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Genetic Complexity of Sinoatrial Node Dysfunction

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17 **calsequestrin-2, GIRK4, HCN4, Nav1.5**

18 **Abstract**

19
20 The pacemaker cells of the cardiac sinoatrial node (SAN) are essential for normal cardiac automaticity.
21 Dysfunction in cardiac pacemaking results in human sinoatrial node dysfunction (SND). SND more
22 generally occurs in the elderly population and is associated with impaired pacemaker function causing
23 abnormal heart rhythm. Individuals with SND have a variety of symptoms including sinus bradycardia,
24 sinus arrest, SAN block, bradycardia/tachycardia syndrome, and syncope. Importantly, individuals
25 with SND report chronotropic incompetence in response to stress and/or exercise. SND may be genetic
26 or secondary to systemic or cardiovascular conditions. Current management of patients with SND is
27 limited to the relief of arrhythmia symptoms and pacemaker implantation if indicated. Lack of effective
28 therapeutic measures that target the underlying causes of SND renders management of these patients
29 challenging due to its progressive nature, and has highlighted a critical need to improve our
30 understanding of its underlying mechanistic basis of SND. This review focuses on current information
31 on the genetics underlying SND, followed by future implications of this knowledge in the management
32 of individuals with sinus node disorders.

33 **1 Introduction**

34 Automaticity is the primary function of the sinoatrial node (SAN). SAN pacemaker cells have the
35 shortest depolarization phase of the action potential, and the fastest firing rate, making the SAN the
36 dominant pacemaker of the heart (Kennedy et al., 2016). The SAN initiates an electrical impulse that

37 propagates throughout the heart, establishing a normal heart rhythm. The sinus node is surrounded by
38 a collagen frame (James, 1977) near the epicardium between the superior vena cava and the right atrium
39 in the posterior right atrial wall (Keith and Flack, 1907). The SAN primarily contains three specialized
40 cell types: pale cells (P cells), transitional cells (T cells), and recently described “fibroblast-like” cells,
41 intertwined in collagen, fibroblasts, fatty tissue, nerves, and capillaries (James, 1977; Balbi et al., 2011;
42 Csepe et al., 2015). P cells are localized to the central region of the SAN, and have a single membrane
43 with very little membrane specialization resulting in poorly defined intercalated discs, few
44 desmosomes, and few gap junctions (Ho and Sánchez-Quintana, 2016). Gap junctions are rare in P
45 cells, and junctions are only formed between P cells or T cells. P cells do not form junctions with other
46 atrial myocytes (James, 1977). Automaticity arises from P cells (James et al., 1966; Woods et al.,
47 1976). Interestingly, transitional cells, located in the periphery of the SA node, act as the distributors
48 of action potentials to atrial myocardium and internodal conduction pathways (Boyett et al., 2000). T
49 cells are a loosely defined, varied group of cells. Many T cells look like P cells with reduced myofibrils,
50 while others closely resemble atrial myocytes (James et al., 1966). Finally, the function of “fibroblast-
51 like” cells has yet to be defined, but presumably, these cells play a role in maintaining the structure of
52 the SAN (Balbi et al., 2011). The SAN connects to the atrioventricular (AV) node via three tracts: the
53 anterior (which splits into two bundles), the middle, and the posterior internodal tracts (James, 1963;
54 Kennedy et al., 2016).

55 The SAN receives blood flow through the sinus node artery, the largest atrial coronary branch, which
56 originates from the left or right coronary artery (Verhaeghe and Van Der Hauwaert, 1967). Of note,
57 parasympathetic modulation of the heart is primarily through the vagal postganglionic pathways along
58 the *Sulcus Terminalis* in the subepicardial region adjacent to the SAN artery (Bluemel et al., 1990).
59 Innervation is both parasympathetic, and sympathetic to the SAN, yet neither innervation pathway has
60 any contact with P or T cells (Balbi et al., 2011).

61 Interestingly, it has been suggested that the total amount of SAN cells is inversely proportional to the
62 age of an individual, suggesting that the number of SAN cells, and the volume of SAN cells, decrease
63 over time (Thery et al., 1977; Shiraishi et al., 1992). However, Alings et al. found that the sinus node
64 does not change in dimensions during an entire adult life span, and that although relative collagen
65 increases from childhood to adulthood, collagen levels do not change once adulthood is reached, but
66 rather the structure of the collagen changes (Alings et al., 1995).

67 Sinoatrial node dysfunction (SND) occurs in one of every 600 cardiac patients above the age of 65
68 (Rodriguez and Schocken, 1990). **SND accounts for about half of the permanent implanted pacemakers
69 in the US (Lamas et al., 2000).** In general, “sick sinus syndrome” (SSS), sinus node dysfunction (SND),
70 and sinoatrial node dysfunction (also SND) are used interchangeably in the literature. SND is usually
71 accompanied by structural abnormalities. Symptoms of SND include sinus bradycardia (heart rate less
72 than 60 beats per minute), sinus arrest (total absence of atrial or ventricular activity), SA block, and
73 bradycardia/tachycardia syndrome (cycling between supraventricular tachyarrhythmias and sinus
74 bradycardia) (Alpert and Flaker, 1983). Syncope has also been reported by about half of patients with
75 SND (Menozzi et al., 1998; Brignole et al., 2013). Although structural defects accompany most forms
76 of SND, syncope and bradycardia associated with SND have also been observed in patients with normal
77 cardiac anatomy (Yabek et al., 1982). SND is generally associated with impaired pacemaker function
78 causing abnormal heart rhythm, but SND can also be associated with impulse transmission issues from
79 apoptosis in the AV node, sinus node, and internodal pathways, that may cause death from complete
80 heart block (James et al., 1996). Further, blockage of the sinus node artery preventing blood flow to
81 the SAN cells can result in SND and sudden death (Jing and Hu, 1997).

82 This review focuses on current information on the genetics underlying SND, followed by a brief
83 overview of the recent update in clinical management of patients with sinus node disorders. A better
84 understanding of the genetics and the molecular mechanisms of the SAN and SND will improve current
85 diagnostic measures and identify alternative therapeutic approaches. The genes currently implicated in
86 human SND are summarized in **table 1**. Although not the focus of this review, SND is also commonly
87 a secondary symptom to other systemic and cardiovascular conditions. Atrial fibrillation (AF) and atrial
88 tachycardia, cardiac transplantation, drug toxicity, hyperinsulinemia and insulin resistance (diabetes
89 type II), sinus node artery obstruction, hyperparathyroidism, intracranial conditions, epilepsy,
90 myxedema coma, cardiac lymphoma, infections, and myocardial infarction are all potential procedures,
91 diseases, or conditions that can result in secondary SND (Lown, 1967; Bexton et al., 1984; Bigger and
92 Sahar, 1987; Wasada et al., 1995; Hasdemir et al., 2003; Shah et al., 2004; Dadlani et al., 2010;
93 Ravindran et al., 2016; Kondo et al., 2020; Kousa et al., 2020; Mesirca et al., 2020).

94 One would be remiss to not specifically mention the recently uncovered link between the novel
95 coronavirus-19 (COVID-19) disease and SND. COVID-19, caused by severe acute respiratory
96 syndrome coronavirus-2 (SARS-CoV-2), may cause various cardiovascular problems even without
97 pre-existing cardiac conditions (Clerkin et al., 2020; Organization, 2020). In May 2020, COVID-19
98 was first reported to cause SND in two patients. Both patients were older than 70 years-of-age, and
99 both experienced sinus bradycardia following intubation for acute hypoxic respiratory failure. Each
100 patient still experienced SND two weeks after onset (Peigh et al., 2020). Two COVID-19 positive
101 patients were further reported to suffer from AV dysfunction and SND without a history of arrhythmias
102 (Babapoor-Farrokhran et al., 2020). The reported cases suggest an interesting association between
103 COVID-19 and SND as a secondary symptom.

104 **2 Genetics of Sinus Node Disease**

105 SND mainly affects the older population, although it can affect people at any age. Most cases of SND
106 are not inherited. However, several genes coding for ion channels such as *HCN4* and *SCN5A*,
107 cytoskeletal proteins, and proteins intricate to cardiac development have been linked to SND (De Ponti
108 et al., 2018). Gene products associated with SND in the SAN are shown in **figure 1**. Major genes linked
109 to SND etiology, as well as human studies and animal models used to characterize the SND phenotype
110 will be discussed below. Genetic complexity and pleiotropy remain a big challenge to proper SND/SSS
111 diagnosis with likely multiple complexities involved including genetic variation, underlying
112 conditions, and environmental factors. With numerous sinoatrial-expressed gene variants associated
113 with SND, and only moderate penetrance, SND can continue within families for generations before
114 diagnosis (Abe et al., 2014). Further, compound variants for prominent SND-implicated genes seem to
115 increase penetrance, emphasizing SND as an oligogenic disease (Baskar et al., 2014; De Filippo et al.,
116 2015; Sacilotto et al., 2017).

