



Review

Inherited bradyarrhythmia: A diverse genetic background

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ABSTRACT

Bradyarrhythmia is a common heart rhythm abnormality comprising number of diseases and is associated with decreased heart rate due to the failure of action potential generation and propagation at the sinus node. Permanent pacemaker implantation is often used therapeutically to compensate for decreased heart rate and cardiac output. The vast majority of bradyarrhythmia cases are attributable either to aging or to structural abnormalities of the cardiac conduction system, caused by underlying structural heart disease. However, there is a subset of bradyarrhythmia primarily caused by genetic defects in the absence of aging or underlying structural heart disease. These include several genes that play principal roles in cardiac electrophysiology, heart development, cardioprotection, and the structural integrity of the membrane and sarcomere. Recent advances in the functional analysis of mutations using a heterologous expression system and genetically engineered animal models have provided significant insights into the underlying molecular mechanisms responsible for inherited arrhythmia. In this review, current understandings of the genetic and molecular basis of inherited bradyarrhythmia are presented.

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1. Introduction

Bradyarrhythmia is a serious electrical disorder of the heart with the potential to be life threatening. The condition is caused by an electrical dissociation in the cardiac conduction system (CCS) comprising the sinus bradycardia, the sinoatrial (SA) exit block and the atrioventricular block (AVB). It often manifests as abnormally suppressed cardiac output in affected individuals, requiring permanent pacemaker implantation in order to compensate for decreased heart rate. The CCS is equipped with a sophisticated histological structure and specialized cellular function in order to maintain proper impulse generation and propagation. The mechanical burden and scars resulting from structural heart disease are a major cause of bradyarrhythmia. Accumulation of connective tissue such as collagen is almost always associated with progression of heart failure, as it promotes dissociation between electrically coupled cardiomyocytes [1]. Collagen deposition is associated with aging and underlying structural heart disease, reflected by the increased incidence and prevalence of bradyarrhythmia associated with these factors [1,2]. In the absence of underlying structural disease or aging, bradyarrhythmia may occur primarily due to genetic defects. In this review, we aim to describe the current understanding of inherited bradyarrhythmia with a focus on diverse genetic backgrounds and molecular physiology (Fig. 1 and Table 1).

2. Modulation mechanisms of heart rate and genetic exacerbation factors: physiological regulation of sinus rhythm

In the CCS, the sinoatrial node (SAN) is the primary pacemaker component and functions as a resource for automaticity; that is, spontaneous depolarization with regular intervals. Histologically, the SAN is intramurally embedded at the junction of the right atrium and the superior vena cava and lies along the crista terminalis [3]. The SAN displays heterogeneous cellular morphology, action potential configuration, and electrophysiological characteristics [4]. The SAN's major pacemaker site is situated at its center, however; this site may shift peripherally depending on various interventional factors such as electrolyte concentrations, autonomic nervous stimuli, and temperature [3]. The underlying mechanisms of this pacemaker shift remain undetermined, however; the pacemaker tends to shift to the site where electrical activity is least suppressed by extrinsic factors [3]. The molecular mechanisms underlying myocyte firing in the central SAN are characterized by the SAN's unique gene expression profile, with minimal expression of *KCNJ2* (inwardly rectifying K channel, Kir2.1) and *SCN5A* (cardiac Na channel, Nav1.5) and higher

expression of *HCN4* (the pacemaker channel). The absence of *KCNJ2* expression allows the resting membrane potential depolarized to enable spontaneous depolarization, while the absence of *SCN5A* expression can prevent rapid upstroke of action potential. Abundant expression of the *HCN4* pacemaker channel promotes spontaneous, slow depolarization in response to phase 4 hyperpolarization. The peripheral SAN, on the other hand, partially shares the gene expression profile and electrophysiological characteristics of the atrial myocytes [3]. The major role of excitation in the peripheral SAN is the rapid transmission of the sinus impulse to surrounding atrial myocytes. An abundant expression of *SCN5A* causes fast upstroke of action potential in phase 0 and this gives rise to rapid electrical conduction in the peripheral SAN. Thus, loss-of-function mutations in *SCN5A* could result in SA exit block, an electrical conduction blockade between the central SAN and surrounding atrial myocytes [5].

The mechanism of cyclic activation in voltage-gated ion channels involves the action of the pacemaker current on the cell membrane and is known as a membrane clock. Recently, a growing body of evidence has implicated the involvement of additional complementary mechanisms in this process, in particular, the rhythmic spontaneous release of Ca^{2+} by the sarcoplasmic reticulum (SR), which is referred to as a calcium clock. The calcium clock functions collaboratively with the membrane clock to form a unified, automatic system, known as a coupled-clock pacemaker system [6]. Genetic defects in the genes involved in membrane and calcium clocks can potentially cause SA disorders.

