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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.

Contents

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1. Introduction

Bradyarrhythmia is a serious electrical disorder of the heart with the potential to be life threating. 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].

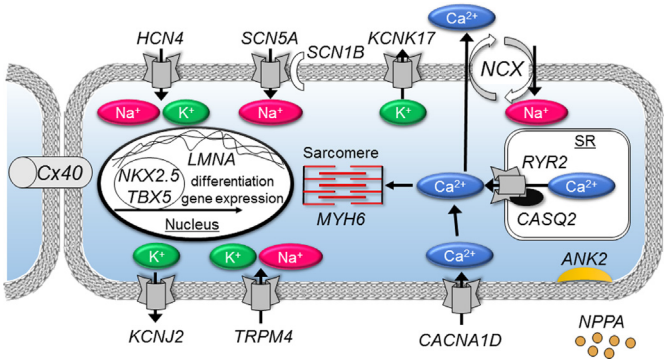

Fig. 1. Molecular modules involved in inherited bradyarrhythmia. Abnormalities in multiple pathways involving membrane ion channels, SR ion channels, sarcomere components, cardiac hormones, and membrane anchor proteins are associated with inherited bradyarrhythmia.

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 Navβ1 and Navβ3 [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

Table 1
Genes responsible for inherited bradyarrhythmia.

Gene name	Protein name	Inheritance mode	Atrial phenotypes	Conduction diseases	Ventricle phenotypes	Additional phenotypes	Function
Ion channels							
HCN4	HCN4	AD	Sinus bradycardia		LVNC, BrS		Loss
SCN5A	Nav1.5	AD, AR	Sinoatrial block, AF, Atrial standstill	PCCD, AVB	LQT3, BrS, DCM		Loss
SCN10A	Nav1.8	AD?	AF?	?	BrS?	Association with conduction parameters in ECG, episodic pain syndrome	?
SCN1B	Navβ1	AD		BBB	BrS	Epilepsy	Loss
KCNJ2	Kir2.1	AD			LQT7(ATS), SQT, BrS	Periodic paralysis, dysmorphic features	
CACNA1D	Cav1.3	AD	Sinus bradycardia			Congenital deafness	Loss
KCNK17	TASK-4	AD		PCCD, AVB, BBB	IVF?		Gain
TRPM4	TRPM4	AD		PCCD, AVB, BBB	BrS		Gain
Ca^{2+} handling proteins on the sarcoplasmic reticulum							
RYR2	Ryanodine receptor 2	AD	Sinus bradycardia		CPVT, ARVC		Loss
CASQ2	Calsequestrin	AR	Sinus bradycardia		CPVT		Loss
Gap junction channel							
GJA5	Connexin40	AD		PCCD, AVB, BBB			Loss
Cardiac hormone							
NPPA	ANP	AD	Atrial standstill, Biatrial dilatation				Loss
Transcription factors							
TBX5	Tbx5	AD	ASD, AF	AVB	VSD	Hand anomalies (heart-hand syndrome)	Loss/gain
Nuclear membrane component							
LMNA	Lamin A/C	AD		PCCD, AVB	DCM	Laminopathies including muscular dystrophy and Hutchinson–Gilford progeria syndrome	Loss
Membrane adaptor protein							
ANK2	Ankyrin-B	AD	Sinus bradycardia	PCCD	LQT4		Loss
Sarcomere protein							
MYH6	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 noncompaction; 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 Ca^{2+}-handling abnormality [18]. Immunohistochemical analysis of cardiomyocytes from these mice showed mislocalization of the Na^+/Ca^{2+} 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^+/Ca^{2+} exchanger and L-type Ca^{2+} 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 Ca^{2+}-handling.

The Cav voltage-gated Ca^{2+} channels, Cav1.2 and Cav1.3, mediate L-type Ca^{2+} 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 calsequestrin *CASQ2*, 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 knockout 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 predominance 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 Navβ1 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 Ca^{2+}-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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