

# Molecular stratification of arrhythmogenic mechanisms in the Andersen Tawil syndrome

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## Abstract

Andersen-Tawil syndrome (ATS) is a rare inheritable disease associated with loss-of-function mutations in *KCNJ2*, the gene coding the strong inward rectifier potassium channel Kir2.1, which forms an essential membrane protein controlling cardiac excitability. ATS is usually marked by a triad of periodic paralysis, life-threatening cardiac arrhythmias and dysmorphic features, but its expression is variable and not all patients with a phenotype linked to ATS have a known genetic alteration. The mechanisms underlying this arrhythmogenic syndrome are poorly understood. Knowing such mechanisms would be essential to distinguish ATS from other channelopathies with overlapping phenotypes and to develop individualized therapies. For example, the recently suggested role of Kir2.1 as a countercurrent to sarcoplasmic calcium reuptake might explain the arrhythmogenic mechanisms of ATS and its overlap with catecholaminergic polymorphic ventricular tachycardia. Here we summarize current knowledge on the mechanisms of arrhythmias leading to sudden cardiac death in ATS. We first provide an overview of the syndrome and its pathophysiology, from the patient's bedside to the protein and discuss the role of essential regulators and interactors that could play a role in cases of ATS. The review highlights novel ideas related to some post-translational channel interactions with partner proteins that might help define the molecular bases of the arrhythmia phenotype. We then propose a new all-embracing classification of the currently known ATS loss-of-function mutations according to their position in the Kir2.1 channel structure and their functional implications. We also discuss specific ATS pathogenic variants, their clinical manifestations, and treatment stratification. The goal is to provide a deeper mechanistic understanding of the syndrome toward the development of novel targets and personalized treatment strategies.

## Keywords

Channelopathies • *KCNJ2* mutations • Kir2.1-Nav1.5 channelosome • Sarcoplasmic reticulum Kir2.1 channels • Kir2.1 mutation classification • Sudden cardiac death

## 1. Introduction

Andersen-Tawil syndrome (ATS) is a rare cardiac disease belonging to the group of inheritable disorders called 'channelopathies' or 'ion channel diseases'. Similar to other channelopathies, ATS associates with mutations in genes encoding ion channels or their regulatory proteins with predisposition to cardiac arrhythmias and sudden cardiac death (SCD), usually encountered in children and young adults.<sup>1</sup> Mutations in *KCNJ2*, the gene encoding the inward rectifier potassium channel Kir2.1, are thought to cause around 60% of ATS cases catalogued as ATS type 1 (ATS1).<sup>2</sup> All ATS1 mutations result in loss-of-function in Kir2.1 but may also alter the function of other

channels or their interacting proteins, with variable pathophysiological consequences. Approximately 40% of patients who present mutations in genes other than *KCNJ2* are grouped as ATS type 2 (ATS2).<sup>3</sup>

Similar to other 'monogenic' ion channel diseases, the study of the molecular mechanisms of arrhythmias in ATS1 has traditionally relied on *in vitro* experiments in heterologous expression systems, and more recently on human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) platforms. These systems assume that there must be a direct relationship between the ion channel mutation and the disease phenotype. However, ion channels do not function in isolation, but are part of large multi-protein complexes, comprising not only

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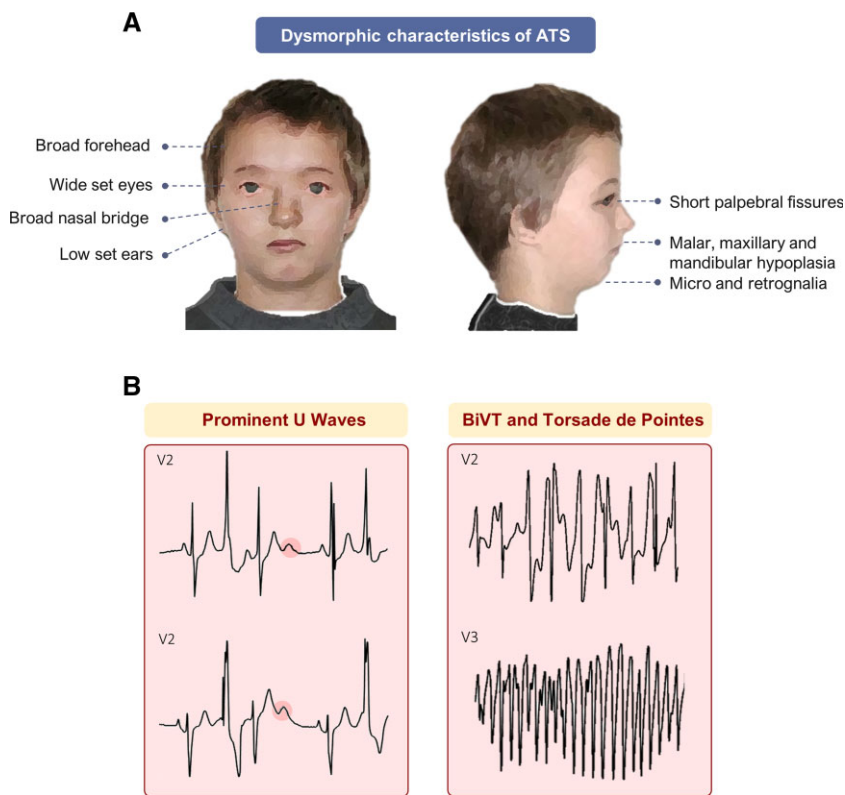
their auxiliary subunits, but also components of the cytoskeleton, regulatory kinases, and phosphatases, trafficking proteins, extracellular matrix proteins, and even other ion channels.<sup>4–6</sup> Therefore, there is a significant discrepancy between what is known about the disease and what should be known in terms of its molecular mechanisms for a more accurate treatment of the patients. Such a gap in mechanistic knowledge hampers understanding of the cardiac arrhythmia phenotype and prevents progress in disease treatment and SCD prevention.

The objective of this review article is to summarize current knowledge on the mechanisms of arrhythmias in ATS1 and offer a novel perspective for future research. We first provide a general overview of the disease and its pathophysiology, from the patient's bedside to the protein. We then propose a classification of the Kir2.1 mutations that have been reported to date, according to their position in the different domains that conform the channel structure, as well as the functional implications in terms of defects in Kir2.1 channel trafficking, biophysics, functional regulation, and type of membrane localization. Throughout the discussion, we address novel ideas related to some of the post-translational channel interactions with partner proteins that might help us better define the arrhythmic phenotype for some of the genetic variants. We then move on to classify the ATS1 mutations that are known to date, with emphasis on pathogenic variants, clinical manifestations and treatment stratification. As we see it, such a stratified approach should help develop a deeper mechanistic understanding of the syndrome's sub-groups, hopefully leading to the identification of novel targets and personalized treatment strategies.

## 2. Andersen-Tawil syndrome Type 1

ATS1 is an extremely rare, inherited cardiac disorder (frequency estimated at < 1 in 1 000 000) with a particular phenotype marked by a triad of periodic paralysis, cardiac arrhythmias, and dysmorphic features first reported by Andersen et al.<sup>7</sup> in 1971. The disease was further characterized by Tawil et al.<sup>8</sup> Mutations in *KCNJ2* that cause ATS1 are inherited in an autosomal dominant way. Nevertheless, the rate of occurrence of *de novo* mutations at the embryonic stage is high. In a recent study including 118 patients with ATS1 from 57 families, about 35% of the mutations were reported as *de novo*.<sup>1</sup> Plaster et al.<sup>9</sup> first described the linkage between ATS1 and multiple mutations in the *KCNJ2* gene. Most ATS1 mutations result in a dominant-negative loss-of-function of Kir2.1 channels, a fact that directly affects the strong inward rectifier potassium current ( $I_{K1}$ ), leading to its substantial downregulation.

Along with a range of other phenotypic manifestations, the triad of symptoms that characterizes the syndrome includes sustained periodic paralysis, muscular weakness, extrasystoles (often present in the form of bigeminy), bidirectional ventricular tachycardia (BiVT), multiple developmental anomalies and dysmorphic features, such as broad forehead, hypoplastic mandible, hypertelorism, low-set ears and digit clinodactyly, among others (Figure 1A).<sup>10,11</sup> In published reports, about 67–75% of ATS1 patients showed dysmorphic features, and 35–50% presented recurrent episodes of muscular weakness.<sup>12,13</sup> On the other hand, 97% of patients presented one or more of the cardiac defects and 90% displayed a prominent U-wave



**Figure 1** Phenotypical and electrocardiographic diagnosis for ATS. (A) Schematic representation of the typical dysmorphic features usually found in a patient with ATS. Note the low-set ears, micrognathia (undersized lower jaw) and hypertelorism (wide interpupillary distance). (B) Characteristic electrocardiographic alterations including the presence of prominent U waves (left panel), BiVT, and TdP.

on the electrocardiogram (ECG). Ventricular arrhythmias have been reported in 60–90% of ATS patients, with a high prevalence (48%) of polymorphic ventricular tachycardia (PVT), and BiVT (44%).<sup>14</sup>

The pleiotropic phenotype of ATS1 may be heterogeneous due to variable expressivity of *KCNJ2* mutations. Thus, patients belonging to the same kindred with reduced or non-functional Kir2.1 current may present different signs and symptoms.<sup>9,11,15</sup> Therefore, the clinical variability of ATS1 can make the diagnosis difficult, as some patients may be non-penetrant, while others within the same family may show the classic triad of symptoms completely or partially in variable combinations.<sup>12</sup> In addition, considering that some causative mutations appear *de novo*, family history is improbably of significant help in the early-onset forms of the disease.<sup>1</sup> Currently, diagnosis is made according to the criteria formulated by Venance and co-workers. These authors mainly consider isolated periodic paralysis, polymorphic ventricular ectopy and typical ATS dysmorphias to define ATS1 pathophysiology.<sup>16</sup> Nevertheless, recognition of the disease is sometimes difficult and the actual number of cases may be underestimated. Hereafter, unless otherwise indicated, we focus on the cardiac electrical manifestations of the disease and their possible mechanisms.