2.1. *HCN4*

In mammals, the hyperpolarized-activated cyclic nucleotide-gated channel (HCN) family is comprised of four distinct genes, *HCN1*, 2, 3 and 4; that are expressed in a wide variety of excitable cells (*HCN4* is predominantly expressed in the central SAN) [7]. *HCN4* slowly becomes permeable for K^+ and Na^+ in response to hyperpolarization, thus giving rise to slow diastolic depolarization resulting in automaticity [7]. Since the first description of an *HCN4* mutation in familial sick sinus syndrome (SSS) [8], twenty-two further mutations have been reported. Patch-clamp analysis of these mutations using a heterologous expression system with *Xenopus oocytes* or cultured cell lines have shown that reduced peak current densities or a hyperpolarizing shift of the voltage-dependence of activation are the major causes of disease [9,10]. Indeed, these loss-of-function properties decrease the slope of diastolic depolarization, resulting in sinus bradycardia. Some *HCN4* mutations disrupt the cyclic-nucleotide binding domain (cNBD) to which cyclic nucleotide cAMP and cGMP bind directly in response to β -adrenergic stimuli [8,9,11]. However, the molecular mechanisms of *HCN4* mutations are not yet fully elucidated; for example, G482R has been reported in multiple families associated with sinus bradycardia and left ventricular noncompaction cardiomyopathy [7,12]; however, the molecular mechanism underlying left ventricular noncompaction remains unknown.

2.2. *SCN5A*

The cardiac Na channel α subunit Nav1.5 encoded by *SCN5A* is associated with auxiliary β -subunits Nav β 1 and Nav β 3 [13]. Activation of the sodium channel initiates a rapid influx of Na^+ , giving rise to the phase 0 upstroke of cardiac action potential, which in turn triggers depolarization of neighboring cardiomyocytes [13]. As this Na^+ influx determines the slope and amplitude of phase 0, mutations in *SCN5A* may affect cardiac conduction velocity. The genetic defects in *SCN5A* are associated with multiple diverse inherited arrhythmias referred to as cardiac sodium channelopathy and include type-3 long QT

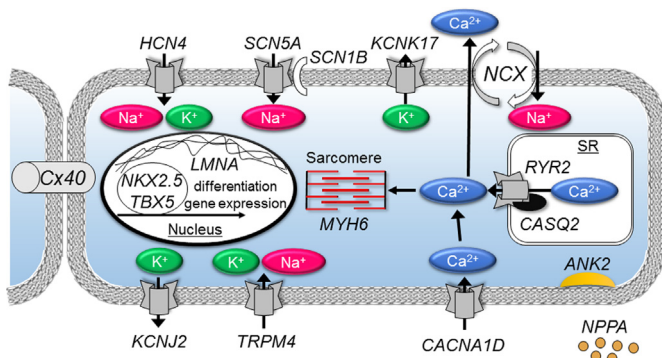


Fig. 1. Molecular modules involved in inherited bradyarrhythmia. Abnormalities in multiple pathways involving membrane ion channels, SR ion channels, sarcomere components, cardiac hormones, and membrane anchor proteins are associated with inherited bradyarrhythmia.

Table 1
Genes responsible for inherited bradyarrhythmia.

Gene name	Protein name	Inheritance mode	Atrial phenotypes	Conduction diseases	Ventricle phenotypes	Additional phenotypes	Function
Ion channels							
<i>HCN4</i>	HCN4	AD	Sinus bradycardia		LVNC, BrS		Loss
<i>SCN5A</i>	Nav1.5	AD, AR	Sinoatrial block, AF, Atrial standstill	PCCD, AVB	LQT3, BrS, DCM		Loss
<i>SCN10A</i>	Nav1.8	AD?	AF?	?	BrS?	Association with conduction parameters in ECG, episodic pain syndrome	?
<i>SCN1B</i>	Navβ1	AD		BBB	BrS	Epilepsy	Loss
<i>KCNJ2</i>	Kir2.1	AD			LQT7(ATS), SQT, BrS	Periodic paralysis, dysmorphic features	
<i>CACNA1D</i>	Cav1.3	AD	Sinus bradycardia			Congenital deafness	Loss
<i>KCNK17</i>	TASK-4	AD		PCCD, AVB, BBB	IVF?		Gain
<i>TRPM4</i>	TRPM4	AD		PCCD, AVB, BBB	BrS		Gain
Ca²⁺ handling proteins on the sarcoplasmic reticulum							
<i>RYR2</i>	Ryanodine receptor 2	AD	Sinus bradycardia		CPVT, ARVC		Loss
<i>CASQ2</i>	Calsequestrin	AR	Sinus bradycardia		CPVT		Loss
Gap junction channel							
<i>GJA5</i>	Connexin40	AD		PCCD, AVB, BBB			Loss
Cardiac hormone							
<i>NPPA</i>	ANP	AD	Atrial standstill, Bialtrial dilatation				Loss
Transcription factors							
<i>TBX5</i>	Tbx5	AD	ASD, AF	AVB	VSD	Hand anomalies (heart-hand syndrome)	Loss/gain
Nuclear membrane component							
<i>LMNA</i>	Lamin A/C	AD		PCCD, AVB	DCM	Laminopathies including muscular dystrophy and Hutchinson–Gilford progeria syndrome	Loss
Membrane adaptor protein							
<i>ANK2</i>	Ankyrin-B	AD	Sinus bradycardia	PCCD	LQT4		Loss
Sarcomere protein							
<i>MYH6</i>	Atrial myosin heavy chain	AD	Sinus bradycardia, AF, ASD		HCM, DCM		Loss

