors lead to excitable cell disease

Nearly 20 years ago, Schott and colleagues described a French cohort displaying an atypical form of long QT syndrome [57]. Affected family members displayed abnormal T-wave morphology, sinus node bradycardia, and atrial fibrillation [57]. Notably, certain individuals displayed stress- and exercise-induced sudden cardiac death [57]. Using linkage analysis, Schott identified that this fourth locus for congenital LQT was associated with an 18 cm region corresponding to the *ANK2* locus (ankyrin-B) [57]. Further analysis revealed a single *ANK2* missense variant, A4274G, resulting in E1425G [58].

Since this initial discovery, a number of additional ankyrin-B human gene variants have been identified [59–63]. Patients with *ANK2* loss-of-function variants may display sinus node dysfunction, atrial fibrillation, polymorphic ventricular arrhythmias, and/or conduction defects [58,60,61]. Studies in mice that are haploinsufficient in ankyrin-B (ankyrin-B$^{+/-}$) have been invaluable in the evaluation of molecular mechanisms underlying the role of ankyrin-B in excitable cell physiology. Ankyrin-B$^{+/-}$ mice demonstrate common phenotypes with the original French LQT4 kindred, including sinus bradycardia, conduction defects, and catecholamine-induced polymorphic ventricular arrhythmia with associated syncope/death [58]. Isolated primary cardiac myocytes from ankyrin-B$^{+/-}$ mice were used to conclude that the E1425G variant was a loss-of-function variant of ankyrin-B. Ankyrin-B$^{+/-}$ cardiomyocytes displayed reduced spontaneous contraction rates, aberrant localization of the Na^+/Ca^{2+} exchanger (NCX), and abnormal calcium transients [58,64,65]. Rescue experiments using exogenous ankyrin-B successfully restored wild type myocyte phenotypes [58,64,65]. Conversely, overexpression of exogenous ankyrin-B E1425G in ankyrin-B$^{+/-}$ myocytes was unable to restore abnormal phenotypes [58].

Further evaluation revealed that ankyrin-B$^{+/-}$ myocytes expressed significantly less Na^+/Ca^{2+} exchanger (NCX), Na^+/K^+ ATPase (NKA), and inositol 1,4,5 trisphosphate receptor ($InsP_3R$) [58]. Additionally, it was determined that the NCX and NKA were reduced preferentially at the transverse tubule [58]. Biochemical analyses demonstrated at ankyrin-B directly associates with the NCX, NKA, and $InsP_3R$ [66]. Furthermore, it was established that exogenous expression of ankyrin-B cDNA in ankyrin-B$^{+/-}$ cardiomyocytes restored proper expression and localization of the NCX, NKA, and $InsP_3R$ [58,60,61,64,66,67]. Eventually it was determined that loss of ankyrin-B-dependent localization of NCX and NKA resulted in increased SR Ca^{2+} load and elevated SR Ca^{2+} transients, essentially mimicking the effects of cardiac glycosides [68,69]. These studies have demonstrated that reduced expression/dysfunction of ankyrin-B can be an underlying cause for human arrhythmia susceptibility by altering myocyte electrical activity.

5.2. Ankyrin-B and the K_{ATP} channel: a new component in glucose homeostasis

The K_{ATP} channel is a hetero-octameric structure of four Kir6x pore-forming subunits and four SUR regulatory subunits [70]. Although K_{ATP} channels were originally identified in heart, it is the pancreatic K_{ATP} channels that have been most thoroughly investigated. In the pancreatic beta cell, the K_{ATP} channel functions as a metabolic sensor, closing the K_{ATP} channel in response to increased blood glucose. Closure of the K_{ATP} channel results in beta cell depolarization, activation of L-type Ca^{2+} channels, and stimulation of insulin granule release [71]. Not surprisingly, mutations in K_{ATP} channel genes (*KCNJ11* and *ABCC8*) are associated with both diabetes mellitus and hyper-insulinemia syndromes (reviewed in [72]). In 2004, a mutation in the Kir6.2 subunit, E322K, was identified in a case of permanent neonatal diabetes mellitus [73]. Several years later, it was determined that this mutation resided in the region of Kir6.2 that associated with ankyrin-B (Fig. 3) [74]. Specifically, it was determined that the E322K variant disrupts the association of Kir6.2 with ankyrin-B in vivo and in vitro and also decreases the sensitivity of the channel to ATP [74]. Based on modeling studies, the disruption of the Kir6.2/ankyrin-B interaction was proposed to result in two seemingly opposing phenotypes: a loss-of-function due to loss of K_{ATP} channel density at the membrane and a gain-of-function due to decreased ATP sensitivity [74]. It is suggested that the combination of the two phenotypes actually results in a diabetic phenotype less severe than either a gain-of-function mutation or loss-of-function mutation alone. These data suggest that the disruption of the ankyrin-B/Kir6.2 complex via the Kir6.2 E322K mutation results in aberrant localization and regulation of the K_{ATP} channel. Further, these data illustrate the complexity of pathways underlying ion channel regulation in health and disease.