Electrocardiographic findings unique to ATS1 are illustrated in *Figure 1B*. They include delayed cardiac repolarization with prolongation of the T wave down slope, wide T–U junction with high amplitude and broad U waves, all of which are harbingers of focal arrhythmias, including bigeminy, ventricular ectopy, BiVT, and PVT in the form of Torsades de Pointes.<sup>12,13</sup> This last life-threatening arrhythmia can be self-limited or degenerated into ventricular fibrillation (VF) leading to cardiac arrest (CA) with syncope or SCD.<sup>17</sup> Mazzanti *et al.*<sup>1</sup> reported SCD in 9.3% of ATS1 patients, whereas Delannoy *et al.*<sup>15</sup> reported 2.3%. Notably, SCD is a major risk factor in young individuals.

Some patients manifest conduction disturbances, such as first-degree atrioventricular (AV) block, right or left bundle branch block, and non-specific intraventricular conduction delay.<sup>13</sup> To our knowledge, only one case of complete AV block has been described in a patient suffering from ATS,<sup>18</sup> which might need to be considered as a possible related sign when clinicians decide on the appropriate treatment. On the other hand, in some patients (~40%),<sup>19</sup> ATS clearly overlaps with catecholaminergic polymorphic ventricular tachycardia (CPVT), a disease mostly associated with mutations in the human cardiac Ryanodine Receptor 2 (*RyR2*) gene encoding the cardiac *RyR2* channel.<sup>20,21</sup> Such an overlap might be explained by the recently observed functional expression of Kir2.1 in the sarcoplasmic reticulum (SR) and its possible role as a countercurrent to sarcoplasmic calcium reuptake.<sup>22</sup>

Although ATS1 was initially described as part of the congenital long QT syndrome (LQTS) family of channelopathies, namely LQT type 7, the QT prolongation is more related to the presence of a pronounced U-wave than an inherent corrected QT (QTc) prolongation.<sup>13,23</sup> Moreover, only 15% of the phenotypic manifestation corresponds to QT prolongation, which argues against the idea that ATS1 belongs to the LQTS family.<sup>1</sup> Recently, Alder *et al.* showed that only 3 of 17 (18%) genes related to LQTS (e.g. *KCNQ1*, *KCNH2*, and *SCN5A*) have been conclusively linked to typical LQTS, whereas the level of evidence supporting a link between *KCNJ2* and LQTS was classified as limited.<sup>24</sup> Therefore, since QT interval prolongation is not representative in the majority of ATS1 patients, and the mutations result in a wide range of cardiac and muscle-skeletal alterations, rather than a LQTS subtype, ATS1 should be considered a separate inheritable channelopathy.

## 3. Source of arrhythmogenesis in ATS1

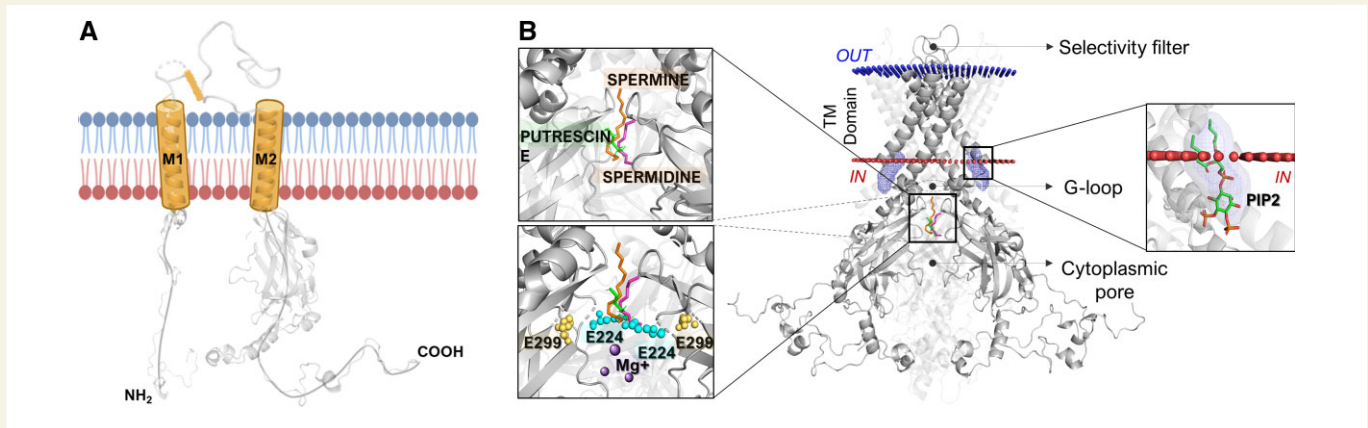
### 3.1 The Kir2.1 channel

*KCNJ2*, the gene coding Kir2.1, is a member of the inwardly rectifying potassium channel family, subfamily J, that also includes *KCNJ12*, *KCNJ4*, *KCNJ14*, *KCNJ17* and *KCNJ18*, coding for Kir2.2, Kir2.3, Kir2.4, Kir2.5 and Kir2.6, respectively.<sup>25</sup> Kir2.4 is expressed in neurons, pulmonary smooth muscle cells and aortic endothelial cells<sup>26,27</sup>; Kir2.6 expression is restricted to skeletal muscle.<sup>28,29</sup> The remaining four Kir2.x isoforms are widely expressed in the mammalian heart<sup>25,28</sup> but are also found in brain, as well as skeletal and vascular muscle.<sup>28</sup>

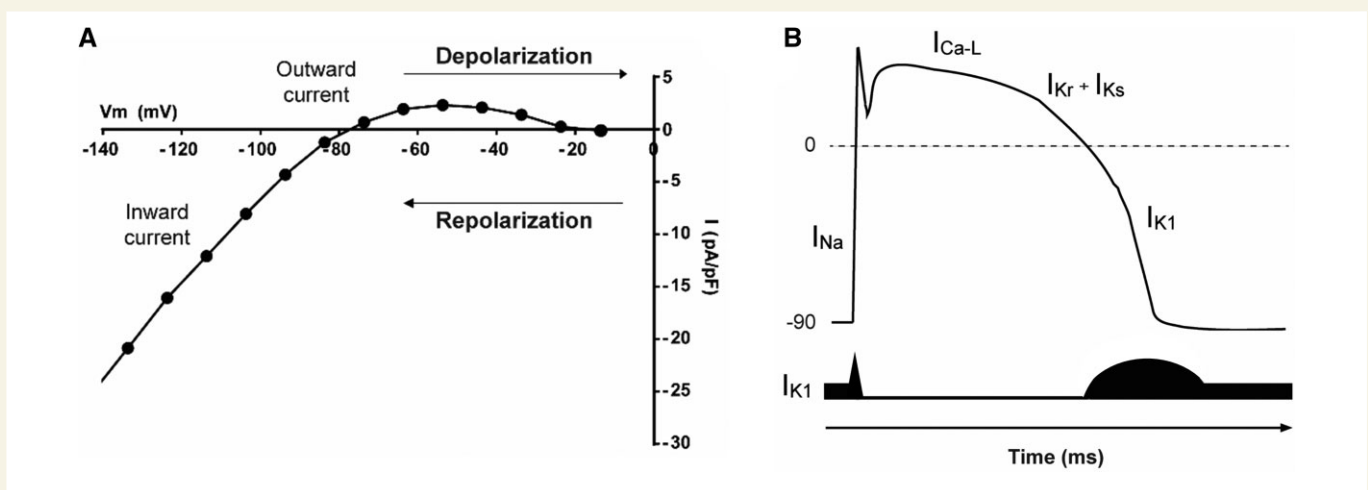
Similar to all other Kir channels, Kir2.1 features two transmembrane helix domains, M1 and M2, an ion-selective pore-forming loop between M1 and M2, and cytoplasmic amino (NH<sub>2</sub>) and carboxyl (COOH) terminals (*Figure 2A*).<sup>30</sup> Functional Kir2.x channels are composed of subunits that co-assemble in a homo- or heterotetrameric manner (*Figure 2B*)<sup>30,31</sup> and are distributed differently in the atria and ventricles. In humans, Kir2.1 is the main isoform forming inwardly rectifier channels alone or in combination with Kir2.2 in the ventricles, whereas Kir2.3 is primarily found in the atria.<sup>4,32,33</sup> This is important because *I<sub>K1</sub>*, the inwardly rectifying potassium current conducted by these channels, has different functional properties depending on the stoichiometry of the monomers forming the final tetramer.<sup>34</sup> Moreover, the consequences of ATS1-associated mutations (homozygous or heterozygous genetic condition) should depend on the configuration of individual Kir2.x channel tetramers, whose specific properties should modify the syndromic phenotype. Such a heterogeneous nature of the channels explains in part the pleiotropy that characterizes ATS1 patients.