AD, autosomal dominant; AR, autosomal recessive; LQT, long QT; AVB, atrioventricular block; BrS, Brugada syndrome; BBB, bundle branch block; LVNC, left ventricular non-compaction; CPVT, catecholaminergic ventricular tachycardia; ATS, Andersen–Tawil syndrome; ASD, atrial septal defect; VSD, ventricular septal defect; PCCD, progressive cardiac conduction defect; AF, atrial fibrillation; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; IVF, idiopathic ventricular fibrillation.

syndrome, Brugada syndrome, SSS, atrial fibrillation (AF), progressive cardiac conduction defect (PCCD), dilated cardiomyopathy (DCM) and sudden infant death syndrome [13]. Patients with *SCN5A* mutations often display mixed arrhythmic phenotypes of cardiac sodium channelopathy, known as overlap syndrome [14]. As mentioned above, the molecular basis for SSS resulting from *SCN5A* mutations is an exit block at the peripheral SAN, caused by decreased conduction velocity from the central SAN [5]. Likewise, impaired sodium channel function may cause a conduction block within the CCS, referred to as AVB or bundle branch block (BBB). The presence of *SCN5A* mutations may distinctly affect the clinical outcomes associated with several arrhythmias. In Brugada syndrome, *SCN5A* mutations are associated with prolonged interatrial conduction times and AF induction; however, they do not appear to be related to spontaneous AF episodes, among other clinical variables [15]. In SSS, *SCN5A* mutation carriers exhibit significantly early onset as well as profound male predominance, thus resembling Brugada syndrome with a considerably earlier age of onset [16].

2.3. Mutations in genes responsible for calcium regulation

The third gene responsible for SSS is *ANK2*, which encodes the anchor protein ankyrin-B, thus linking integral membrane proteins to the underlying spectrin–actin cytoskeleton of cardiomyocytes [17]. Genetically engineered *Ank2* heterozygote knockout mice develop sinus bradycardia and exercise-induced aberrant ventricular tachycardia due to a Ca²⁺-handling abnormality [18]. Immunohistochemical analysis of cardiomyocytes from these mice showed mislocalization of the Na⁺/Ca²⁺ exchanger, Na⁺/K⁺-ATPase, and the IP3 receptor [19,20]. Biophysical analysis of SAN cells using a patch-clamp identified reduced currents in the Na⁺/Ca²⁺ exchanger and L-type Ca²⁺ channels [17]. These observations suggest that human *ANK2* mutations may predispose individuals to SAN dysfunction as a result of the biophysical disturbance of multiple proteins involved in Ca²⁺-handling.

The Cav voltage-gated Ca²⁺ channels, Cav1.2 and Cav1.3, mediate L-type Ca²⁺ current essential for normal cardiac pacemaker activity and conduction in both the SAN and the atrioventricular node. Cav1.3 activates more rapidly and under more

hyperpolarized membrane potentials when compared with Cav1.2 [21]. These properties allow Cav1.3 to contribute more significantly to the diastolic depolarization of SAN cells. Loss-of-function mutations in the *CACNA1D* encoding Cav1.3 cause SAN dysfunction with congenital deafness, attributable to the loss of rapid activation kinetics and negative activation thresholds of Cav1.3 in humans [22], which is consistent with the phenotypes observed in mice with genetic inactivation of *CACNA1D* [23].

Genes responsible for sinus bradycardia via abnormal Ca^{2+} regulation include the ryanodine receptor *RYR2* and the calmodulin-dependent protein kinase II *CACNA1D*, both of which are known to be causative genes for catecholaminergic polymorphic ventricular tachycardia (CPVT) [24–26]. CPVT-related mutations in these genes affect Ca^{2+} regulation by disrupting its storage and release from the SR during periods of exercise or emotional stress, resulting in sinus bradycardia and fatal ventricular tachyarrhythmia [27]. Postma et al. found a markedly lower resting heart rate in CPVT probands and their family members with *RYR2* mutations when compared with those of non-carrier family members [25]. They further reported that CPVT patients with *CASQ2* mutations develop sinus bradycardia, consistent with observations in *Casq2* homozygote knock-out mice [24]. The identification of gene mutations contributing to Ca^{2+} release and storage in the SR served to reinforce the critical role of calcium clocks in the maintenance of normal sinus rhythm.