117 **2.1 Calsequestrin-2 (CASQ2) and ryanodine receptor 2 (RYR2)**

118 *Background:*

119 Cardiac calsequestrin, calsequestrin-2 (Casq2), localized to the sarcoplasmic reticulum (SR), is a low-
120 affinity high-capacity Ca^{2+} -binding protein involved in the ability of the sarcoplasmic reticulum to
121 preserve and release Ca^{2+} in cardiac myocytes (Murphy et al., 2011). Voltage-gated Ca^{2+} channels (L-
122 type calcium channels) create an initial Ca^{2+} influx into the cell, which causes RyR2 receptors to release
123 more calcium from the SR in a process called calcium induced-calcium release. Calsequestrin, the
124 major Ca^{2+} binding protein in the SR, is anchored to the membrane of the SR by RyR2 (either directly,

125 or indirectly via triadin and junctin), and regulates the release of Ca^{2+} through the RyR2 channel (Beard
126 et al., 2004). Importantly, calsequestrin, the ryanodine receptor, junctin, and triadin are all necessary
127 for proper calcium cycling (Zhang et al., 1997).

128 *Human studies:*

129 Loss of function variants in *CASQ2* can result in catecholaminergic polymorphic tachycardia (CPTV),
130 bradycardia, and atrial arrhythmias (Glukhov et al., 2015). CPVT was first identified in 1975 as a case
131 report of “bidirectional tachycardia” induced by physical effort and stress in a 6 year old female with
132 no structural abnormalities (Reid et al., 1975). A later follow-up of 21 children over 7 years properly
133 emphasized CPVT as a separate entity of ventricular tachycardia in children, emphasizing the dangers
134 of misdiagnosis with the lurking potential for spontaneous sudden cardiac death (SCD) without
135 immediate therapy. Leenhardt et al. showed the adrenergic-induced ventricular arrhythmias to be
136 preventable with proper β -blocker treatment (Leenhardt et al., 1995).

137 The CPVT phenotype from *CASQ2* variants is attributed to an increase in expression of calreticulin
138 and RyR2 (Song et al., 2007). CPVT is also commonly induced by *RYR2* variants, and has been highly
139 associated with bradycardia (Miyata et al., 2018). An exon 3 deletion of *RYR2* (c.169-353_273+657del)
140 was found in a family with a history of SND and CPVT (Dharmawan et al., 2019). Exon 3 deletions
141 were identified in two additional families that showed symptoms of SND (Bhuiyan et al., 2007).
142 Further, a RyR2 (R420Q) missense mutation was associated with sinus bradycardia and atrial
143 arrhythmias (Domingo et al., 2015). Although CPVT is well documented across *CASQ2* and *RYR2*
144 variants, SAN dysfunction and atrial arrhythmias have been identified in CPVT patients, signifying the
145 importance of calsequestrin-2 and ryanodine receptor 2 in proper SAN functioning (Sumitomo et al.,
146 2007). Additionally, a study across 303 patients with lone AF found rare *CASQ2* variants, implicating
147 *CASQ2* in atrial fibrillation as well (Weeke et al., 2014).

148 *Mouse models:*

149 Glukhov et al. described the first *Casq2*-null (*Casq2*^{-/-}) mouse model with SAN defects. Phenotype
150 included bradycardia, RR interval variability, SAN conduction abnormalities, and abnormally high
151 atrial ectopic activity resulting in AF, implicating calsequestrin-2 in SAN functioning. Increased
152 fibrosis in the pacemaking complex was observed in *Casq2*-deficient mice. At the cellular level, lack
153 of functioning *Casq2* caused abnormal calcium release from the sarcoplasmic reticulum and increased
154 diastolic calcium concentration, resulting in a delay between action potential and transient calcium
155 upstrokes (Glukhov et al., 2015). This disruption in intracellular calcium cycling explains the SAN,
156 atrial myocyte, and ventricular myocyte dysfunction seen in individuals with *CASQ2* loss of function
157 variants.

158 Atrial burst pacing induced atrial flutter and AF in *Casq2*^{-/-} mice versus wild-type (WT) mice, and
159 isolated *Casq2* deficient hearts showed ectopic foci from the pulmonary vein region when visualized
160 with atrial optical voltage maps. Isolated hearts from *Casq2*-null mice also experienced diastolic
161 subthreshold spontaneous Ca elevations (SCaEs) and delayed afterdepolarizations (DADs) when AF
162 was not reached, but R-propafenone (RyR2 and Na⁺ channel blocker) was shown to prevent AF, DADs,
163 and SCaEs in these mice. Authors attributed this success mostly to the inhibition of RyR2, as an
164 equipotent Na_v channel inhibitor did not reach the same result (Faggioni et al., 2014). Notably, atrial
165 overdrive pacing suppressed ventricular arrhythmias in a *Casq2*-null mouse model, and therefore could
166 provide a new therapeutic option for CPVT (Faggioni et al., 2013). Interestingly, a RyR2 R4496C
167 homozygous mouse showed sinus pausing, atrial arrhythmias, and reduced SAN automaticity. This

168 variant is associated with enhanced RyR2 activity, and supports a link between *RYR2* variants in
169 humans with SND (Zhang et al., 2013).

170 **2.2 G protein-activated inward rectifier potassium channel 4 (*KCNJ5*)**

171 *Background:*

172 Activation of G protein via a transmitter is a common type of cell-cell communication. In general, a
173 neurotransmitter binds to a seven transmembrane receptor on the outside of the cell, that results in the
174 exchange of GDP for GTP on the inward side of the receptor, allowing the dissociation of the
175 heterotrimeric G-protein subunits, that then further act as effectors (Gilman, 1987; Neer, 1995; Mark
176 and Herlitze, 2000). For heart rate modulation, the G $\beta\gamma$ (as opposed to the G α) subunit specifically
177 activates the K_{Ach} channel by binding to the N- and C-termini of GIRK1 (G protein-regulated inwardly
178 rectifying K⁺) and GIRK4 subunits directly (Logothetis et al., 1987; Huang et al., 1995). The
179 muscarinic acetylcholine K⁺ channel (K_{Ach}) is composed of GIRK1 and GIRK4 (Kir3.4, *KCNJ5* gene)
180 subunits in the atria, and contributes to heart rate regulation in mammals (Wickman et al., 1998; Mark
181 and Herlitze, 2000). During parasympathetic stimulation, K_{Ach} channels are activated, slowing the heart
182 rate and the contractile force of the heart (Wickman et al., 1997). *Girk4* knockout mice not only lacked
183 *I_{KAch}*, but also *Girk1* expression, reinforcing that *Girk4* plays a leading role in the expression and
184 localization of *Girk1* to the cell membrane (Wickman et al., 1998; Kennedy et al., 1999). The K_{Ach}
185 channel is rapidly and reversibly inhibited upon membrane stretch that allows mechano-electrical
186 regulation of the atria (Ji et al., 1998). Although *Girk4* is necessary for proper *Girk1* membrane
187 expression, *Girk4* homotetramers have also been identified in the atria (Corey and Clapham, 1998;
188 Bender et al., 2001). Regulation of the *Girk1/Girk4* heterotetrameric channel occurs via
189 phosphorylation by cyclic AMP dependent protein kinase (PKA) (upregulation) and via
190 dephosphorylation by protein phosphatase 2A (PP2A) (downregulation) (Müllner et al., 2003).

191 Remarkably, the guanine nucleotide-binding protein subunits 2 and 5 (GNB2 and GNB5), that create
192 the beta subunits of the G protein that interacts with GIRK1 and GIRK4, also play a role in SND. *GNB5*
193 variants have been reported in a patient with sinus bradycardia and cognitive disability (Lodder et al.,
194 2016). A *GNB2* variant was more strictly associated with cardiac conduction abnormalities resulting
195 in SND and AV block (Stallmeyer et al., 2017).