Functionally, in both atria and ventricles, the current/voltage (*I/V*) relationship of *I<sub>K1</sub>* has a reversal potential close to the resting membrane potential (RMP; -80 to -90 mV in the ventricles) and shows a negative slope conductance (inward rectification) at depolarized potentials between -50 and 0 mV (*Figure 3A*). Thus, while *I<sub>K1</sub>* represents the largest conductance at the RMP, it approaches zero during the action potential (AP) plateau.<sup>35</sup> Therefore, in the heart, such a non-linear *I/V* relationship reveals how *I<sub>K1</sub>* helps control the RMP, the depolarization toward threshold, the shape, and duration of the plateau and the final phase of AP repolarization (*Figure 3B*).<sup>36,37</sup>

Inward rectification of Kir2.x channels results from blockage by intracellular Mg<sup>2+</sup> and polyamines, which upon membrane depolarization penetrate the cytoplasmic pore of the channel, binding to negatively charged residues and obstructing the outward flow of K<sup>+</sup>.<sup>38,39</sup> Kir2.1 channels possess multiple Mg<sup>2+</sup> and polyamine binding sites, particularly at the D172, E224, and E299 positions (*Figure 2B*).<sup>40</sup> Mutations at any of these residues modify *I<sub>K1</sub>* producing a defective inwardly rectifying response of the channel. Specifically, amino acidic replacements at residues D172 and E299 (D172N and E299V, respectively) are Kir2.1 gain-of-function mutations causing short QT syndrome (SQTS). Both mutations abbreviate the action potential duration (APD) in single cells and the QT interval on ECG.<sup>41,42</sup> Similarly, class 1c antiarrhythmic drugs (e.g. flecainide and propafenone) have been shown to bind to the Cysteine 311 (Cys311) residue of the Kir2.1 channel and to reduce the polyamine-induced inward rectification increasing the outward *I<sub>K1</sub>*.<sup>43,44</sup> Thus, in addition to β-blockers, which counteract the proarrhythmic effects of adrenaline, a common treatment for ATS1 contemplates the use of propafenone/flecainide alone or in combination.<sup>45–48</sup>



**Figure 2 The Kir2.1 channel structure.** (A) Kir2.1 channel monomer. (B) **Centre**, Homotetrameric Kir2.1 channel structure pointing at some relevant regions for the movement of  $K^+$  from intracellular to extracellular spaces. **Left**, Kir2.1 with residues E224 and E299, polyamines (spermine, putrescine, and spermidine) and  $Mg^{2+}$  localized at the cytoplasmic entrance to disrupt the passage of  $K^+$  across the channel. **Right**, Kir2.1 channel conformation with phosphatidylinositol 4,5-bisphosphate (PIP2) at the Kir2.1 binding pocket close to the more cytoplasmic transmembrane plane.



**Figure 3 Inward rectifier potassium current and the AP.** In the mammalian ventricles, Kir2.1 is the main protein forming the inward rectifier  $K^+$  channels responsible for  $I_{K1}$ , the current that controls the RMP and the final phase of repolarization. (A)  $I_{K1}$  current/voltage ( $I/V$ ) relation shows a reversal potential at  $-80$  mV and strong rectification at voltages between  $-50$  and  $0$  mV. (B) **Top**, representation of the human ventricular AP with main outward ( $I_{Kr}$ ,  $I_{Ks}$  and  $I_{K1}$ ) and inward ( $I_{Na}$  and  $I_{CaL}$ ) ionic currents indicated. **Bottom**, temporal changes in  $I_{K1}$  in relation to the cardiac AP.  $I_{K1}$  increases prior to the AP upstroke, goes to zero during the plateau and increases again during the final phase of repolarization.

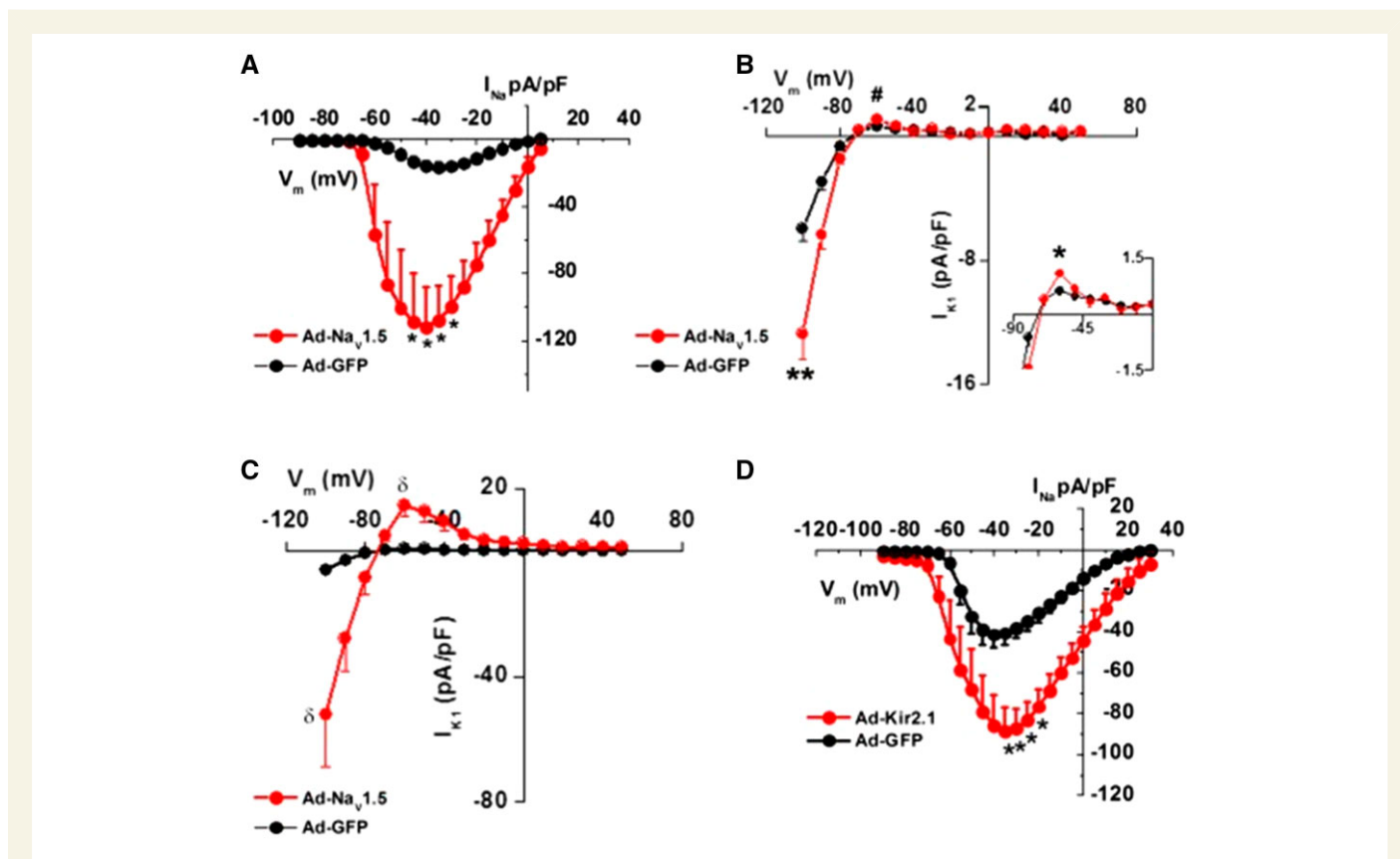
Kir2.1 function is also regulated by other mechanisms. Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2 or PIP2) is a membrane-anchored phospholipid bound to the cytoplasmic leaflet of the Kir2.x channels, which serves as a second messenger to regulate channel gating (Figure 2B).<sup>49,50</sup> PIP2 selectively activates the Kir2.1 channel, while other PIPs have the opposite competitive function.<sup>51</sup> On the other hand, phosphorylation of Kir2.1 channels enables channel function and protein–protein interaction, primarily by the action of protein kinase pathways, such as protein kinases A and C (PKA and PKC, respectively).<sup>52,53</sup> Adrenergically induced PKA phosphorylation (e.g. serine at position 425) results in channel inactivation.<sup>54</sup> In canine Purkinje myocytes, guinea pig and mouse ventricular myocytes, isoproterenol-induced PKA activation inhibited Kir2.1 function, leading to arrhythmogenesis.<sup>55</sup> Therefore, it is important to highlight that adrenergically induced loss of  $I_{K1}$  may affect

AP repolarization, leading to early afterdepolarizations and triggered activity.<sup>54–56</sup>

### 3.2 The Kir2.1 channelosome

The two most important ionic currents controlling ventricular excitability are  $I_{K1}$  and the sodium inward current  $I_{Na}$ . Our understanding of their relationship derives from basic and clinical studies on arrhythmogenesis, demonstrating that changes in the peak density of either ionic current alter both cell excitability and conduction velocity (CV). In addition, it has been amply demonstrated that the balance between  $I_{Na}$  and  $I_{K1}$  is a key determinant of the frequency and stability of the ventricular rotors that generate cardiac fibrillation and lead to SCD.<sup>57</sup> In this section, we review the main features of these two fundamental ion channels and their interactions in the context of ATS1 pathophysiology.