3. Genetic basis for atrial standstill

3.1. *SCN5A*

SCN5A is abundantly expressed throughout the ventricular working myocardium and the CCS, as well as in the atrium [13]. Certain *SCN5A* mutations cause conduction block in the entire atrium, leading to atrial standstill and SSS [16,28]. A retrospective study of patients who experienced cardiac device-lead capture issues, including atrial standstill, showed a high prevalence of loss-of-function *SCN5A* mutations [29].

3.2. *NPPA*

Mutations in *NPPA*, the gene encoding atrial natriuretic peptide (ANP), are associated with certain atrial arrhythmias [30,31]. A deletion mutation in *NPPA* has been identified in an AF family spanning three generations. Affected members exhibited a transition from paroxysmal to chronic AF accompanied by atrial arrest in their forties [30]. Another mutation, R150Q, has previously been described in six AF families and is characterized by progressive, extreme biatrial dilatation and atrial standstill [31]. ANP is a circulating hormone that, via stimulation of the intracellular second messenger cGMP, plays a primary physiological role in the regulation of intravascular blood volume and vascular tone by means of natriuresis, diuresis, and vasodilatation. Moreover, cGMP signaling triggered by ANP has been shown to shorten both atrial conduction times and the effective refractory period, thus providing an arrhythmia substrate by direct modulation of cardiac ion channel properties [32,33]. However, the electrophysiological effect of these *NPPA* mutations on the cardiomyocytes themselves remains elusive.

4. Genetic basis of conduction block

4.1. *LMNA*

The *LMNA* gene encodes the ubiquitous inner-nuclear membrane protein lamin A/C, responsible for maintaining the structural

integrity and stability of the nuclear envelope. *LMNA* is further involved in various nuclear functions such as gene replication and chromatin organization [34]. Mutations in *LMNA* result in laminopathy, a wide spectrum of phenotypes with at least eleven distinct diseases [34]. Of these, progressive conduction block with DCM is the most frequently described cardiac phenotype [35]. *LMNA*-related DCM leads to severe and progressive damage to the heart, resulting in a higher risk of sudden cardiac death [36]. Male carriers have a worse prognosis due to the high prevalence of malignant ventricular arrhythmias and end-stage heart failure [37,38]. Knock-in mice for H222P-*LMNA* display male pre-dominance for high mortality and progression of heart failure and provide a satisfactory mouse model for laminopathy [39].

4.2. Mutations in sodium channel complex genes

SCN1B mutations have been reported in patients with cardiac conduction abnormalities associated with Brugada syndrome [40]. *SCN1B* encodes the auxiliary Na^+ channel subunit Nav β 1 that increases the current density of Nav1.5 [13].

A new gene responsible for cardiac conduction is *SCN10A* that encodes the neuronal Na^+ channel Nav1.8. Several genome-wide association studies (GWAS) have demonstrated that variation of *SCN10A* has a significant impact on resting heart rate, PR duration, and QRS intervals in the general population [41] despite the extremely low level of *SCN10A* expression in the heart. The precise mechanisms underlying *SCN10A* variation modulation of cardiac conduction properties and arrhythmia triggers, such as BrS and AF, are not fully elucidated. A possibility is that mediation could be directed by the activities of the autonomic nervous system, in which *SCN10A* is predominantly expressed [42–44].

4.3. *GJA5* (Cx40)

Additional electrical modulators for rapid electrical propagation in the CCS are gap junction channels formed by connexins (Cx) [45]. In the heart, three major Cx subtypes are expressed; namely Cx40, Cx43, and Cx45; that together form a hexameric Cx complex (connexon) at the cell membrane [45]. Gap junction channels are composed of two connexons between two adjacent cardiomyocytes and allow for rapid electrical conduction by passing signal molecules and ions. Of the three Cx subtypes, the high conductance Cx40 is exclusively expressed in the atrium and CCS [45]. A *GJA5* gene mutation, Q58L, has been reported to be associated with progressive familial conduction block and sudden cardiac death [46]. Heterologously expressed mutant Cx40 shows a profound reduction in gap junction conductance, as well as defective formation of membrane plaques. When the structural analysis of Cx26 is compared with Cx40, residue Q58 of Cx40 is expected to form symmetric hydrogen bonds to the same residue of the opposite monomer in parallel [47]. Therefore, Q58L-Cx40 in all likelihood has a structural abnormality that prevents assembly of two Cx40 hexamers.

4.4. *KCNJ2*

KCNJ2 is the gene responsible for encoding the inward rectifier potassium channel Kir2.1 and is the major regulator of excitability and resting membrane potential in most cardiomyocytes, with the exception of nodal cells [48]. To date, over 40 loss-of-function mutations in *KCNJ2* have been identified in approximately 70% of patients with Andersen–Tawil syndrome, a condition diagnosed using the clinical triad of periodic paralysis, dysmorphic features, and ventricular arrhythmia [49]. However, *KCNJ2* mutation carriers do not always present with the clinical triad [50] and conduction

abnormalities, such as first-degree AVB and BBB, have been documented in 23% of cases [51].