196 *Human studies:*

197 Dobrev et al. compared 24 patients with chronic AF and aberrant atrial pacemaking function to 39
198 patients with normal sinus rhythm. The density of *I_{KAch}* in patients with chronic AF was only 50% of
199 the density of *I_{KAch}* in the sinus rhythm group. GIRK4 mRNA expression was also decreased,
200 potentially implicating reduced GIRK4 expression in increased atrial excitability (Dobrev et al., 2001).
201 C171T and G810T variants in GIRK4 have now been identified as risk factors for lone paroxysmal AF
202 in Chinese populations (Zhang et al., 2009). Further, A G387R heterozygous dominant variant in
203 GIRK4 was found in a large Chinese kindred with clinical long QT syndrome (LQTS), a hereditary
204 disorder that leads to sudden cardiac death. In 2019, A GIRK4 variant (W1010C) was first identified
205 in a 3-generation family with SND. The W1010C variant in GIRK4 interestingly resulted in increased
206 *I_{KAch}*. This gain-of-function variant caused an enhanced parasympathetic tone, causing familial SND
207 and hyperpolarization of the pacemaker cells (Kuß et al., 2019). Overall, loss-of-function *Girk4*
208 variants seem to induce atrial arrhythmias, while gain-of-function variants may be implicated in SND.

209 *Mouse Models*

210 SAN pacemaker cells from *Kcnj5*^{-/-} mice completely lacked *I_{KAch}* and showed about 50% reduction in
211 cholinergic regulation of heart rate (cite Wickman et al. Neuron 1998 and Mesirca et al. J Gen Physiol,
212 2013), further supporting the importance of the GIRK4 in parasympathetic modulation. In addition,
213 *Kcnj5* knockout mice had a 10% increase in resting heart rate with this decreased parasympathetic tone.
214 The ability of these mice to recover to resting heart rate after stress (exercise or pharmacological
215 stimulation) was significantly prolonged (Mesirca et al., 2013).

216 2.3 Voltage-gated sodium channel alpha subunit 5 (*SCN5A*)

217 *Background:*

218 Atrial, ventricular, and Purkinje myocyte depolarization, which causes a complete contraction of the
219 heart, is initially regulated by the cardiac sodium channel. Na_v1.5 (the pore-forming, ion-conducting
220 α-subunit of the cardiac sodium channel) is encoded by *SCN5A*, and variants in this gene have been
221 implicated in a wide range of cardiac diseases such as Brugada syndrome, long QT syndrome, atrial
222 fibrillation, sick sinus syndrome, dilated cardiomyopathy, and others (Wilde and Amin, 2018). Butters
223 et al. analyzed electrophysiological mathematical models of SAN cells, 2D models of the intact SAN-
224 atrium tissue, and actual recordings of activation patterns from isolated intact rabbit SAN-atrium tissue
225 to elucidate the mechanism of *SCN5A*^{+/-} variants on SA node function. Isolated SAN cells from adult
226 rabbits harboring a heterozygous *SCN5A* variant display slower pacemaking rates in the peripheral
227 cells, but not in the SAN central cells, yet 2D models show intact atrium-SAN tissue to have a
228 decreased pacemaking rate overall with AP conduction issues, that may potentially cause SA node exit
229 block and sinus arrest, as seen in SND (Butters et al., 2010).

230 *Human studies:*

231 A *SCN5A* variant associated with long QT syndrome type 3 (LQTS3) and Brugada syndrome was first
232 identified in a large family in 2001. Individuals carriers within the family that harbored the variant
233 displayed a lower heart rate, and experienced marked QT prolongation during episodes of bradycardia,
234 that resulted in sudden death within the family (van den Berg et al., 2001). Further, *SCN5A* human
235 variants (usually autosomal recessive inheritance patterns) were linked to SND (Benson et al., 2003),
236 dilated cardiomyopathy, conduction disorders (McNair et al., 2004; Freyermuth et al., 2016; Yang et
237 al., 2017), and infant death syndrome (Denti et al., 2018).

238 A loss-of-function E161K Na_v1.5 variant was identified in two unrelated individuals with family
239 history of bradycardia, SND, conduction disease, and Brugada syndrome. The reduced functional
240 Na_v1.5 protein expression caused atrial, ventricular, and SAN conduction slowing. Diastolic
241 depolarization rate and upstroke velocity were both reduced in E161K computational models (Smits et
242 al., 2005). An L1821fs/10 *SCN5A* variant causing a c-terminus truncation was identified in a 12-year-
243 old male diagnosed with SSS, and when expressed in HEK-293 cells, Na_v1.5 current density was
244 decreased by 90% (Tan et al., 2007). The SND phenotype identified in *SCN5A* variants seems to
245 generally come secondary to Brugada syndrome and LQTS3. For example, a loss-of-function E1784K
246 variant was identified in 41 individuals, and 39% of individuals had SND, while nearly all of them
247 (93%) had LQT3 (Makita et al., 2008). However, some *SCN5A* variants have shown a SND phenotype
248 without the Brugada-type ST elevation (Nakajima et al., 2013; Wilders, 2018; Alkorashy et al., 2020).
249 An I230T homozygous Na_v1.5 variant was found in four children with SND, yet heterozygous carriers
250 of the variant showed normal conduction (Neu et al., 2010). Additionally, a case report found a R121W
251 Na_v1.5 novel variant in an individual diagnosed with SND (Holst et al., 2010). Finally, D349N and

252 D1790N autosomal recessive variants in $Na_v1.5$ were associated with pediatric sinus arrest and SND
253 (Kodama et al., 2013).

254 *Mouse models:*

255 Mice heterozygous for functional $Na_v1.5$ ($Scn5a^{+/-}$) demonstrated bradycardia and sinoatrial block due
256 to slowed pacemaker rates and slower SA conduction, particularly in larger peripheral SAN cells (Lei
257 et al., 2005). Interestingly, $Scn5a^{+/-}$ mice showed sex-dependent effects on SAN functioning in older
258 mice, particularly shown as PR interval prolongation (in old males), RR interval prolongation (longer
259 in old males), QTc prolongation (similar across both genders), and T-wave prolongation (longer in old
260 males) (Jeevaratnam et al., 2010). $Scn5a^{+/-}$ mice also had decreased heart rate variability, reduced SA
261 node automaticity, slowed SA conduction, increased fibrosis, and increased fibroblasts as
262 consequences of the decreased $Na_v1.5$ expression, particularly in older age mice (Hao et al., 2011).

263 **2.4 Hyperpolarization activated cyclic nucleotide gated potassium channel 4 (*HCN4*)**

264 The ability of the SAN cells to spontaneously initiate electrical impulse comes from the funny current
265 (I_f) activation, a Na^+/K^+ depolarization current. Funny current channels have inward current at diastolic
266 voltages that are then activated by membrane hyperpolarization (“membrane clock” hypothesis for
267 pacemaker automaticity) via binding to the intracellular cAMP that can be modified by sympathetic
268 and parasympathetic transmitters, therefore modulating the heart rate (DiFrancesco, 1993; Accili et al.,
269 2002; Altomare et al., 2003). I_f may also be regulated by cAMP-activated protein kinase (PKA) in the
270 SAN (Liao et al., 2010). While there are four members of the HCN channel family, many studies
271 proposed that only HCN2 and HCN4-based channels are expressed in the murine SAN, with HCN4-
272 based channels having a higher level of expression, confirming the role of HCN4-based channels in
273 driving cardiac pacemaker activity (Moosmang et al., 2001; Xiao et al., 2010). However, Fenske et al.
274 found HCN1-based channels highly expressed in the SAN, and reported HCN1-based channels as
275 critical for the stabilization of the lead pacemaker region in mice (Fenske et al., 2013). HCN4 channels
276 and beta-2 adrenergic receptors (β_2 -AR) form a complex that is essential for HCN4 channel regulation
277 (Greene et al., 2012). Interestingly, the expression of HCN2 and HCN4 channels were shown to
278 decrease at the SAN, and increase in the atria and pulmonary vein in older age in dogs, which could
279 account for the disproportionately older population afflicted by SND (Li et al., 2014; Du et al., 2017).