**Figure 4** Reciprocal regulation of  $\text{Nav}1.5$  and  $\text{Kir}2.1$  in ARVMs. (A) and (B)  $\text{Nav}1.5$  overexpression increases both  $I_{\text{Na}}$  and  $I_{\text{K}1}$  densities. (A) Superimposed  $I_{\text{Na}}$  density/voltage relationships (5 mmol/L  $[\text{Na}^+]_o$ ) for Ad-GFP and Ad- $\text{Nav}1.5$ -infected cells. (B) Superimposed  $I_{\text{K}1}$  density/voltage relationship for Ad-GFP and Ad- $\text{Nav}1.5$ -infected cells. **Inset**, magnification of the outward component of the  $I_{\text{K}1}$  I–V relationship. (C) and (D),  $\text{Kir}2.1$  overexpression increases both  $I_{\text{K}1}$  and  $I_{\text{Na}}$  densities. (C) Superimposed  $I_{\text{K}1}$  density/voltage relationships for Ad-GFP and Ad- $\text{Kir}2.1$ . (D) Superimposed  $I_{\text{Na}}$  density/voltage relationships (20 mmol/L  $[\text{Na}^+]_o$ ) for Ad-GFP and Ad- $\text{Kir}2.1$ . \* $P < 0.005$ ; # $P < 0.05$ ;  $\delta P < 0.01$ . Reproduced by permission from Milstein, M.L., et al., *Dynamic reciprocity of sodium and potassium channel expression in a macromolecular complex controls cardiac excitability and arrhythmia*. Proc Natl Acad Sci U S A, 2012. **109**(31): p. E2134–43.<sup>58</sup>

The consequences of a **molecular** interplay between  $\text{Nav}1.5$ , responsible for  $I_{\text{Na}}$ , and  $\text{Kir}2.1$  channels in heart diseases had not been investigated until very recently, and it seems to be much more complex than previously thought.<sup>58</sup> Reciprocal regulation between them was demonstrated in (i) single adult rat ventricular myocytes (ARVMs), in which adenoviral transfer of  $\text{Nav}1.5$  increased both  $I_{\text{Na}}$  and  $I_{\text{K}1}$  (Figure 4A and B) and adenoviral transfer of  $\text{Kir}2.1$  also increased both  $I_{\text{K}1}$  and  $I_{\text{Na}}$  (Figure 4C and D); (ii) in neonatal rat ventricular myocyte monolayers, where co-overexpression of  $\text{Nav}1.5$  with  $\text{Kir}2.1$  increased CV, abbreviated APD and increased rotor frequency beyond those produced by  $\text{Kir}2.1$  overexpression alone; and (iii) in transgenic mice overexpressing  $\text{Kir}2.1$ , where peak  $I_{\text{Na}}$  density is twice as large as the control. On the other hand, in heterozygous  $\text{Kir}2.1$  knockout mice,  $\text{Nav}1.5$  protein was significantly reduced.<sup>58</sup> Further, data in mice showed that a reduction of the cardiac sodium channel  $\text{Nav}1.5$  was accompanied by a concomitant reduction in  $I_{\text{K}1}$ .<sup>59</sup> In addition, experimental proximity ligation assays have confirmed that the distance between both channels at the membrane is narrow enough ( $<40$  nm) to allow their positive interaction.<sup>60</sup> Importantly, the finding that co-expression of  $\text{Nav}1.5$  may reduce internalization of  $\text{Kir}2.1$  was a central mechanistic observation.<sup>58</sup>

Altogether, the evidence suggests that in cardiac cells there is model independent co-regulation of  $\text{Kir}2.1$  and  $\text{Nav}1.5$ . What controls the

$\text{Kir}2.1$ - $\text{Nav}1.5$  co-dependence is just the beginning to be elucidated. Notably, the processes of trafficking, distribution and function of both types of channel are highly dependent on the help of multiple auxiliary proteins and protein–protein interactions, which contribute to incorporation of the fully folded proteins into macromolecular complexes that empower each of the channel's respective role in the control of cardiac excitability.<sup>36,58</sup> In the case of  $\text{Kir}2.x$ , each of the four cardiac monomers that conform a given channel contains a sequence motif of three amino acids (SEI for  $\text{Kir}2.1$  and  $\text{Kir}2.2$ , and SAI for  $\text{Kir}2.3$ ) that allows the interaction with PDZ (postsynaptic density protein, *Drosophila* disc large tumour suppressor, and zonula occludens-1 protein) domain proteins.<sup>58,61</sup> An example of this is the synapse-associated protein-97 (SAP97), an important anchoring/adaptor protein for cardiac ion channels located at the intercalated discs and transverse tubules (*t-tubules*) of cardiomyocytes co-localizing with  $\text{Nav}1.5$  and  $\text{Kir}2.1$ .<sup>58,62,63</sup> *In vitro* experiments have shown that silencing SAP97 decreases both  $I_{\text{K}1}$  and  $I_{\text{Na}}$  implying a close interaction among all three proteins.<sup>63,64</sup> However, while cardiac-specific silencing of SAP97 in a murine model did decrease  $\text{Kir}2.1$ , it did not alter functional  $I_{\text{Na}}$  in isolated cardiomyocytes.<sup>64</sup> On the other hand, in a more recent study, cardiac-specific ablation of SAP97 increased  $I_{\text{Na}}$ .<sup>65</sup> Settling this important controversy will require additional experiments ensuring adequate control of membrane protein levels and channel function.

Another important scaffolding protein interactor of Kir2.x channels is  $\alpha$ 1-syntrophin, a member of the dystrophin-associated protein complex (DAPC).<sup>66</sup> The DAPC is involved in mechanoprotection of the plasma membrane<sup>67</sup> acting as a linker between the intracellular space and the extracellular matrix.<sup>62</sup> In the heart, the dystrophin-associated protein  $\alpha$ 1-syntrophin acts as a scaffold for numerous signalling and ion channel proteins that control cardiac excitability.<sup>57,66,67</sup> Similar to SAP97,  $\alpha$ 1-syntrophin is also a PDZ domain protein that co-localizes with Kir2.1 and  $\text{Na}_v1.5$  at the sarcolemma.<sup>58,62,63,68,69</sup> Interestingly, Matamoros *et al.* demonstrated the existence of a PDZ-binding-like domain at the  $\text{NH}_2$ -terminal of  $\text{Na}_v1.5$  where  $\alpha$ 1-syntrophin also modifies  $I_{\text{Na}}$  and  $I_{\text{K}1}$  by enhancing  $\text{Na}_v1.5$  and Kir2.1 membrane levels.<sup>69</sup> More recently, Jimenez-Vazquez *et al.*<sup>70</sup> demonstrated that iPSC-CMs from DMD patients with cardiomyopathy have a dysfunctional  $\text{Na}_v1.5$ -Kir2.1 channelosome, which they rescued by expressing *SNTA1*, the gene coding  $\alpha$ 1-syntrophin, to restore excitability and prevent arrhythmias.

### 3.3 The Kir2.1 interactome

From the foregoing, it is evident that at least some fractions of the strong inward rectifier potassium channel Kir2.1 and the main cardiac voltage-gated sodium channel  $\text{Na}_v1.5$  are part of macromolecular complexes, called *channelosomes*, that keep them functioning together at the lateral membrane, the t-tubules and the intercalated disks to control cardiac excitability under normal physiological conditions and in disease states.<sup>4,6,71</sup> Recently, a comprehensive map of the Kir2.1 interactome constructed in T-REx 293 cells using BioID,<sup>72</sup> identified a total of 218 high-confidence novel interactions controlling various molecular mechanisms of Kir2.1 function (Figure 5), ranging from intracellular trafficking to crosstalk with the insulin-like growth factor receptor signalling pathway and lysosomal degradation.<sup>72</sup> In fact, the Kir2.1 BioID interactome dataset represents a repository for numerous, novel biological hypotheses for genes and molecular mechanisms implicated in Kir2.1-associated cardiac diseases. For example, out of the 218 high-confidence Kir2.1 interactors identified in our BioID screen, 36 of them have been associated (mutation and/or GWAS hit) with one or several heart-related traits or diseases, e.g. atrial fibrillation or systolic/diastolic blood pressure.<sup>72</sup> However, the Kir2.1 interactome remains to be fully validated in cardiomyocytes. Yet, from the point of view of ATS1, the possibility of such a remarkable number of interactors suggests that the arrhythmogenic mechanisms of at least some cases of ATS1 are likely to be more complex than currently thought, requiring more sophisticated experimentation for their elucidation. This highlights the need to understand the role of macromolecular complexes in the mechanisms that regulate Kir2.1 in both normal and disease contexts toward helping uncover novel targets for therapeutic intervention in ATS1 and other Kir2.1-associated channelopathies.

Altogether, the evidence supports the idea that Kir2.1 and  $\text{Na}_v1.5$  proteins form *channelosomes* that enable these two important channels to reciprocally modulate each others' function. In these macromolecular complexes, multiple accessory proteins likely take part in the trafficking, distribution, location, and turnover in the microdomain of both channels. However, until recently, the reports on consequences of ATS1 mutations have been reserved to the functional analysis of  $I_{\text{K}1}$ , which leaves a wide-open door to ask whether other Kir2.1 interactors, particularly  $\text{Na}_v1.5$ , may be affected one way or another by such mutations.