4.5. *TRPM4*

TRPM4 encodes the Ca²⁺-activated transient receptor potential cation channel subfamily M member 4 and is preferentially expressed in Purkinje fibers and the right ventricle [52]. The first responsible loci for progressive familial conduction block was found in 19q13 [53] and was identified as *TRPM4* [54]. Further genetic screening of various conduction disturbances has shown a high prevalence of *TRPM4* mutations in the right BBB (26%; 5 of 19 probands) and AVB (12%; 3 of 26 probands) [55]. Mutations in *TRPM4* have been further identified in cases of Brugada syndrome (4.4%; 11 in 248 probands) [56].

4.6. *KCNK17*

A mutation in the *KCNK17* gene encoding the pH-sensitive cardiac two-pore domain potassium channel (K2P) TASK-4 has been identified as a contributor to progressive and severe cardiac conduction disorder combined with idiopathic ventricular fibrillation by whole exome sequencing [57]. Mutant TASK-4 channels generated a three-fold increase in currents, while surface expression unchanged. Overexpression of the mutant TASK-4 leads to hyperpolarization and strong inhibition of the upstroke velocity in the spontaneously beating cardiomyocyte cell line HL-1. Strong expression of *KCNK17* has been observed in human Purkinje cells. These results support the likelihood that TASK-4 is functionally relevant for cardiac conduction disorders [57]. However, no specific TASK-4 blockers are available and mice do not functionally express the *KCNK17* gene; thus, little is known regarding the function and role of TASK-4 in the heart.

5. Genes involved in cardiac development and bradycardia

Development of the CCS is a complex biological process with the potential to be wrought with problems. Several transcription factors, including homeodomain proteins and T-box proteins, are essential for CCS morphogenesis and the activation or repression of key regulatory genes [58]. Of the cardiogenic transcription factor genes; *GATA4*, *NKX2-5*, *TBX3*, and *TBX5* play key roles in the development of the primary and second heart fields, while mutation results in congenital heart diseases such as patent foramen ovale, itself often associated with conduction disorders [59]. Holt–Oram syndrome is an inherited, multi-organ anomaly caused by *TBX5* mutation [60]. As *TBX5* promotes the expression of several genes involved in the development of the upper limbs, varying degrees of upper limb abnormalities have been recognized in Holt–Oram syndrome cases. Approximately 75% of probands have cardiac anomalies, whereas about 40% of affected family members present only with ECG abnormalities and without heart malformations [61]. Common ECG abnormalities include first degree AVB and bradycardia [61], which is in line with the preferential expression of *TBX5* in the endocardial cushion region during the developmental stage. The vast majority of *TBX5* mutations in Holt–Oram syndrome are truncation mutations that often delete the T-box domain and result in haplo-insufficiency of T-box activity. In contrast, most missense mutations result in less severe anomalies as the full protein structure is well preserved. A missense mutation, G125R, has been identified in a family suffering from faint digit abnormalities and a higher prevalence of AF without heart malformation [62]. AF is believed to be associated with the increased expression of *NPPA*, *GJA5*, *KCNJ2*, and *TBX3* [62].

6. Advanced genetic and genomic technologies

Many of the causative genes described here were identified using a candidate gene approach, in which genes are selected based on findings of preceding genetic linkage analysis or molecular pathway information [63]. Considering that the human genome encodes at least 20,000 protein-coding genes, the candidate gene approach focuses only on a small fraction of the genome with the remainder unanalyzed. Genome-wide association studies (GWAS) using single nucleotide polymorphisms (SNPs) can significantly expedite linkage analysis by narrowing the regions of interest for further directed sequencing. GWAS has been used in the cardiac electrophysiological field and has resulted in the identification of several new loci involved in long QT syndrome, a key role for calcium signaling pathways in myocardial repolarization [64], and many other ECG parameters [41,65].