280 *Human studies*

281 In 2003, an *HCN4* variant (573X) causing truncation of HCN4 c-terminus was first identified in a
282 patient with SND, presented as sinus bradycardia and chronotropic incompetence (Schulze-Bahr et al.,
283 2003). Importantly, familial sinus bradycardia linked to c-terminus truncation and loss of cAMP
284 dependent regulation of HCN4 was documented in 2010 (Schweizer et al. PMID: **20693575**). A D553N
285 HCN4 missense variant was identified in an individual with recurring syncope, QT prolongation,
286 polymorphic VT, and torsade de pointes. The individual variant, when transfected in COS7 cells,
287 displayed a reduction in HCN4 expression at the cellular membrane, correlating the variant and the
288 loss of functional HCN4 with SND (Ueda et al., 2004). Sixteen family members carrying a G480R
289 missense variant in HCN4 (autosomal dominant) experienced sinus bradycardia. Molecular studies
290 showed reduced synthesis and trafficking of G480R variant HCN4 to the membrane (Nof et al., 2007).
291 A G482R HCN4 variant was reported in a family presenting with bradycardia and left ventricular non-
292 compaction cardiomyopathy. Interestingly, this was the first study to link HCN4-associated SND with
293 structural abnormalities of the myocardium (Milano et al., 2014). Further, HCN4- R393H loss-of-
294 function, c.1737+1 G>T splice site, I1479V loss-of-function, A485E loss-of-function, R375C loss-of-

295 function, and V759I loss-of-function variants have all been identified in individuals with SND and
296 other related cardiac conduction disorders (Hategan et al., 2017; Ishikawa et al., 2017; Servatius et al.,
297 2018; Alonso-Fernández-Gatta et al., 2020; Erlenhardt et al., 2020).

298 *Mouse models*

299 Hcn2-deficient mice presented spontaneous absence seizures and abnormal cardiac sinus rhythm,
300 implicating the HCN family in SAN function (Ludwig et al., 2003). Mice expressing the dominant
301 negative 573X HCN4 isoform lacking cAMP regulation present with SAN bradycardia, similar to that
302 observed in the SND individual (Alig et al., PNAS 2009, PMID: **19570998**). Conditional knockout of
303 *hcn4* have produced variable outcome phenotype, from sinus pauses (Hermann et al. EMBO 2007;
304 PMID: **17914461**), to severe heart block and death ((Baruscotti et al., 2011)). A murine tamoxifen-
305 inducible, cardiac-specific knockout model of exon 2 of *Hcn4* channels showed remarkable
306 bradycardia (50% reduction in heart rate), AV block, and death on day 5 on average.. Importantly, I_f
307 was reduced by about 70% in these mice (Baruscotti et al., 2011). A similar pattern of strong
308 bradycardia and AV block was observed in mice expressing a dominant negative HCN4 subunit lacking
309 channel conductance and completely lacking I_f . However, no mortality was observed in these mice,
310 despite the presence of recurrent ventricular arrhythmia (Mesirca P. et al. Nat commun 2014). Although
311 HCN4 expression is essential for proper SA node functioning, extreme activation of I_f in cardiac cells
312 can lead to initiation of ectopic foci, resulting in atrial and ventricular arrhythmias (Stieber et al., 2004).
313 Morris et al. isolated atrial pacemaker cells from rats, and found that overexpression of Hcn2 via
314 adenovirus-mediated gene transfer resulted in pacing acceleration. This potentially implicates gain-of-
315 function variants in *HCN2* and *HCN4* with increased pacemaker activity resulting in ectopic foci and
316 cardiac arrhythmia (Morris et al., 2013). Interestingly, an Hcn1-deficient mouse model also showed
317 bradycardia, slowed SAN conduction, sinus dysrhythmia, and sinus pauses. This study suggests a role
318 of *HCN1* in SAN function and human SND (Fenske et al., 2013).

319

320 **2.3 Sodium/calcium exchanger 1 precursor (*SLC8A1*)**

321 *Background:*

322 Spontaneous action potentials in SAN cells provide the primary pacemaking activity for the entire
323 heart, and are important for proper cardiac functioning. The cardiac Na^+ - Ca^{2+} exchanger (NCX1) plays
324 an integral role in diastolic depolarizations that trigger these recurrent action potentials. Following
325 diastolic SR Ca^{2+} release from ryanodine receptors, increased cytosolic Ca^{2+} causes an inward current
326 via NCX, which accelerates late diastolic depolarization to the action potential threshold (“calcium
327 clock” model for pacemaker automaticity) (Bogdanov et al., 2001) , (Lakatta et al. Circ Res 2010;
328 PMID: **20203315**). Further, NCX1 inactivation has the ability to completely halt SA node firing.
329 (Groenke et al., 2013) by generating intermittent burst firing induced by intracellular Ca^{2+} overload
330 (Torrente et al., 2015: PMID: **26195795**). NCX1 has 10 transmembrane helices, and four ion-binding
331 sites, one for Ca^{2+} and three for Na^+ (Liao et al., 2012; Secondo et al., 2015). This NCX1 structural
332 study agrees with current stoichiometric studies showing a mostly 3 Na^+ to 1 Ca^{2+} exchange rate (Bers
333 and Ginsburg, 2007). NCX1 can function to facilitate either inward or outward current depending on
334 the membrane potential (Bers, 2002). NCX1 is the predominant pathway for calcium extrusion in
335 cardiomyocytes during resting membrane potential; the high extracellular sodium concentration allows
336 NCX1 to exchange calcium out of the cell. Overall, NCX promotes myocytes to relax, therefore
337 implicating a role of NCX1 in contractility. Further, spontaneous pacemaker release of Ca^{2+} by RyR2

338 activates the NCX1 on the sarcoplasmic reticulum membrane, which then pushes the cell to the
339 minimum threshold for triggering an action potential (Shattock et al., 2015).

340 *Human studies:*

341 Genetic variants in *SLC8A1*, the gene encoding NCX1, are associated with numerous
342 electrocardiographic traits due to changes in calcium cycling. Polymorphisms in *SLC8A1* across human
343 populations were first associated with hypertension. Seven NCX1 polymorphisms with a high minor
344 allele frequency of more than 4% were identified in 1865 individuals, with 787 being hypertensive
345 (Kokubo et al., 2004). Hong et al found a single-nucleotide poly-morphism on the *SLC8A1* locus
346 correlated with PR interval prolongation by a genome-wide association study using the Korea
347 Association Resource database (Hong et al., 2014). Kim et al. also found a common *SLC8A1* variant
348 associated with prolongation in QT interval, suggesting the predisposition of these populations to
349 ventricular arrhythmias and sudden cardiac death (Kim et al., 2012). Further, patients homozygous for
350 allele rs13017968 in *SLC8A1* had higher rates of coronary artery abnormalities, predisposing these
351 populations to Kawasaki disease. At present, there are no human data correlating mutations on *SLC8A1*
352 with sinus node dysfunction. However, animal models showed major phenotypes of sinus node
353 dysfunction when experiencing loss of NCX1 (Gao et al., 2013; Groenke et al., 2013; Torrente et al.,
354 2017). Comment on the high MAF?

355 *Mouse models:*

356 The role of NCX1 in calcium cycling, and its importance to the SAN, have been studied through
357 multiple *Slc8a1*-null mouse models. A global *SLC8A1* knockout resulted in abnormal myofibrillar
358 organization and severe electrical defects that caused embryonic lethality (Fu et al., 2010); therefore,
359 cardiac specific knockouts are required to study the role of NCX specifically in the heart. Gao et al.
360 used a global myocardial and SAN-targeted knockout of *SLC8A1* to study the role of NCX1 in
361 pacemaker activity. Although isolated SAN cells showed similar basal contractility rates in *SLC8A1*
362 knockout versus WT mice, *Slc8a1* knockout mice showed inability to respond to isoproterenol,
363 implicating a role of NCX1 in the sympathetic response of the heart (Gao et al., 2013). Several studies
364 have established the role of NCX1 in calcium efflux, however, the role of NCX1 in triggering an action
365 potential is still not completely understood. Groenke et al. showed pacemaker activity to be completely
366 ablated in an atrial-specific *Slc8a1* knockout mouse model (Groenke et al., 2013). The *Slc8a1* atrial
367 specific knockout mouse also lacked P waves, and had arrhythmic depolarizations in the SAN.
368 Although SAN automaticity still occurred without NCX1, the automaticity came in bursts similar to
369 tachycardia-bradycardia syndrome and SND (Torrente et al., 2015). Torrente et al. further used their
370 atrial-specific *Slc8a1* knockout, and found severe cellular Ca^{2+} accumulation during SA nodal
371 pacemaker activity, leading to intermittent hyperactivation of small conductance K^+ (SK) channels,
372 subsequently resulting in arrhythmias. This data identified the potential influence of intracellular Ca^{2+}
373 on SK channels and overall SAN repolarization, and signified SK channels as potential therapeutic
374 targets for SAN dysfunction if presented alongside Ca^{2+} cycling issues (Torrente et al., 2017).