### 3.4 ATS1 causative mutations

Mutations described as causing ATS1 are distributed throughout the Kir2.1 channel structure (Figure 6A). For example, the D71V mutation

was described to reside at the  $\text{NH}_2$ -terminal and to markedly reduce the  $I_{\text{K}1}$  current amplitude.<sup>9</sup> Several mutations have been reported to happen at the pore domain of the channel, including substitutions and deletions, such as  $\Delta 95-98$ , S136F and G144S,<sup>9</sup> each one leading to ATS1 by affecting a particular function of the channel. Some other mutations can occur at the COOH-terminal residues of the channel. Tinker *et al.* first described the alterations produced by the R218W, R218Q substitutions related to ATS1,<sup>73</sup> and later the traffic deficient  $\Delta 314-315$  Kir2.1 channel deletion.<sup>9,74</sup> To date, more than 90 ATS1 mutations have been identified along the Kir2.1 structure, as summarized on [Supplementary material online, Table S1](#).

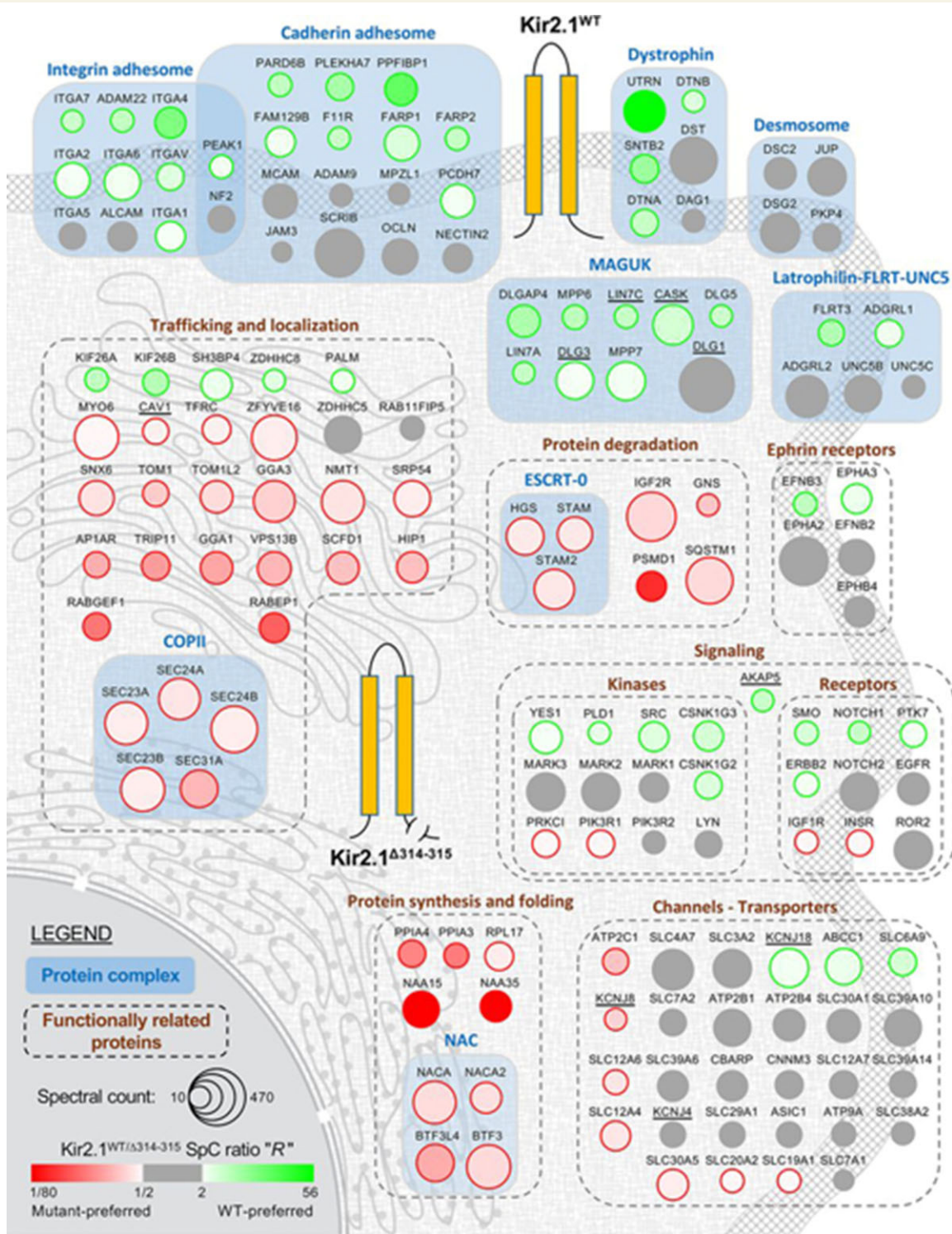
Apart from classifying the Kir2.1 mutations according to their position in the different domains that conform the channel, we catalogue them by their functional implications, which hopefully will help rise other investigators' interest toward increasing mechanistic understanding of the syndrome. In our view, a deeper understanding of mutation-specific molecular alterations likely will result in a more detailed comprehension of the disease and personalized treatments. We propose an ATS1 classification focused on the mutation's nature and its consequences. Thus, we discriminate among ATS1-**A** for trafficking-deficient mutations; ATS1-**B** for mutations related to PIP2-binding defects; ATS1-**C** for mutations with reported changes in channel conformation; and, finally, ATS1-**D** for mutations with defects in two or more such mechanisms ([Supplementary material online, Table S1](#)). In the following lines, we discuss details for a representative example of each of the four groups of mutations and stratify ATS1 according to the type of causal mutation.

#### 3.4.1 Kir2.1 <sup>$\Delta 314-315$</sup> : an example of ATS1 type A trafficking defective mutation

Among the mutations producing disruption of membrane trafficking (classified as ATS1-A), Kir2.1 <sup>$\Delta 314-315$</sup>  was one of the first to be identified as causing ATS1, where deletion of residues 314 and 315 (Figure 6B), which are essential to ensure Golgi export toward the plasma membrane,<sup>9</sup> demonstrated substantial loss-of-function of both  $I_{\text{K}1}$  and  $I_{\text{Na}}$  currents.<sup>36,58-60,69</sup> Such data suggested that  $\text{Na}_v1.5$  and Kir2.1 channels pre-assemble early in the forward trafficking pathway enabling them to traffic together more efficiently to the membrane as part of a  $\text{Na}_v1.5$ -Kir2.1 complex, rather than alone.<sup>36,69</sup>

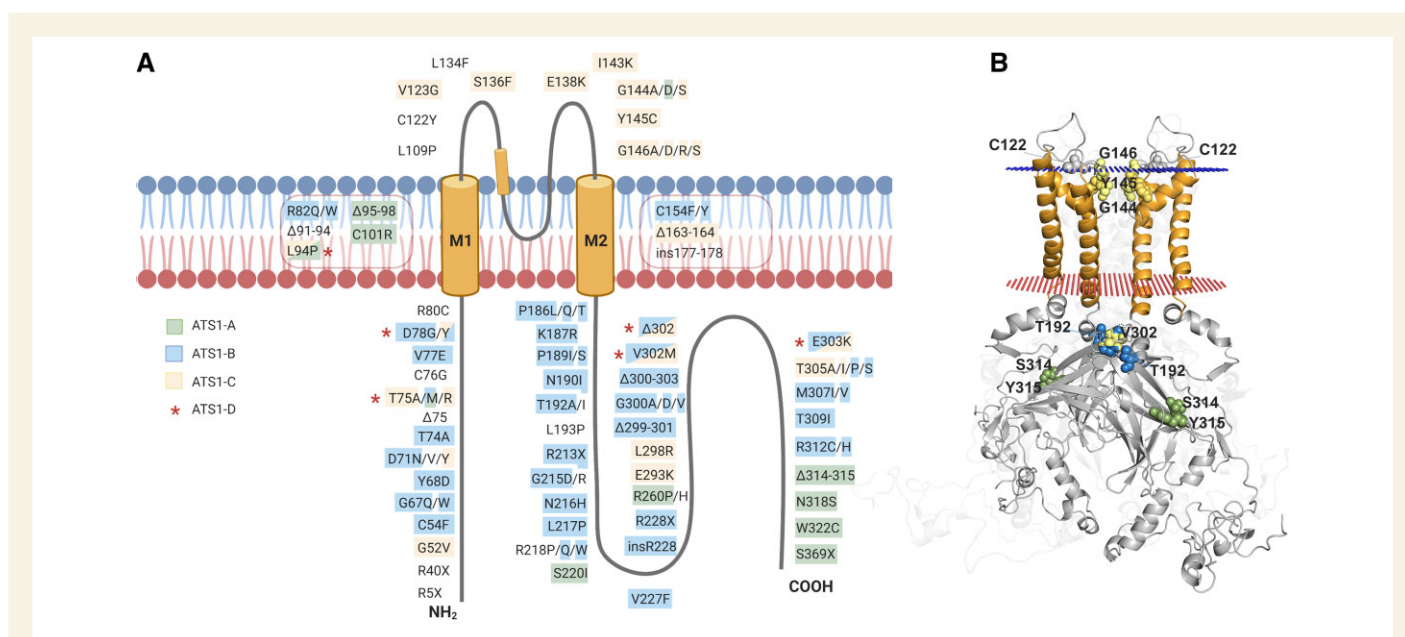
The assembly of the coat protein complex II (COPII) subunits after cargo recognition allows Kir2.1 channels to leave the SR towards the Golgi apparatus, through the formation of COPII-coated vesicles.<sup>75</sup> Only when Kir2.1 folds properly, a recognition motif is exposed that enables channel transport to the cell surface.<sup>74</sup> Such a motif is made possible by the interaction between part of the  $\text{NH}_2$ -terminal and the middle zone of the COOH-terminal allowing the association with Adaptin Protein 1 (AP-1), which leads to the assembly of clathrin-coated vesicles.<sup>76-78</sup> It has been revealed that silencing the AP1  $\gamma$ -subunit reduces  $I_{\text{Na}}$  and  $I_{\text{K}1}$  and modifies the AP characteristics, which confirms that both  $\text{Na}_v1.5$  and Kir2.1 are in Golgi and traffic together following the canonical anterograde pathway (CAP) to reach the plasmalemma. Conceivably, after post-translational modification at the Golgi,<sup>36</sup> a portion of the formed Kir2.1 channels returns to the SR via a purported COPI-mediated potential retrograde pathway (PRP) (Figure 7; left).