GWAS on heart rate revealed the genetic heterogeneity of heart rate regulation and 21 loci were identified; including *HCN4*, gap junction gene *GJA1*, and the atrial α -myosin heavy chain (α -MHC) gene *MYH6* [41]. A rare *MYH6* variant, R721W, that predisposes individuals to SSS susceptibility has been previously identified [66]; however, the disease-causing *MYH6* mutations for familial SSS and their underlying mechanisms remain unknown. We screened nine genotype-negative probands with SSS families for mutations in *MYH6* and identified an in-frame 3-bp deletion that was predicted to delete one residue (delE933) at the highly conserved coiled-coil structure within the binding motif of myosin-binding protein C in one patient [66]. Irregular fluorescent speckles retained in the cytoplasm with substantially disrupted sarcomere striation have been observed in neonatal rat cardiomyocytes transfected with α -MHC mutants carrying delE933 or R721W. In addition to sarcomere impairments, delE933 α -MHC exhibited electrophysiological abnormalities both in vitro and in vivo. The atrial cardiomyocyte cell line HL-1 stably expressing delE933 α -MHC showed a significantly slower conduction velocity on multielectrode array when compared with those of wild-type α -MHC or control plasmid transfected cells. Furthermore, targeted morpholino knockdown of *MYH6* in zebrafish resulted in significantly reduced heart rate that could be rescued by co-expressed wild-type human α -MHC and not by delE933 α -MHC. These data reinforces the relevance of *MYH6* in sinus node function and suggests that structural damage to the sarcomere and functional impairment of atrial action potential propagation may underlie familial SSS with *MYH6* mutations [66].

7. Conclusions

It is now clear that a number of genes are involved in inherited bradyarrhythmia. Recent genetic studies have demonstrated that inherited arrhythmia is attributable to many genes with diverse functions. While the precise underlying mechanisms remain to be elucidated; these genetic defects may disrupt important cardiac functions including electrophysiological properties, development, cardioprotection, and the structural integrity of the membrane and sarcomere, ultimately leading to bradyarrhythmia. However, there are a large number of patients suffering from bradyarrhythmia whose etiologies remain unknown. As we have recently identified a novel *MYH6* mutation based on the most advanced genomic findings using GWAS to investigate SSS [66], new technologies such as next generation sequencing may provide the opportunity to identify new genes for inherited bradyarrhythmia as well as novel insights into the molecular mechanisms behind cardiac rhythm regulation.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