375

376 **2.6 Ankyrin-2 (ANK2)**

377 Ankyrin-B (AnkB, encoded by *ANK2*) is a membrane adapter protein critical in the recruitment,
378 organization, and stabilization of the ion channels and transporters underlying the excitation-
379 contraction coupling, particularly in the SAN. Loss-of-function variants in *ANK2* are associated with

380 a complex cardiac phenotype including heart rate variability, CPVT, conduction defects, atrial
381 fibrillation, sinus node bradycardia, sudden cardiac death, and recently, arrhythmogenic
382 cardiomyopathy (Roberts et al., 2019). Two families characterized with severe sinus node disorder
383 (SND) were found to have *ANK2* allele variants, making AnkB the first non-ion channel protein
384 associated with human sinus node disease. SAN cells from *Ank2*^{+/-} mice showed reduction in
385 membrane expression of NCX1, Na⁺-K⁺-ATPase (NKA), and voltage-dependent L-type calcium
386 channel alpha1D subunit (Cav1.3), causing abnormal intracellular Na⁺ and Ca²⁺ cycling, which
387 generated various cardiac arrhythmic events (Curran and Mohler, 2011).

388 Optical mapping was further used to analyze the complete, intact, atrial pacemaker complex. *Ank2*^{+/-}
389 mice had greater RR variability due to multiple competing pacemaker sites between the SAN and the
390 atrioventricular junction (AVJ), further highlighting the role of AnkB in cardiac automaticity, yet
391 suggesting some unknown mechanisms of compensation (Glukhov et al., 2010). Computational models
392 have further analyzed the role of AnkB in the generation of lethal arrhythmias. The loss of functional
393 NCX and NKA specifically allows Ca²⁺ overload in the sarcoplasmic reticulum, therefore initiating
394 afterdepolarizations, and introducing variability and inconsistency in the SAN firing. Loss of Cav1.3 in
395 the SA node slows the overall pace of firing, explaining the bradycardia seen in families with AnkB
396 dysfunction (Wolf et al., 2010; Wolf et al., 2013). Interestingly, individuals with atrial fibrillation have
397 reduced levels of AnkB expression and increased levels of miR-34a (a microRNA associated with
398 cardiac fibrosis). Of note, the 3' untranslated region of *ANK2* also contains the binding site to miR-
399 34a, implicating a potential role of miR-34a in the electrical remodeling of the atria and in the
400 regulation of AnkB expression (Zhu et al., 2018). Although the culmination of cardiac AnkB studies
401 implicate loss-of-function *ANK2* variants in numerous cardiac diseases, the lack of family history in
402 many of these cases, and overall incomplete penetrance of AnkB-associated disease, strongly implies
403 that additional genetic and/or environmental factors must be involved in the development of the severe
404 "AnkB syndrome" phenotype. Notably, intense endurance exercise or other genetic variants likely
405 play a role in the development of cardiac disease associated with loss-of-function *ANK2* variants
406 (Roberts et al., 2019).

407 **2.7 Myosin heavy chain 6 (*MYH6*)**

408 *MYH6* encodes the alpha myosin heavy chain subunit of myosin (MHC- α), a major component of the
409 sarcomere - a necessary component of muscle fiber for proper contraction in the heart (Epp et al., 1993;
410 Squire, 1997). A MHC- α R721W missense variant has been identified in Icelandic populations (0.38%
411 allelic frequency) and is associated with SND, with 50% of the carriers for this variant being diagnosed
412 with SND. Carriers of the variant that were not diagnosed with SND still showed reduced heart rate
413 and PR interval prolongation (Holm et al., 2011). Interestingly, another R654W heterozygous MYH- α
414 variant was identified in an Australian family with severe yet diverse cardiac arrhythmias, including
415 SND and cardiac arrest due to ventricular fibrillation, resulting in sudden cardiac death or sinus node
416 dysfunction (Lam et al., 2015).

417 **2.8 Lamin A (*LMNA*)**

418 Nuclear lamins (Lamins A, B1 and B2) are the major components of nuclear lamina, which plays a
419 vital structural role in the nuclear envelope (Mounkes et al., 2001). *LMNA* variants are associated with
420 numerous cardiac conditions, particularly dilated cardiomyopathy (MacLeod et al., 2003; Lin et al.,
421 2018; Yokokawa et al., 2019). A c.357-2A>G heterozygous splice site variant in *LMNA* was identified
422 in a proband diagnosed with SND, who a family history of cardiac arrhythmia and dysfunction. This
423 novel variant was predicted to cause haploinsufficiency, as aberrant mRNA from the mutant allele

424 would likely decay by nonsense-mediated mRNA decay (Zaragoza et al., 2016). Although no
425 population-wide variant in *LMNA* has been identified in relation to SND, the numerous familial
426 variants identified and connected with conduction disorders provide a rationale for further exploration
427 of the role of lamin A in SND.

428 **2.9 L-type calcium channel subunit Ca_v1.3 (*CACNA1D*)**

429 Ca_v1.2 (alpha1C) and Ca_v1.3 (alpha1D) subunits make up the cardiac L-type voltage-activated calcium
430 channel (Matthes et al., 2004). Ca_v1.3 is expressed mostly in the SAN, AV node, and atrial myocytes
431 (Mangoni et al., 2003). In the SAN, Cav1.3 plays a major role in pacemaker activity by driving inward
432 current during diastolic depolarization and regulating diastolic SR Ca²⁺ release (Mangoni et al., 2003),
433 (Torrente et al: Cardiovasc Res 2016, PMID: 26786159). *CACNA1D*^{-/-} mice have severe SND and
434 atrioventricular 2nd degree block (Platzer et al., 2000) (Zhang et al., Circ Res 2002, PMID: **12016264**)
435 (Mesirca et al. PNAS 2016: PMID: 26831068). In addition, Ca_v1.3 also plays an important role in
436 calcium homeostasis in the ear, as *CACNA1D*^{-/-} mice experience deafness along with SND (Chu et al.,
437 2007). The phenotype of SND-associated deafness was later identified in two related families with a
438 Ca_v1.3 variant (G403_V404insG). This rare combination of symptoms is termed sinoatrial node
439 dysfunction and deafness (SANDD) (Baig et al., 2011). This *CACNA1D* variant was also identified in
440 four additional families with SANDD, along with an A376V missense variant (Liaqat et al., 2019).

441 **2.10 Short-stature homeobox 2 (*SHOX2*)**

442 Short-stature homeobox 2 is a transcription factor encoded by *SHOX2*. Shox2 is essential for the proper
443 development of the sinoatrial node (Puskaric et al., 2010). *SHOX2*^{-/-} mice are embryonically lethal due
444 to lack of SAN development, but *SHOX2*^{-/-} zebrafish survive with bradycardia (Hoffmann et al., 2013).
445 Loss-of-function variants in *SHOX2* cause early-onset and familial atrial fibrillation (Hoffmann et al.,
446 2016; Li et al., 2018). A heterozygous missense P33R Shox2 variant was also identified recently in a
447 patient with SND (Hoffmann et al., 2019). Screening should continue for *SHOX2* variants in patients
448 with SND, as this gene has only been recently implicated in sinoatrial node function in humans.

449 **2.11 Transient receptor potential cation channel subfamily C member 3 (*TRPC3*)**

450 TRPC channels (non-selective Ca²⁺-permeable cation channels) are thought to play an important role
451 in store-operated Ca²⁺ entry (SOCE), described as Ca²⁺ influx to the sarcolemma after Ca²⁺ depletion,
452 although the pathway is currently poorly understood (Wu et al., 2004; Ju et al., 2007). TRPC channels
453 are activated when diacylglycerol (DAG) is released from the plasma membrane via agonist binding
454 to G protein-coupled receptors (Wu et al., 2004). All TRPC subtypes (1-7) are expressed in the SAN
455 except subtype 5, but TRPC3 is the only subtype expressed on the membrane surface of the pacemaker
456 cells (Ju et al., 2007).