Residues 314 and 315 in the Kir2.1 sequence are key to achieve the conformation that allows Golgi to plasma membrane trafficking. When these amino acids are deleted, the tertiary structure of the Kir2.1 <sup>$\Delta 314-315$</sup>  mutant does not fold correctly, and the channels are retained at the Golgi.<sup>74</sup> Owing to the interaction of trafficking-deficient monomers with wild-type (WT) subunits, Kir2.1 <sup>$\Delta 314-315$</sup>  expression exerts a dominant-negative effect on



**Figure 5** Graphical representation of the Kir2.1 BioID interactome. Protein complexes and groups of functionally related proteins encompassing 152 of the 218 high-confidence Kir2.1 BioID hits are depicted in a cell. Major organelles in the cell (nucleus, endoplasmic reticulum, Golgi apparatus, and cytoplasmic membrane) are shown in the background to roughly indicate the approximate subcellular localization of the proteins in the cell. The Kir2.1<sup>WT</sup>-preferred interactors, Kir2.1<sup>Δ314-315</sup>-preferred interactors and Kir2.1<sup>WT/Δ314-315</sup>-neutral interactors are represented. The colour intensity of each circle is an indicator of the strength of the normalized Kir2.1<sup>WT/Δ314-315</sup> spectral count (SpC) ratio 'R' value. The size of each circle represents the average SpC counts observed in either the Kir2.1<sup>WT</sup> or Kir2.1<sup>Δ314-315</sup> BioID experiment (whichever is the largest is represented in the FIG.). Reprinted by permission from Park SS et al, Kir2.1 Interactome Mapping Uncovers PKP4 as a Modulator of the Kir2.1-Regulated Inward Rectifier Potassium Currents. *Molecular & Cellular Proteomics*, 19, 1436–1449; 2020.<sup>72</sup>





**Figure 6** Kir2.1 subunit with mutations associated with ATS1 along of the channel structure. (A) In a simplified monomeric way, membrane trafficking mutations (ATS1-A); residues interacting with PIP2 binding and causing ATS1-B when are mutated; mutations producing channel conformation alterations (ATS1-C); and mutations that alter more than one Kir2.1 functional mechanism (ATS1-D) are marked and highlighted. (B) Dimeric Kir2.1 with punctual residues G144, Y145, G146, T192, V302, S314 and Y315 are highlighted along the structure (the rest of monomers are discarded to facilitate visualization).

the function of Kir2.1<sup>WT</sup> channels. However, Kir2.1<sup>Δ314-315</sup> expression reduces not only  $I_{K1}$  but also  $I_{Na}$ , indicating that at least portions of both channels will fail to traffic together from the Golgi to the cell surface<sup>36,69</sup> (Figure 7; right). The residual currents, together with the fact that both Kir2.1 and  $Na_v1.5$  are essential channels for maintaining cardiac cell excitability and contractility,<sup>79</sup> suggest that additional pathways may lead these channels to the membrane. As a matter of fact, unconventional traffic routes have been proposed.<sup>80</sup> Recently, Pérez-Hernández et al.<sup>59</sup> provided evidence for an unconventional traffic pathway for the transport of Kir2.1 and  $Na_v1.5$  to the cell surface. On this alternative route, the channels would interact with Golgi Reassembly and Stacking Protein (GRASP) at the SR and traffic together to specific subdomains of the plasma membrane without passing through the Golgi. It seems likely that this pathway allows reciprocal modulation between Kir2.1 and  $Na_v1.5$  channels, which may result in sarcolemmal channelosomes (Figure 7).

The possible existence of alternative trafficking pathways opens new possibilities toward addressing the treatment of ATS1; by pharmacologically promoting transport routes independent of the 314 and 315 Kir2.1 residues, as long as the channels are functionally active, phenotypic manifestation of ATS1 might be prevented. Nevertheless, research on possible non-conventional trafficking route(s) mediating the transport of ion channels opens a wide range of new questions. These will need to be addressed to understand the intracellular dynamics of membrane channels like Kir2.1 and  $Na_v1.5$ , which are key for normal cardiac electrical function and whose loss-of-function mutations underlie arrhythmogenic diseases such as ATS1 and Brugada syndrome.<sup>36,59</sup>

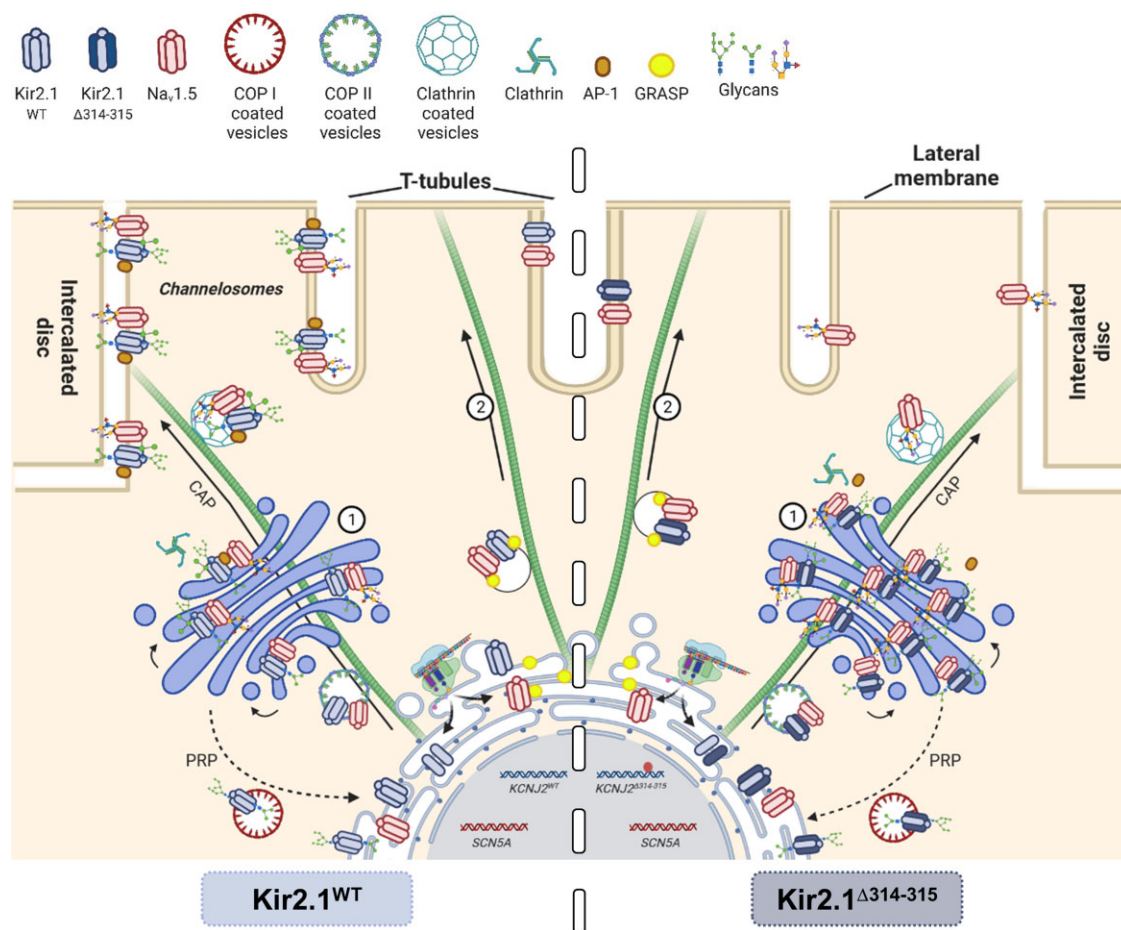
### 3.4.2 Kir2.1<sup>T192A</sup>: an example of ATS1 Type B PIP2-binding altered mutation

Among the multiple ATS1 mutations that have been discovered (see Supplementary material online, Table 1), a substantial fraction (around

51%) impact strongly on Kir2.1 channel-PIP2 interactions. PIP2 binding is required to stabilize the open state of Kir2.1 at the plasma membrane.<sup>81</sup> Therefore, dysfunctional Kir2.1 channel-PIP2 interactions may represent a major pathogenic mechanism for ATS1.<sup>82</sup> Proarrhythmic activity related to ATS1 might be given by a reduced availability of open Kir2.1 channels leading to RMP depolarization, APD prolongation and increased frequency of spontaneous APs. At the same time, polyamines, the molecules that mediate Kir2.1 rectification, may act as cofactors for Kir2.1 PIP2-binding.<sup>83</sup> As such, a PIP2-binding defect might result in Kir2.1 dysfunction by itself or by changing the interactions with other essential modulators of the channel. Interestingly, in general, ATS1 mutations of residues related to PIP2 induce marked susceptibility to malignant cardiac events, which highlights them as pathogenic variants of this disease.<sup>84</sup> Several mutations in plenty of cytoplasmic PIP2-binding domains have been described in the literature (Supplementary material online, Table 1). Their primary effect might be the disruption of necessary gating transitions that lead to channel opening following PIP2 binding.<sup>85</sup>