- [1] Karagueuzian HS. Targeting cardiac fibrosis: a new frontier in antiarrhythmic therapy? *Am J Cardiovasc Dis* 2011;1:101–9.
- [2] Gazoti Debessa CR, Mesiano Maifirino LB, Rodrigues de Souza R. Age related changes of the collagen network of the human heart. *Mech Ageing Dev* 2001;122:1049–58.
- [3] Boyett MR, Honjo H, Kodama I. The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc Res* 2000;47:658–87.
- [4] Chandler NJ, Greener ID, Tellez JO, et al. Molecular architecture of the human sinus node: insights into the function of the cardiac pacemaker. *Circulation* 2009;119:1562–75.
- [5] Butters TD, Aslanidi OV, Inada S, et al. Mechanistic links between Na⁺ channel (SCN5A) mutations and impaired cardiac pacemaking in sick sinus syndrome. *Circ Res* 2010;107:126–37.
- [6] Lakatta EG, Maltsev VA, Vinogradova TM. A coupled SYSTEM of intracellular Ca²⁺ clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ Res* 2010;106:659–73.
- [7] Milano A, Vermeer AM, Lodder EM, et al. HCN4 mutations in multiple families with bradycardia and left ventricular noncompaction cardiomyopathy. *J Am Coll Cardiol* 2014;64:745–56.
- [8] Schulze-Bahr E, Neu A, Friederich P, et al. Pacemaker channel dysfunction in a patient with sinus node disease. *J Clin Invest* 2003;111:1537–45.
- [9] DiFrancesco D. Funny channel gene mutations associated with arrhythmias. *J Physiol* 2013;591:4117–24.
- [10] Verkerk AO, Wilders R. Pacemaker activity of the human sinoatrial node: effects of HCN4 mutations on the hyperpolarization-activated current. *Europace* 2014;16:384–95.
- [11] Xu X, Marni F, Wu S, et al. Local and global interpretations of a disease-causing mutation near the ligand entry path in hyperpolarization-activated cAMP-gated channel. *Structure* 2012;20:2116–23.
- [12] Schweizer PA, Schroter J, Greiner S, et al. The symptom complex of familial sinus node dysfunction and myocardial noncompaction is associated with mutations in the HCN4 channel. *J Am Coll Cardiol* 2014;64:757–67.
- [13] Remme CA. Cardiac sodium channelopathy associated with SCN5A mutations: electrophysiological, molecular and genetic aspects. *J Physiol* 2013;591:4099–116.
- [14] Makita N, Behr E, Shimizu W, et al. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. *J Clin Invest* 2008;118:2219–29.
- [15] Kusano KF, Taniyama M, Nakamura K, et al. Atrial fibrillation in patients with Brugada syndrome relationships of gene mutation, electrophysiology, and clinical backgrounds. *J Am Coll Cardiol* 2008;51:1169–75.
- [16] Abe K, Machida T, Sumitomo N, et al. Sodium channelopathy underlying familial sick sinus syndrome with early onset and predominantly male characteristics. *Circ Arrhythm Electrophysiol* 2014;7:511–7.
- [17] Mohler PJ, Schott JJ, Gramolini AO, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003;421:634–639.
- [18] Mohler PJ, Splawski I, Napolitano C, et al. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. *Proc Natl Acad Sci USA* 2004;101:9137–9142.
- [19] Mohler PJ, Davis JQ, Davis LH, et al. Inositol 1,4,5-trisphosphate receptor localization and stability in neonatal cardiomyocytes requires interaction with ankyrin-B. *J Biol Chem* 2004;279:12980–7.
- [20] Mohler PJ, Davis JQ, Bennett V. Ankyrin-B coordinates the Na/K ATPase, Na/Ca exchanger, and InsP3 receptor in a cardiac T-tubule/SR microdomain. *PLoS Biol* 2005;3:e423.
- [21] Koschak A, Reimer D, Huber I, et al. alpha 1D (Cav1.3) subunits can form L-type Ca²⁺ channels activating at negative voltages. *J Biol Chem* 2001;276:22100–6.
- [22] Baig SM, Koschak A, Lieb A, et al. Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. *Nat Neurosci* 2011;14:77–84.
- [23] Christel CJ, Cardona N, Mesirca P, et al. Distinct localization and modulation of Cav1.2 and Cav1.3 L-type Ca²⁺ channels in mouse sinoatrial node. *J Physiol* 2012;590:6327–42.
- [24] Postma AV, Denjoy I, Hoorntje TM, et al. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. *Circ Res* 2002;91:e21–6.
- [25] Postma AV, Denjoy I, Kamblock J, et al. Catecholaminergic polymorphic ventricular tachycardia: RYR2 mutations, bradycardia, and follow up of the patients. *J Med Genet* 2005;42:863–70.
- [26] Glukhov AV, Kalyanasundaram A, Lou Q, et al. Calsequestrin 2 deletion causes sinoatrial node dysfunction and atrial arrhythmias associated with altered sarcoplasmic reticulum calcium cycling and degenerative fibrosis within the mouse atrial pacemaker complex. *Eur Heart J* 2015;36:686–97.
- [27] Venetucci L, Denegri M, Napolitano C, et al. Inherited calcium channelopathies in the pathophysiology of arrhythmias. *Nat Rev Cardiol* 2012;9:561–575.
- [28] Baskar S, Ackerman MJ, Clements D, et al. Compound heterozygous mutations in the SCN5A-encoded Nav1.5 cardiac sodium channel resulting in atrial standstill and His-Purkinje system disease. *J Pediatr* 2014;165:1050–2.
- [29] Chiang DY, Kim JJ, Valdes SO, et al. Loss-of-function SCN5A mutations associated with sinus node dysfunction, atrial arrhythmias, and poor pacemaker capture. *Circ Arrhythm Electrophysiol* 2015;8(5):1105–12. <http://dx.doi.org/10.1161/CIRCEP.115.003098>.
- [30] Hodgson-Zingman DM, Karst ML, Zingman LV, et al. Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. *N Engl J Med* 2008;359:158–165.
- [31] Disertori M, Quintarelli S, Grasso M, et al. Autosomal recessive atrial dilated cardiomyopathy with standstill evolution associated with mutation of natriuretic peptide precursor A. *Circ Cardiovasc Genet* 2013;6:27–36.
- [32] Crozier I, Richards AM, Foy SG, et al. Electrophysiological effects of atrial natriuretic peptide on the cardiac conduction system in man. *Pacing Clin Electrophysiol* 1993;16:738–42.
- [33] Kecskemeti V, Pacher P, Pankucsi C, et al. Comparative study of cardiac electrophysiological effects of atrial natriuretic peptide. *Mol Cell Biochem* 1996;160–161:53–9.
- [34] Capell BC, Collins FS. Human laminopathies: nuclei gone genetically awry. *Nat Rev Genet* 2006;7:940–52.
- [35] Fatkin D, MacRae C, Sasaki T, et al. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N Engl J Med* 1999;341:1715–24.
- [36] Becane HM, Bonne G, Varnous S, et al. High incidence of sudden death with conduction system and myocardial disease due to lamins A and C gene mutation. *Pacing Clin Electrophysiol* 2000;23:1661–6.
- [37] van Rijsingen IA, Nannenberg EA, Arbustini E, et al. Gender-specific differences in major cardiac events and mortality in lamin A/C mutation carriers. *Eur J Heart Fail* 2013;15:376–84.
- [38] Arimura T, Onoue K, Takahashi-Tanaka Y, et al. Nuclear accumulation of androgen receptor in gender difference of dilated cardiomyopathy due to lamin A/C mutations. *Cardiovasc Res* 2013;99:382–94.
- [39] Arimura T, Helbling-Leclerc A, Massart C, et al. Mouse model carrying H222P-Lmna mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies. *Hum Mol Genet* 2005;14:155–69.
- [40] Watanabe H, Koopmann TT, Le Scouarnec S, et al. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest* 2008;118:2260–8.
- [41] Holm H, Gudbjartsson DF, Arnar DO, et al. Several common variants modulate heart rate, PR interval and QRS duration. *Nat Genet* 2010;42:117–22.
- [42] Verkerk AO, Remme CA, Schumacher CA, et al. Functional Nav1.8 channels in intracardiac neurons: the link between SCN10A and cardiac electrophysiology. *Circ Res* 2012;111:333–43.
- [43] Hu D, Barajas-Martinez H, Pfeiffer R, et al. Mutations in SCN10A are responsible for a large fraction of cases of Brugada syndrome. *J Am Coll Cardiol* 2014;64:66–79.
- [44] Le Scouarnec S, Karakachoff M, Gourraud JB, et al. Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome. *Hum Mol Genet* 2015;24:2757–2763.
- [45] Virginijus V, Peter Brink R. Biophysical properties of gap junctions. In: Douglas P, Zipes Jose J, editors. *Cardiac electrophysiology*. 6th ed. Elsevier; 2014. p. 151–60.
- [46] Makita N, Seki A, Sumitomo N, et al. A connexin40 mutation associated with a malignant variant of progressive familial heart block type I. *Circ Arrhythm Electrophysiol* 2012;5:163–72.
- [47] Maeda S, Nakagawa S, Suga M, et al. Structure of the connexin 26 gap junction channel at 3.5 Å resolution. *Nature* 2009;458:597–602.
- [48] Jeanne Nerbonne M. Voltage-regulated potassium channels. In: Douglas P, Zipes Jose J, editors. *Cardiac electrophysiology*. 6th ed. Elsevier; 2014. p. 23–32.
- [49] Nguyen HL, Pieper GH, Wilders R. Andersen-Tawil syndrome: clinical and molecular aspects. *Int J Cardiol* 2013;170:1–16.
- [50] Kimura H, Zhou J, Kawamura M, et al. Phenotype variability in patients carrying KCNJ2 mutations. *Circ Cardiovasc Genet* 2012;5:344–53.