457 Ca²⁺ entry through TRPC3 in the SAN seems to play a role in AF and sinus arrhythmia. *TRPC3*^{-/-} mice
458 treated with angiotensin II had reduced incidence of AF compared to WT control mice during AF
459 pacing (Ju et al., 2015). Trpc3 specifically has been shown to increase local Ca²⁺ release (LCR) and
460 NCX current (*I_{NCX}*) in mouse embryonic stem cell-derived cardiomyocytes, which resulted in increased
461 spontaneous action potentials (Qi et al., 2016). Both of these studies support the hypothesis that Ca²⁺
462 entry via TRPC3 is a pro-arrhythmic pathway which potentially causes increased pacemaker activity.
463 TRPC3 is upregulated in AF patients and AF animal models (Harada et al., 2012). Finally, TRPC-3
464 channel upregulation also has been shown to cause an increased accumulation of collagen consistent
465 with atrial fibrosis in mice (Han et al., 2020).

466 **Stromal interaction molecule 1 (*STIM1*)**

467 Store-operated calcium entry (SOCE) is an important pathway for Ca^{2+} reentry after calcium depletion,
468 particularly in cardiac pacemaker cells. *STIM1* is an endoplasmic reticulum Ca^{2+} sensor essential to
469 the SOCE pathway, implicating *STIM1* in the calcium clock of SAN cells. Following Ca^{2+} depletion
470 in the SAN cells, *STIM1* localizes to the cell periphery, along with calcium release-activated calcium
471 channel protein 1 (*Orai1*) (Liu et al., 2015). *STIM1* and *Orai1* channels are selectively expressed in
472 SAN cells, and *STIM1* is essential in SAN functioning. *Stim1* cardiac knockout mice showed SR
473 calcium store depletion in SAN cells, resulting in SAN dysfunction (Zhang et al., 2015). *STIM1*
474 deficient mice experienced slowed heart rate after stimulation, sinus arrest, and extreme autonomic
475 response to cholinergic signaling. Further, they also showed reduction in L-type Ca^{2+} current, and
476 enhanced NCX activity, linking *STIM1* to more regulatory pathways in Ca^{2+} cycling in SAN cells than
477 previously anticipated (Zhang et al., 2015). Beyond the SA node, *STIM1* has been found to play an
478 essential role in interatrial conduction via its expression in sinus cardiomyocytes from the SAN to the
479 coronary sinus. Deletion of *Stim1* from coronary sinus cardiomyocytes slowed conduction across the
480 atria and increased susceptibility to atrial arrhythmias in *Stim1* cardiac specific knockout mice (Zhang
481 et al., 2020).

482 **Potassium two pore domain channel subfamily K member 2 (*KCNK2*)**

483 TREK-1, or K2p2.1, (encoded by *KCNK2* gene) is a K^+ channel with four transmembrane segments
484 and two pore domains that is activated via membrane stretch and arachidonic acid, among other
485 mechanisms (Fink et al., 1996; Lesage and Lazdunski, 1998; Maingret et al., 1999). K^+ channels in
486 cardiac tissue open to cause hyperpolarization, and close during depolarization, therefore playing a
487 crucial role in selecting the duration of an action potential (Snyders, 1999). Beyond membrane stretch
488 and arachidonic acid, TREK-1 is regulated by polyunsaturated fatty acids, temperature, receptor-
489 coupled second messenger systems, volatile anesthetics, neuroprotectant agents, and selective
490 serotonin reuptake inhibitors (Goonetilleke and Quayle, 2012).

491 TREK-1 is expressed ubiquitously in porcine heart, with elevated expression in atrial tissue. Atrial
492 burst pacing (a simulation for AF) has shown the ability to reduce TREK-1 expression by 70% after
493 just 7 days in the atria. (Schmidt et al., 2014). Cardiac-specific TREK-1-deficient mice experienced
494 bradycardia and sinus pauses following induced stress. Moreover, isolated SAN cells in TREK-1
495 deficient mice showed decreased background K^+ current that caused abnormal cell excitability,
496 confirming the role of TREK-1 in the cardiac action potential. Our group has showed that βIV -
497 spectrin/TREK-1 complex expression was decreased in a canine model with pacing-induced heart
498 failure and SAN dysfunction (Unudurthi et al., 2016).

499 **Transient receptor potential melastatin 4 (*TRPM4*)**

500 TRPM4 is a selective monovalent cation channel that allows flow of Na^+ , K^+ , Cs^+ , and Li^+ (Guinamard
501 et al., 2010), which is activated by intracellular Ca^{2+} (Launay et al., 2002). A study in 160 unrelated
502 probands identified multiple *TRPM4* variants associated with right-bundle branch block and isolated
503 atrioventricular block, signifying the role of *TRPM4* in cardiac conduction. Surprisingly, none of the
504 patients with SND harbored *TRPM4* variants (Stallmeyer et al., 2012). However, TRPM4 is expressed
505 in mouse sinoatrial node cells, and is potentially implicated in heart rate rhythm regulation (Demion et
506 al., 2007). Further, TRPM4 inhibition showed the ability to reduce the action potential rate by
507 modulation of Ca^{2+} -activated nonselective cation current in isolated mammalian right atrial cells (Hof

508 et al., 2013). Therefore, patients with SND should continue to be screened for *TRPM4* variants in the
509 future considering the involvement of TRPM4 involvement in cardiac conduction.

510 **3 Clinical Management of Sinoatrial Node Dysfunction**

511 Pharmacologic Approach

512 Many genetic forms of SND are chronic, yet symptoms are mild. Acute SND, however, can appear
513 secondary to another condition, procedure, or disease, as mentioned previously. Acute SND presented
514 as bradycardia or bradycardia-tachycardia syndrome is dangerous, and can be managed with a small
515 selection of pharmacological agents. Therapeutic control of SND is ideal because of the reduced cost
516 and avoidance of surgical intervention. Current pharmacological options for acute management of
517 bradycardia associated with SND include atropine, isoproterenol, aminophylline, or theophylline
518 according to the ACC/AHA/HRS updated guidelines in 2019 (Kusumoto et al., 2019).

519 Atropine may successfully reverse an acute SND condition to a normal sinus rhythm (Schweitzer and
520 Mark, 1980). Atropine shortens the sinus cycle length and the sinus recovery time of the SAN, therefore
521 potentially treating bradycardia (Dhingra et al., 1976). The SAN response to atropine is bimodal,
522 however, with slowing of the heart rate in small doses (< 0.4 mg), and acceleration of heart rate in
523 higher doses (Das et al., 1975). Atropine is also a suitable therapeutic approach for myocardial
524 infarction induced bradyarrhythmia at (< 0.8 mg). Overall, atropine intravenous treatment of 0.5 mg –
525 2 mg total (only up to 1 mg at a time per 3 – 5 minutes) seems to be the best therapy for treatment of
526 bradycardia attributable to acute SND (Kusumoto et al., 2019). Atropine, however, should not be used
527 to treat bradycardia in patients who have undergone heart transplant, as atropine treatment resulted in
528 sinus arrest or AV block in 20% of heart transplant patients from one study (Bernheim et al., 2004).
529 Importantly, isoproterenol does not have any clinical trials as a pharmacological therapy for SND
530 patients, and therefore should be used with caution, particularly when there is a concern for coronary
531 ischemia (Kusumoto et al., 2019). There are some positive case studies exploring isoproterenol as a
532 therapeutic measure for bradycardia (Sodeck et al., 2007; Herman and Zhou, 2011), but the outcomes
533 have reported incidence of supraventricular tachycardia (Cossú et al., 1997). In summary, atropine is
534 generally the best pharmacological treatment for acute bradycardia.

535 Acute bradycardia following heart transplant is a common outcome. As mentioned earlier, atropine is
536 a poor choice for bradycardia therapy after heart transplant due to the potential for complete AV block
537 and sinus arrest (Bernheim et al., 2004). In this scenario, theophylline has displayed the ability to
538 reverse bradycardia in the majority of post-transplant patients, averting the need for pacemaker
539 implantation (Bertolet et al., 1996; Kertesz et al., 2003). After heart transplant, relative bradycardia is
540 often considered as a heart rate of less than 80 beats per minute (bpm), as postoperative patients have
541 higher hemodynamic demand. The lack of parasympathetic input in a donor heart often results in SND
542 (Woo et al., 2008). Bertolet and colleagues showed the mean heart rate of 29 patients suffering from
543 bradyarrhythmias post-heart transplant was improved from (62 +/- 7) to (89 +/- 10) bpm when treated
544 with theophylline (300mg intravenous, 474 +/- 99 mg/day orally) after 24 hours of treatment (Bertolet
545 et al., 1996). Aminophylline has also some supportive data, but theophylline is more supported across
546 the literature (Kusumoto et al., 2019). Theophylline shows some promise in treating chronic SND as
547 well (Alboni et al., 1991; Saito et al., 1993), but theophylline has numerous situational restrictions that
548 prevent it from becoming the gold-standard for chronic SND therapy. Theophylline should specifically
549 be avoided in cases of bradycardia-tachycardia syndrome or in patients experiencing any ventricular
550 ectopy (Ling and Crouch, 1998).