Cytoplasmic residues 175 to 206 constitute one of the three Kir2.x PIP2-binding domains. Mutations at these sites reduce PIP2-binding affinity and abolish channel activity.<sup>81</sup> The case of T192, a highly conserved amino acid residue, is of interest. In Kir2.1, T192 is located at the COOH-terminal immediately after the M2 domain (Figure 6). Ai et al. (2002) reported the first clinical cases for mutation Kir2.1<sup>T192A</sup>. Despite lacking the characteristic dysmorphic features of ATS1, the patients presented premature ventricular complexes, PVT, bigeminy and large U waves.<sup>86</sup> Functional assays demonstrated that the presence of homomeric Kir2.1<sup>T192A</sup> mutant channels yielded unmeasurable  $K^+$  currents, whereas the expression of heteromeric Kir2.1<sup>WT</sup>/Kir2.1<sup>T192A</sup> channels reduced  $I_{K1}$ .<sup>86</sup> Weaker channel-PIP2 interactions at this site caused a reduction in current due to a reduced open-state probability. A similar ECG phenotype accompanied by a family history of periodic paralysis, abnormal QT-U complex, and non-sustained VT was later observed in a 19-year-old patient reported by Nagase et al.<sup>87</sup>





**Figure 7 Possible Kir2.1 trafficking pathways.** After translocation into the sarcoplasmic reticulum (SR), Kir2.1<sup>WT</sup> (left) and Kir2.1<sup>Δ314-315</sup> (right), along with Nav1.5 channels, are folded and packaged into COPII-coated vesicles to reach the Golgi following the CAP. Protein sequence recognition by AP-1 at the Golgi enables trafficking to the sarcolemma (1). However, absence of Kir2.1 residues 314 and 315 prevents recognition by AP-1 and the channels are retained at the Golgi. Kir2.1<sup>WT</sup> and Kir2.1<sup>Δ314-315</sup> channels could also interact with GRASP at the SR, a pathway that appears to positively regulate the interaction between Kir2.1 and Nav1.5 to reach the membrane and form *channelosome*-like complexes (2). A fraction of Kir2.1 proteins may remain at the SR to form functional channels without going through the Golgi. Alternatively, these channels might return to the SR via a COPI-mediated PRP after post-translational modification at the Golgi.

### 3.4.3 Selectivity filter: hotspot of ATS1 Type C structural alterations

About 28% of the known Kir2.1 mutations alter the channel conformation, half (~50%) of those affecting the pore-forming region located at the extracellular side of the channel. Owing to its essential role in K<sup>+</sup> conductance and selective permeation, this domain is highly conserved in mammals and among other potassium channels.<sup>88–90</sup> Consequently, mutations affecting these residues and altering the selectivity filter of Kir2.1 channels are highly pathogenic.

As summarized in [Supplementary material online, Table 1](#), there are several genetic alterations occurring in the selectivity filter zone and adjacent to the 'GYG' sequence. Therefore, we refer to the Kir2.1 pore-forming domain (amino acids 129–147) as a potential hotspot of ATS1 causative mutations that modify the channel conformation and its normal function.

G144A<sup>91</sup> and Y145C<sup>92</sup> are mutations that disrupt the selectivity filter by altering the signature sequence 'GYG' of Kir2.1. Patients with either of these mutations manifest the classical triad of symptoms, ranging from arrhythmogenic episodes and ventricular ectopy to muscular

weakness and facial dysmorphias.<sup>91,92</sup> In each case, mutations are inherited in an autosomal dominant form, where only one of the parents is a carrier with weak symptoms related mainly to arrhythmias and periodic paralysis. For mutations involving substitutions of G146 (pertaining also the conserved 'GYG' sequence motif), with the exception of G146S (which served to confirm the loss-of-function of the mutated channels),<sup>93</sup> only clinical cases without functional analyses have been reported. In general, probands with mutations in this essential residue presented paralysis attacks, dysmorphias and arrhythmogenic electrocardiographic defects like ST segment depression and prominent U waves.<sup>82,93–95</sup> However, it is important to highlight that, among the probands, the same mutation resulted in different severity. From the foregoing, it is clear that substitutions of the same amino acid may cause different phenotypes due to the heterogeneity and variable penetrance that characterizes the mutations causing ATS1.

On the other hand, one should also consider mutations in residues that, while not belonging to the critical region of the pore-forming domain (residues 129–147), may have relevance in its conformation by altering the Kir2.1 tridimensional structure due to the gain or loss of relevant molecular

interactions. This could be the case of Cys122 and Cys154, which through the creation of a disulfide bond, are important in maintaining an extracellular loop located next to the selectivity filter (Figure 6B).<sup>96</sup> Experiments using *Xenopus laevis* oocytes have shown that disulfide bonds are required for function of the Kir2.1 and Kir2.3 channels but not for processing into the cell membrane.<sup>96,97</sup> Therefore, mutations affecting these conserved residues might alter other properties of the channel, resulting in a loss-of-function by mechanisms that are still unknown. These mechanisms could include altered recycling or degradation of the channel, as well as interaction with other effector proteins.

### 3.4.4 Kir2.1<sup>V302M</sup>: an example of ATS1 Type D with more than one alteration

Despite the punctual nature of *KCNJ2* mutations regarding a specific function or mechanism, genetic variants that impact more than one such characteristics can be also found. The V302 residue, located in the Kir2.1 G-loop (Figure 6B), which provides a diffusion barrier for the potassium conduction pathway between the cytoplasmic and transmembrane pore regions,<sup>98,99</sup> constitutes an example of this. It is also suggested that the V302 side chain is close to some other residues (e.g. R218) that participate in PIP2 binding.<sup>100</sup> Consequently, genetic modifications affecting position 302 could indirectly alter PIP2 binding to the channel. In addition, mutations in this residue would also dramatically affect potassium conduction at the G-loop.<sup>101</sup>

A substitution described in this position is V302M, a missense loss-of-function mutation in the final portion of the COOH-terminal of Kir2.1<sup>23,73</sup> that produces defects in channel regulation by PIP2 as well as channel conformation.<sup>102</sup> However, while earlier studies suggested this mutation was involved in channel assembly and membrane trafficking, Ma et al.<sup>101</sup> provided evidence that V302M does not disrupt protein stability nor trafficking. The clinical cases reported for this mutation are heterogeneous. One patient had the full set of ATS1 features and another had only dysmorphias and extrasystoles in the form of bigeminy.<sup>23</sup> An index case presenting the V302M mutation also had the R80C genetic variant in Kir2.1. However, the phenotype was apparently no more severe than in other cases with only one substitution in *KCNJ2*.<sup>12,103</sup> Pegan et al.<sup>98</sup> concluded that this mutation does not provoke misfolding or a protein destabilizing defect. Nevertheless, it seemed to disrupt potassium conduction through the G-loop without reducing channel surface expression. Consequently, residues within the G-loop could act as possible hotspots that present markedly dysfunctional ATS1 related channels.

Further studies are required to elucidate the role of the V302 side chain in gating; whether it acts by directly modifying the G-loop pore opening or by allosterically coupling the PIP2-binding site to the transmembrane potassium conduction pathway remains to be investigated.

## 4. ATS1 – CPVT phenotypic overlap

About 75% of ATS1 patients suffer episodes of BiVT and PVT,<sup>1</sup> which overlaps with the CPVT-specific arrhythmogenic abnormalities, but the mechanism is unknown. Recently, we reported on a discovery of a previously undescribed fundamental function of Kir2.1 channels that may help reveal the mechanism of such an overlap.<sup>22</sup> Specifically, we verified that, in addition to controlling the RMP and excitability at the sarcolemma, a substantial fraction of functional Kir2.1 channels are retained within a novel microdomain in the SR membrane and help control intracellular calcium homeostasis and excitation–contraction (e–c)

coupling.<sup>22</sup> The finding provides direct demonstration of a vital potassium channel activity that has been overlooked by the relevant textbooks, and that contributes countercurrent for SERCA (sarco/endoplasmic reticulum Ca<sup>2+</sup>-ATPase)-mediated Ca<sup>2+</sup> reuptake and to a lesser extent RyR mediated Ca<sup>2+</sup> release. This unique function for Kir2.1 channels opens new possibilities for the understanding of the role of ion channel fluxes across the SR in muscle contraction, and the molecular mechanisms responsible for arrhythmias, muscular weakness and paralysis produced by mutations in *KCNJ2*. Revealing such mechanisms should lead to novel, more effective targets in the treatment of overlapping inheritable disorders related to calcium dynamics alterations in both ATS1 and CPVT. It is important to differentiate ATS1 from CPVT correctly because their respective prognosis and mortality are different. Inoue et al. recently suggested that exercise testing can reveal differences between these two syndromes: whereas the incidence of ventricular arrhythmias at baseline and recovery from exercise in ATS1 patients is relatively high, CPVT patients frequently present arrhythmias at peak exercise, being completely suppressed after recovery. Moreover, the morphology of the arrhythmogenic events in each group of patients is different and may allow physicians to establish a careful differential diagnosis.<sup>19</sup>