- [51] Zhang L, Benson DW, Tristani-Firouzi M, et al. Electrocardiographic features in Andersen–Tawil syndrome patients with KCNJ2 mutations: characteristic T–U-wave patterns predict the KCNJ2 genotype. *Circulation* 2005;111:2720–6.
- [52] Liu H, El Zein L, Kruse M, et al. Gain-of-function mutations in TRPM4 cause autosomal dominant isolated cardiac conduction disease. *Circ Cardiovasc Genet* 2010;3:374–85.
- [53] Brink PA, Ferreira A, Moolman JC, et al. Gene for progressive familial heart block type I maps to chromosome 19q13. *Circulation* 1995;91:1633–40.
- [54] Kruse M, Schulze–Bahr E, Corfield V, et al. Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. *J Clin Invest* 2009;119:2737–44.
- [55] Stallmeyer B, Zumhagen S, Denjoy I, et al. Mutational spectrum in the Ca(2+)–activated cation channel gene TRPM4 in patients with cardiac conductance disturbances. *Hum Mutat* 2012;33:109–17.
- [56] Liu H, Chatel S, Simard C, et al. Molecular genetics and functional anomalies in a series of 248 Brugada cases with 11 mutations in the TRPM4 channel. *PLoS One* 2013;8:e54131.
- [57] Friedrich C, Rinne S, Zumhagen S, et al. Gain-of-function mutation in TASK-4 channels and severe cardiac conduction disorder. *EMBO Mol Med* 2014;6:937–51.
- [58] Hatcher CJ, Basson CT. Specification of the cardiac conduction system by transcription factors. *Circ Res* 2009;105:620–30.
- [59] Holt M, Oram S. Familial heart disease with skeletal malformations. *Br Heart J* 1960;22:236–42.
- [60] Basson CT, Bachinsky DR, Lin RC, et al. Mutations in human TBX5 [corrected] cause limb and cardiac malformation in Holt–Oram syndrome. *Nat Genet* 1997;15:30–5.
- [61] Newbury–Ecob RA, Leanage R, Raeburn JA, et al. Holt–Oram syndrome: a clinical genetic study. *J Med Genet* 1996;33:300–7.
- [62] Postma AV, van de Meerakker JB, Mathijssen IB, et al. A gain-of-function TBX5 mutation is associated with atypical Holt–Oram syndrome and paroxysmal atrial fibrillation. *Circ Res* 2008;102:1433–42.
- [63] Tabor HK, Risch NJ, Myers RM. Candidate–gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 2002;3:391–7.
- [64] Arking DE, Pulit SL, Crotti L, et al. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. *Nat Genet* 2014;46:826–36.
- [65] Sotoodehnia N, Isaacs A, de Bakker PI, et al. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet* 2010;42:1068–76.
- [66] Holm H, Gudbjartsson DF, Sulem P, et al. A rare variant in MYH6 is associated with high risk of sick sinus syndrome. *Nat Genet* 2011;43:316–20.