551 Beyond therapeutic purposes, atropine and isoproterenol can be used to evaluate the severity of
552 asymptomatic sinus bradycardia. Interestingly, the chronotropic response to atropine or isoproterenol
553 can identify patients in need of preventative pacemaker implantations (Vavetsi et al., 2008).

554 Finally, a study of 192 patients with SND showed that cilostazol was able to prevent permanent
555 pacemaker implantation. Only 20.4% of patients receiving cilostazol needed a pacemaker implant,
556 while 55.8% of patients not receiving cilostazol required a pacemaker. By increasing heart rate,
557 cilostazol shows promise to be the first long-term pharmacological therapeutic for SND as many of the
558 pharmacological options discussed earlier are only effective short-term, and have negative long-term
559 side effects (Sonoura et al., 2019). With Sonoura et al. publishing this study in 2019, long-term studies
560 showing longer-term survivability over 5 or 10 years in patients using cilostazol would further support
561 the use of this drug to manage sinus node disorders.

562 Temporary Pacemaking

563 When pharmacological therapeutics fail, temporary pacemakers are an alternative approach and can be
564 implemented via a multitude of methods for care of acute bradycardia. Transcutaneous cardiac pacing
565 is an external pacing method (prevents the risk of infection and other surgical complications), and is
566 commonly used in response to patients with life-threatening bradycardia (Dalsey et al., 1984).
567 Transcutaneous pacing is often used for patients in cardiac arrest, and can be successful if used within
568 the early phases of cardiac arrest (Noe et al., 1986). Transcutaneous pacing does, however, show
569 success in reverting bradycardia in patients not in cardiac arrest too (Clinton et al., 1985). Transvenous
570 pacemaker therapy is another temporary pacemaker option. Transvenous pacemakers can be implanted
571 in the internal and external jugular, subclavian, brachial, and femoral vein, although the right internal
572 jugular is the most preferred location for best access to the right ventricle (Gammage, 2000). The early
573 studies of transvenous pacemaker therapy showed very high rates of pacemaker malfunction (as high
574 as 43%) (Lumia and Rios, 1973). Even today, approaches for temporary transvenous cardiac pacing
575 vary widely, so outcomes are difficult to compare across studies (Diemberger et al., 2020). However,
576 a study in 2018 across more than 360,000 patients found complications for modern-day temporary
577 transvenous pacing to be only about 4%, but with 37.9% of patients still needing permanent pacemaker
578 implantation (Metkus et al., 2019). Overall, the ACC/AHA/HRS guidelines recommend
579 transcutaneous pacing over transvenous pacemaker therapy due to the complications of the transvenous
580 method when a patient is hemodynamically unstable and critically ill due to bradycardia (Kusumoto et
581 al., 2019). This suggestion may be changed in the future with large group studies showing the success
582 of transvenous pacing.

583 Permanent Pacemaker Implant

584 In general, a permanent pacemaker implant should be avoided if possible to avert the potential
585 procedural complications related to the implant and long-term management of the implant.
586 Unfortunately, permanent pacemaker implantation may be necessary to prevent severe and life-
587 threatening arrhythmias, and should be considered with severe symptomatic sinus bradycardia and
588 bradycardia-tachycardia syndrome (Kusumoto et al., 2019). For example, permanent pacemaking after
589 Fontan operation, a surgery for children with only a single functional ventricle, often results in SND
590 and requires permanent atrial pacing in 13-16% of patients (Cohen et al., 1998; Takahashi et al., 2009).
591 Further, patients presenting with atrial fibrillation and significant atrial fibrosis may require a
592 permanent pacemaker implant (Akoum et al., 2012). Atrial flutter, associated with SND, can also be
593 used to predict the need for pacemaker implantation. In 211 patients following ablation, an atrial flutter

594 length of more than 273 milliseconds predicted the need for a pacemaker implantation (Sairaku et al.,
595 2012).

596 Future Implications

597 SND is a disorder caused by the inability of the heart to perform its pacemaking function (Semelka et
598 al., 2013). It is manifested clinically as sinus bradycardia, sinus pause or alternating bradyarrhythmias
599 and tachyarrhythmias. Importantly, patients report chronotropic incompetence in response to stress or
600 exercise (Semelka et al., 2013). Permanent pacemaker implantation is only recommended for
601 symptomatic patients. However, there is no existing therapy to reverse the primary genetic cause in
602 patients diagnosed with primary or chronic SND. Calcium-activated potassium channels (SK4) have
603 been implicated in the automaticity of cardiomyocytes, yet Haron-Khun et al. showed that TRAM-34
604 (a selective blocker of SK4 channels) successfully reduced delayed afterdepolarizations and calcium
605 transients in hiPSC-CMs (human induced pluripotent stem cell-derived cardiomyocytes) from patients
606 with CPVT2 from a CASQ2-D307H variant. When mice with the CASQ2-D307H knock in variant
607 were injected with TRAM-34, electrocardiographic recordings showed reduced arrhythmias (and
608 reduced severity of arrhythmias) at rest, and during exercise. This was also shown in CASQ2 KO mice.
609 TRAM-34 and clortimazole (SK4 inhibitors) therefore hold great therapeutic promise for human
610 populations with loss-of-function CASQ2 variants (Haron-Khun et al., 2017).

611 Nonfunctional cardiac funny current (I_f) causes unusual Ca^{2+} handling as previously discussed,
612 therefore disrupting the pacemaker activity by the sinus node. Remarkably, Mesirca et al. showed how
613 cardiac funny current-deficient mice have impulse generation and conduction defects, which can be
614 rescued with genetic deletion of cardiac muscarinic G-protein-activated channels (GIRK4). Although
615 *HCN4* and *GIRK4* loss of function variants have each been implicated in SND, the combination of
616 silencing both genes seems to repair severe cardiac arrhythmia phenotype of SND associated with
617 atrioventricular block and ventricular arrhythmia (Mesirca et al., 2014). Interestingly, follow-up work
618 has indicated that genetic deletion of GIRK4 also rescues and conduction defects in model mice of
619 Cav1.3 mediated SND (Mesirca et al. PNAS 2016: PMID: 26831068). Furthermore, rescuing of SND
620 in mice models carrying dysfunction in HCN4, Nav1.5 and Cav1.3 channels can be mimicked by acute
621 administration of the I_{KACH} blocker tertiapin-Q (Bidaud et al. Sci Rep 2020: PMID: 32555258).
622 Importantly, the ability of pharmacologic inhibition of I_{KACH} to improve SA node function has been
623 demonstrated recently in human SA nodes with history of SND (Li et al. Sci Transl Med 2017:
624 PMC5775890). Finally, silencing of GIRK4 expression in human atrial myocytes was shown to
625 efficiently decrease I_{KACH} densities and therefore is a great potential tool for treating arrhythmia. (Liu
626 et al., 2009). In conclusion, available results in model mice and human SA node tissue indicate that
627 gene therapy or pharmacologic strategy targeting GIRK4 channels can constitute an important future
628 direction for clinical handling of SND.

629 *HCN4* lentiviral gene transfer has interestingly shown the bioengineering potential to allow for
630 pacemaker cell therapy. Transducing *HCN4* revived autonomous pacemaking and increased
631 responsiveness to autonomic regulation in *HCN4*-transduced myocytes (Boink et al., 2008). Further,
632 myocyte enhancer factor-2 (MEF2) and activator protein-1 (AP1), with binding sequences located on
633 conserved non-coding sequence 13 (CNS13), are involved in HCN4 enhancement via the *HCN4*
634 promoter (Kuratomi et al., 2009), and could be used to upregulate HCN4 to promote pacemaking
635 activity.