5.3. Ankyrin-G and $Na_v1.5$: Brugada syndrome

Voltage-gated Na^+ (Na_v) channels are required for normal electrical activity in a number of cell types, including neurons, skeletal muscle, and cardiomyocytes [75–81]. Ankyrin-G has been firmly established as a critical player in the trafficking of Na_v channels in the central nervous system [82]. In neuronal cells, ankyrin-G is co-expressed with $Na_v1.2$

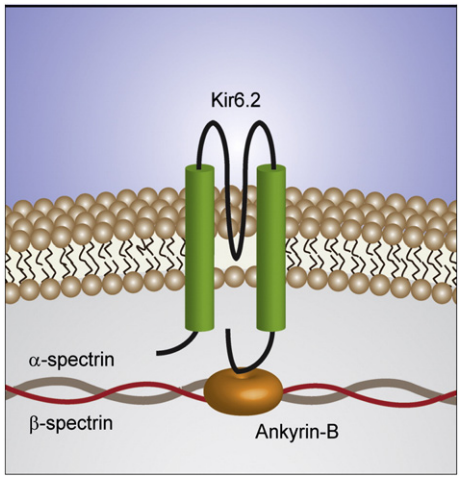

Fig. 3. Regulation of the pancreatic K_{ATP} channel by ankyrin-B. Ankyrin-B associates with the α-subunit of the K_{ATP} channel, Kir6.2, via a C-terminal motif in Kir6.2. Association with ankyrin-B regulates Kir6.2 plasma membrane expression and K_{ATP} channel ATP sensitivity.

and $Na_v1.6$ at the axon initial segments, nodes of Ranvier, and the neuromuscular junction [83–86]. More specifically, it has been demonstrated that ankyrin-G directly associates with Na_v channels at these specialized neuronal membrane domains [85,87]. In 2003, two studies published independently identified a unique ankyrin-G binding sequence (ABS) in the cytoplasmic DII/DII loop of $Na_v1.2$ [88,89]. The ABS is a highly conserved nine amino acid motif [(V/A)P(I/L)AXXE(S/D)] and found in most Na_v channel gene products, including $Na_v1.1$, $Na_v1.2$, $Na_v1.4$, $Na_v1.5$, and $Na_v1.6$. Based on the presence of both ankyrin-G and $Na_v1.5$ at the cardiac intercalated disc, it was proposed that perhaps ankyrin-G filled a similar role in cardiac physiology [61]. Indeed, Bennett and colleagues demonstrated that an ankyrin-G-dependent pathway was necessary for proper targeting of $Na_v1.5$ to the intercalated disc [61]. Specifically, their study demonstrated co-localization of ankyrin-G and $Na_v1.5$ to the cardiac intercalated disc, that $Na_v1.5$ and ankyrin-G co-immunoprecipitated from detergent-soluble cardiac lysates, and that deletion of the ABS rendered $Na_v1.5$ incapable of binding ankyrin-G [61]. Using primary cardiomyocytes deficient in ankyrin-G expression, a follow-up study by Lowe and colleagues demonstrated that loss of ankyrin-G resulted in decreased $Na_v1.5$ protein expression, decreased $Na_v1.5$ membrane targeting, and reduced cardiomyocyte I_{Na} [79].

Brugada syndrome, a cardiac syndrome characterized by ST segment elevation, right bundle branch block, and fatal cardiac arrhythmias [90], had long been attributed to loss of function mutations in *SCN5A* (encoding the $Na_v1.5$ channel) [91]. Specifically, it was believed that these mutations altered $Na_v1.5$ channel biophysics or drastically altered protein structure [92]. However, in 2004, Priori and colleagues screened a large number of Brugada patients for mutations in the ABS region of *SCN5A* [61]. One *SCN5A* missense mutation was identified, E1053K [61]. Work by Bennett et al. determined that E1053K prevented associated of $Na_v1.5$ with ankyrin-G [61]. Moreover, $Na_v1.5$ E1053K was not properly targeted to the intercalated disc, implicating an ankyrin-G-based pathway for $Na_v1.5$ targeting in cardiomyocytes [61]. In summary, findings from the brain, heart, and pancreas identify ankyrin polypeptides as critical nodes for regulation of membrane excitability for normal physiology as well as demonstrate how defects in these pathways underlie human pathophysiology. As ankyrins are expressed far beyond these tissues, future studies will likely yield additional linkages between these genes and other disease etiologies.

6. Caveolin-3 and type 9 long QT syndrome

Caveolae ("little caves") are invaginations of the plasma membrane critical to membrane endocytosis, cholesterol homeostasis, cell signaling, and tumorigenesis [93,94]. The primary coat proteins of caveolae are encoded by three genes: *CAV1* (caveolin-1), *CAV2* (caveolin-2) and *CAV3* (caveolin-3) [95–98]. While caveolin-1 and caveolin-2 are expressed in most cell types, caveolin-3 is primarily expressed in muscle tissue [98]. Structurally, caveolin proteins are similar, with an N-terminal domain, a scaffolding domain, and hydrophobic domain, and a C-terminal domain [99]. Caveolin-3 has been linked with Duchenne muscular dystrophy, rippling muscle disease, and idiopathic hyperCKemia [100–103]. At a molecular level, caveolin co-purifies with a number of cardiac membrane proteins, including $Na_v1.5$, HCN4 pacemaker channel, NCX, $InsP_3R$, $K_v1.5$, PMCA, $Ca_v1.2$, and TRP channels [104–111]. Caveolin-3 knockout mice display abnormal cardiac T-tubule organization, [112,113] moderate cardiomyopathy, [93] and skeletal muscle myopathy [112,114]. Similar to these findings in caveolin-deficient mice, humans harboring *CAV3* variants display parallel phenotypes, [100–103] suggesting a role for caveolin in human cardiac arrhythmias.