### 4.1 Potential SR Kir2.1 correlations

It has been difficult to correlate genotype with phenotype in ATS1 patients due to the low frequency of the disease in the general population and the incomplete penetrance of the more than 90 identified causative mutations (Figure 6 and Supplementary material online, Table 1).<sup>1</sup> In addition, although Kir2.1 is expressed in several tissues, the phenotypic manifestations in ATS1 are only understood by considering the wide range of proteins with which the altered ionic channels interact.<sup>6</sup> Therefore, pleiotropy might occur, at least in part, by alterations in the components of the Kir2.1 macromolecular complexes forming at the SR, plasmalemma or both. For example, although the type A Kir2.1<sup>Δ314–315</sup> mutation leads to defects in cardiac excitability as a result of a defective Kir2.1-Na<sub>v</sub>1.5 channelosome trafficking,<sup>36</sup> its arrhythmogenic effect might be caused also by an alteration in a SR Kir2.1 channelosome that could be different for other mutations, and requires further study. Thus, the mechanisms underlying arrhythmogenic diseases might not be linked to a direct genotype-phenotype correlation. The presence of multiple proteins and signalling pathways involved in different macromolecular complexes at different microdomains of cells in which the channels are expressed makes things much more complex and challenging.

ATS-related structural dysmorphias, such as bone defects during development, could occur by alteration in calcium dynamics. A suitable calcium transient between the cytosol and the extracellular compartment is necessary for the normal physiology of bone cells.<sup>104</sup> Skeletal defects in patients with ATS1 might be understood by considering that Kir2.1 plays an important role providing countercurrent to calcium movements across the SR membrane.<sup>22</sup> Conceivably, calcium mediated transcription defects secondary to loss-of-function mutations in SR Kir2.1 channels might lead to dysmorphias such as hypoplastic mandible, hypotelorism, low-set ears or digit clinodactyly, typical in some individuals with ATS1. Furthermore, osteoclasts also express Kir2.1 in their plasma membrane.<sup>105</sup> This allows the inward flow of K<sup>+</sup> in exchange for protons (H<sup>+</sup>) to stabilize the RMP, which is essential for normal function of these cells.<sup>106</sup> When Kir2.1 is mutated, the reduced potassium inward current in the osteoclasts causes an imbalance between bone formation and resorption.<sup>107</sup> This alteration would also explain the manifestation of

dysmorphic symptoms in ATS1 patients. In addition, the presence of functional Kir2.1 channels at the SR helps understand the as yet unexplained phenotypic overlap between ATS1 and CPVT in some patients, and the arrhythmias and intermittent paralysis seen in skeletal muscle diseases.<sup>108,109</sup>

## 5. From mechanism to therapy

The variety of treatment alternatives in ATS patients highlights the absence of effective therapy available for these patients. Moreover, data pointing to the left ventricular Purkinje fibres as a potential focal origin of ventricular arrhythmias in ATS patients<sup>110</sup> underlie the decision of considering catheter ablation as a possible option. However, radiofrequency catheter ablation has failed whenever it has been chosen as treatment,<sup>14</sup> likely because the genetic defect and abnormal phenotype are not localized at a single spot.<sup>111</sup> In fact, to date there are no established clinical guidelines for ATS, and the unique general consensus is not to recommend medications known to prolong the QT interval for treatment of ATS patients.<sup>19,112–114</sup> Yet, current pharmacologic therapy for arrhythmias in ATS1 remains non-specific and symptoms related. It is based on the use of class Ic antiarrhythmic drugs (e.g., flecainide and propafenone) and amiodarone to reduce arrhythmia susceptibility.<sup>1</sup> Nowadays, even though it is not nearly 100% effective, flecainide is the most widely used therapy in ATS patients,<sup>46,115–117</sup> but without consideration of specific mechanisms. Both flecainide and propafenone bind to the cytoplasmic portion of Kir2.1,<sup>43,44</sup> they also reduce Na<sub>v</sub>1.5 channel function.<sup>118</sup> Similarly, both drugs inhibit KCNH2-encoded 'hERG' potassium channels responsible of the rapid delayed rectifier potassium current (I<sub>K<sub>r</sub>) at clinically relevant concentrations, all of which highlights the need for careful monitoring or avoiding drug administration to patients with other risk factors for acquired LQTS.<sup>119,120</sup> Mazzanti *et al.*<sup>1</sup> reported that the combination of these drugs usually fails to reduce arrhythmias in ATS1, whereas amiodarone increases the risk of cardiac events and should be avoided. Often, an implantable cardiac defibrillator is necessary when cardiac arrhythmias are severe and symptomatic but this does not exclude the risk of SCD.<sup>1,121,122</sup> As such, treatment is empirical and subject to clinical judgment<sup>1</sup> which, unfortunately, often results in proarrhythmia due to poorly understood mechanisms. Therefore, there is an urgent need to improve the understanding and treatment of ATS1.<sup>123</sup></sub>

Design of new drugs should be relevant in the coming years. Focusing on the molecular characterization of targets of possible activating or inhibitory molecules is essential in the identification of a useful pharmacophore.<sup>44,124</sup> Studies on the analysis of the usefulness of already approved drugs and their derivatives to treat diseases such as ATS1 should continue toward improving the stratification and prognosis of affected patients. On the other hand, novel understanding of different arrhythmogenic mechanisms associated with different ATS1 mutations might open new pathways for personalized treatments of patients suffering from this devastating channelopathy. Because the molecular mechanisms that cause ATS1 are different depending on the mutation, pharmacological treatment and clinical management should be different for each patient. For example, as the Kir2.1<sup>Δ314–315</sup> mutation affects both Kir2.1 and Na<sub>v</sub>1.5 membrane trafficking, cardiac excitability will likely be significantly reduced in ATS1 patients carrying the mutation, which should directly exclude treatment with sodium channel blockers like flecainide or propafenone because of the possibility of proarrhythmia. In these patients, molecules capable of optimizing non-conventional transport pathways of trafficking-deficient mutations should be

considered toward reducing pathogenicity. Conversely, patients carrying mutations that affect Kir2.1-PIP2 interactions without affecting sodium channel function might benefit from the use of currently available antiarrhythmics. In other words, while a specific combination of  $\beta$ -blockers and other antiarrhythmics may be appropriate for a patient with a type B ATS1 mutation that alters Kir2.1 channel conformation, the approach might be dangerous for a patient with a type A ATS1 mutation leading to defects in Kir2.1 trafficking. Hence, drug treatment of ATS1 mutation should be personalized.

## 6. Conclusions and future directions

This review article provides an overview of the complexity of Kir2.1 channel biology and its association with the pathological state through mutation-dependent mechanisms. Such complexity is revealed in the ATS1, an extremely rare disease with a particular pleiotropic phenotype and cardiac electrical alterations that often overlap with other arrhythmogenic diseases. Hence, many questions remain. ATS1 associates with mutations in the *KCNJ2* gene encoding Kir2.1 protein, with important side effects on the Na<sub>v</sub>1.5 channel (see *Figure 5* for more details), and possibly other interacting proteins, likely involved in ATS2 through mechanisms that are incompletely understood. Also, the cellular mechanisms governing alternative distribution of Kir2.1 channel through the classical trafficking route vs. the GRASP dependent pathway remains unclear. Similarly, studies are needed to clarify the mechanisms that retain Kir2.1 in the SR, including the possibility of differentiating between a retention in the SR from its translocation or, alternatively, sent from the Golgi through retrograde flow involving COPI-coated vesicles. Such questions highlight the need to study the role of the post-translational processes such as glycosylation acquired in the Golgi in the functional state of Kir2.1. They also call for the need to understanding the biology of the Kir2.1 interactome in the cardiomyocyte, both at the subcellular and tissue levels, and to demonstrate that the molecular mechanisms already described *in vitro* are really transferable to an *in vivo* context. For example, demonstrating that dysfunction of intramolecular Kir2.1-Na<sub>v</sub>1.5 interaction is a significant source of arrhythmogenesis in ATS1 patients would be a potential paradigm shifting discovery that would likely improve arrhythmia therapy and SCD prevention in ATS1 and other cardiac diseases.

Conditions are prime to generate small (mouse) or large (pig) genetic animal, as well as human (e.g., hiPSC-CMs) models to investigate the molecular mechanisms of arrhythmogenesis in ATS1. The *in vivo* experimental models offer a brilliant opportunity to identify the genetic causes of ATS, as well as the mechanisms by which this channelopathy leads to SCD. Such information is crucial if we want to develop precision therapy approaches aimed at directly and effectively treating the disease, which represents the current gold standard in personalized medicine. With this objective in mind, and to facilitate treatment adequacy, here we propose a realistic stratification of ATS1 according to the specific type of causal mutation. The information we have collected should allow scientists to broaden their vision about the multiplicity of possible targets available to correct the molecular consequences of ion channel mutations in ATS1 and other arrhythmogenic cardiac diseases.

## Supplementary material

Supplementary material is available at *Cardiovascular Research* online.



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## Data availability

Data available upon request. All the data commented here have been analysed after extracting relevant information from the original articles included in the bibliography section. Any specific data, less those originally generated by us, will be provided by the original authors upon request.

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