636 In summary, SND is a disorder that more commonly affects the elderly population and impacts the
637 pacemaking function of the heart resulting in arrhythmia and chronotropic incompetence. The

638 complexity of SND is partially attributed to the complexity of genetic abnormalities, and partially
 639 attributed to gene pleiotropy. Many variants of SND-associated genes can exhibit multiple unrelated
 640 phenotypic traits. Currently, clinical management of SND patients is restricted to the treatment or relief
 641 of arrhythmia symptoms. However, there is no widely-available therapeutic option that targets or
 642 reverses the primary genetic cause in patients with chronic SND. Understanding the complexity of
 643 genetics that contribute to disease progression is critical to developing new therapeutic strategies for
 644 this complex, life threatening disorder.

645 **4 Conflict of Interest**

646 The authors declare no conflict of interest.

647 **5 Author Contributions**

648 All authors listed have made substantial direct and intellectual contribution to the work, and approved
 649 it for publication. All authors have read and agreed to the published version of the manuscript.

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655 **7 Tables and Figures**

656 **Table 1. Proteins implicated in human SND.**

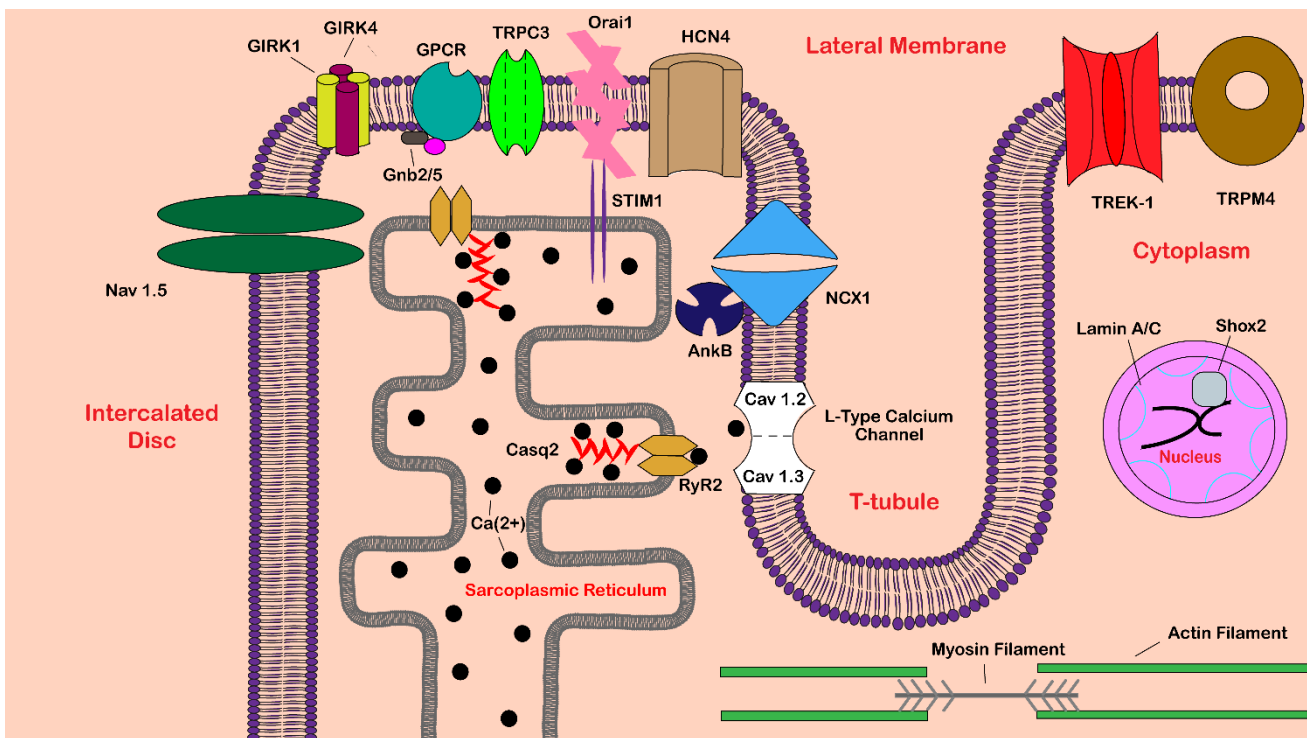
Protein	Gene	Associated Cardiac Diseases	References
Calsequestrin-2	<i>CASQ2</i>	SND/bradycardia, CPVT, atrial arrhythmias	(Reid et al., 1975; Leenhardt et al., 1995; Sumitomo et al., 2007; Weeke et al., 2014; Glukhov et al., 2015)
Ryanodine receptor 2	<i>RYR2</i>	SND/bradycardia, CPVT, atrial arrhythmias	(Sumitomo et al., 2007; Domingo et al., 2015; Miyata et al., 2018; Dharmawan et al., 2019)
G protein-activated inward rectifier potassium channel 4	<i>KCNJ5</i>	SND/bradycardia, atrial arrhythmias, long QT syndrome	(Dobrev et al., 2001; Zhang et al., 2009; Yang et al., 2010; Wang et al., 2013;

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		type 13, Anderson-Tawil syndrome	Kokunai et al., 2014; Kuß et al., 2019)
Guanine nucleotide-binding protein subunit beta-2/5	<i>GNB2/GNB5</i>	SND/bradycardia, cognitive disability, cardiac conduction abnormalities	(Lodder et al., 2016; Stallmeyer et al., 2017; Fukuda et al., 2020)
Sodium/calcium exchanger 1	<i>SLC8A1</i>	Conduction disorders (PR and QT prolongation), ventricular arrhythmias, Kawasaki disease	(Kim et al., 2012; Hong et al., 2014)
Sodium voltage-gated channel alpha subunit 5	<i>SCN5A</i>	SND/bradycardia, long QT syndrome type 3, Brugada syndrome, dilated cardiomyopathy, conduction disorders, infant death syndrome	(van den Berg et al., 2001; Benson et al., 2003; McNair et al., 2004; Smits et al., 2005; Nakajima et al., 2013; Freyermuth et al., 2016; Yang et al., 2017; Denti et al., 2018)
Hyperpolarization activated cyclic nucleotide gated potassium channel 4	<i>HCN4</i>	SND/bradycardia, ventricular arrhythmias, left ventricular noncompaction	(Schulze-Bahr et al., 2003; Ueda et al., 2004; Nof et al., 2007; Milano et al., 2014)
Ankyrin-B	<i>ANK2</i>	SND/bradycardia, CPVT, atrial arrhythmias, arrhythmogenic cardiomyopathy	(Mohler et al., 2003; Le Scouarnec et al., 2008; Roberts et al., 2019)
Myosin heavy chain 6	<i>MYH6</i>	SND/bradycardia, aorta coarctation, ventricular arrhythmias	(Holm et al., 2011; Lam et al., 2015; Bjornsson et al., 2018)
Lamin A	<i>LMNA</i>	SND/bradycardia, dilated	(MacLeod et al., 2003; Zaragoza et al., 2016; Lin et al., 2018;

		cardiomyopathy, conduction disorders	Yokokawa et al., 2019)
L-type calcium channel subunit $Ca_v1.3$	<i>CACNA1D</i>	Sinoatrial node dysfunction and deafness (SANDD)	(Baig et al., 2011; Liaqat et al., 2019)
Short-stature homeobox 2	<i>SHOX2</i>	SND/bradycardia, atrial arrhythmias	(Hoffmann et al., 2016; Li et al., 2016; Hoffmann et al., 2019)

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659 **Figure 1. Schematic of proteins implicated in sinoatrial node dysfunction.** Shown is a partial SA
 660 pacemaker cell. Proteins are labeled in black text, while various cellular locations are labeled in red
 661 text. Abbreviations include: calsequestrin-2 (Casq2), ryanodine receptor 2 (RyR2), G protein-activated
 662 inward rectifier potassium channel 1/4 (GIRK1/4), Guanine nucleotide-binding protein subunit beta-
 663 2/5 (Gnb2/5), G-protein coupled receptor (GPCR), sodium/calcium exchanger 1(NCX1), sodium
 664 voltage-gated channel alpha subunit 5 (Na_v 1.5), hyperpolarization activated cyclic nucleotide gated
 665 potassium channel 4 (HCN4), ankyrin-b (AnkB), short-stature homeobox 2 (Shox2), transient receptor
 666 potential cation channel subfamily C member 3 (TRPC3), stromal interaction molecule 1 (STIM1),
 667 calcium-release-activated calcium channel protein 1 (Orai1), potassium two pore domain channel
 668 subfamily K member 2 (TREK-1), and transient receptor potential melastatin 4 (TRPM4).

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