In 2006, Vatta and colleagues screened "genotype negative" LQTS probands for potential variants in *CAV3* [115]. These individuals displayed a variety of cardiac phenotypes, including non-exertional syncope, sinus bradycardia, and prolonged QT_c intervals [115]. Four mutations were identified and termed LQT9 [115]. Prior to these findings, it had been demonstrated that caveolin-3 associated with Na_v channels, [111] leading to further investigation of caveolin-3 and $Na_v1.5$ interactions in the cardiomyocyte. Studies by Vatta and others demonstrated that caveolin-3 and $Na_v1.5$ co-localize and co-immunoprecipitate in vivo [111]. Furthermore, it was determined that the N-terminal domain of caveolin-3 was required for interaction with $Na_v1.5$ [115]. Heterologous overexpression of both $Na_v1.5$ and caveolin-3 in HEK cells produced a two- to three-fold increase in late Na^+ current, likely contributing to the QT prolongation and cardiac arrhythmias observed in *CAV3* mutation carriers [115]. It remains to be determined whether *CAV3* mutations result in abnormal subcellular targeting (since *CAV3* mutations do not disrupt association with $Na_v1.5$ [115]), whether *CAV3* mutations allosterically affect Na_v channels, or whether cardiac dysfunction results from altered localization of other caveolin-3-associated ion channels to the sarcolemma. Of note, two recent studies have found an associated between *CAV3* mutations and sudden infant death syndrome [116,117].

7. α-syntrophin and type 12 long QT syndrome

Syntrophins are a family of cytoplasmic adapter proteins that link the actin-based cytoskeleton (via the dystrophin-associated complex) to the extracellular matrix. Syntrophins have an internal PDZ domain that has been demonstrated to interact with the C-terminal region of Na_v channels, including $Na_v1.4$ and $Na_v1.5$ [118,119]. Interestingly, *mdx* mice (lacking dystrophin) display loss of cardiomyocyte $Na_v1.5$ expression from the peripheral sarcolemma, [119] presumed to result from dysregulation of syntrophin. In addition to $Na_v1.5$, syntrophins have been linked to a number of ion channels; namely, the plasma membrane calcium ATPase (PMCA) and neuronal nitric oxide synthase (nNOS) [120–122]. Since nitric oxide (NO) directly affects late I_{Na} in LQT3 (where pathogenic mutations in *SCN5A* accentuate late I_{Na}), this connection is relevant to topic [123]. Additionally, variants of CAPON (*NOS1AP*), a nNOS regulatory protein, have been linked with QT interval regulation in genome-wide association studies [124,125]. Direct sequencing of α-1 syntrophin (*SNTA1*) in a number of "genotype negative" LQTS patients (see above) identified a novel missense mutation, A390V, in a patient presenting with syncope and QT_c prolongation (now termed LQT12) [123]. This mutation was found to decrease association of α-1 syntrophin with PMCA4b and nNOS. Additionally, A390V increased $Na_v1.5$ nitrosylation, as well as increased persistent I_{Na} in cardiomyocytes [123]. It is believed that this $Na_v1.5$ dysregulation is the underlying cellular mechanism for LQT12. Considering the number of ion channels known to associate with α-1 syntrophin, it is possible that other forms of cardiac dysfunction may be attributable to dysfunction in α-1 syntrophin.

8. Conclusion

Cardiovascular therapies including anti-platelet drugs, statins, renin–angiotensin system antagonists, beta-blockers, and thrombolytic agents developed in this past century have decreased cardiovascular-based mortality over the past fifty years. However, cardiovascular disease remains the leading cause of death in the United States. Cardiac ion channels and transporters regulate membrane excitability and therefore would appear to represent a critical family of targets for anti-arrhythmia therapy. Unfortunately, ion channel antagonists have been unsuccessful in decreasing mortality in patients at risk for cardiac sudden death — and in select cases have actually increased mortality [126]. It is well established that macromolecular signaling complexes, composed of adaptor proteins, ion channels, and signaling molecules, are essential the creation, maintenance, and regulation of local microenvironments in a number of cell types. The identification of mutations and variants within a larger ion channel complex has provided valuable insights into the molecular basis of human disease. Based on the data discussed in this review, we propose that non-conventional cellular

pathways that indirectly regulate cardiac membrane ion channels and transporter activities (ankyrins, spectrins, and syntrophins) may serve as exciting new routes to tune cellular excitability in both congenital and acquired forms of cardiovascular disease.

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