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Review

Recent understanding of clinical sequencing and gene-based risk stratification in inherited primary arrhythmia syndrome

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ABSTRACT

Inherited primary arrhythmia syndromes (IPAS) may result in ventricular tachycardia or ventricular fibrillation by some genetic disorders, leading to sudden cardiac death. IPAS are also called "channelopathies" since many of these are caused by an abnormality in myocardial ion channels. Congenital long-QT syndrome (LQTS) is the most well documented IPAS, which may be seen in 0.1% of the general population. More than 15 disease-causing genes have been identified in almost 70% of LQTS patients and genetic testing is well applied to not only clinical diagnosis but also risk stratification and gene-based therapeutic strategy for each person with LQTS. Thus, in LQTS, gene-based personalized medicine can be realized.

Unlike the LQTS, genetic testing for the Brugada syndrome (BrS) is still controversial since only 20% of patients can be identified with the causing gene mutations, most of which are in *SCN5A*. Furthermore, even in the *SCN5A* mutation-positive carriers, their phenotypes are not completely consistent with BrS, but may cause other IPAS including LQTS, cardiac conduction defect, sick sinus syndrome, and dilated cardiomyopathy. On the other hand, a recent Japanese BrS registry demonstrated that the pore-region mutations in *SCN5A* are significantly associated with a risk of lethal cardiac events. Furthermore, a genome-wide association study revealed that a common variant in *SCN10A* or *HEY2* in addition to *SCN5A* is associated with BrS, thus, BrS may not be a monogenic Mendelian disease but probably an oligogenic disease.

The purpose of this review is to describe the basic genetic and pathophysiological findings of the IPAS, particularly LQTS and Brugada syndrome, and to outline a rational approach to genetic testing, management, and family screening.

Contents

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Introduction

Inherited primary arrhythmia syndromes (IPAS) may result in ventricular tachycardia (VT) or ventricular fibrillation (VF) by some genetic disorders, leading to sudden cardiac death (SCD). IPAS are also called "channelopathies" since many of these are caused by an abnormality in myocardial ion channels. Congenital long-QT syndrome (LQTS) is the most well observed IPAS, which may be seen in 0.1% of the general population. More than 15 disease-causing genes have been identified in almost 70% of LQTS patients and most of them are *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3) genes (Fig. 1). Therefore, genetic testing is well applied to LQTS not only for a clinical diagnosis but also gene-based risk stratification and therapeutic strategies for LQTS [1].

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is rare but a highly penetrant autosomal dominant disease with SCD in young people, in which the cardiac ryanodine receptor (*RYR2*) gene is identified in more than 60% of CPVT. On the other hand, genetic test for the Brugada syndrome (BrS) is still controversial since only 20% of responsible genes could be identified, most of which are *SCN5A* [2]. Thus, recent understanding for genotype-phenotype correlation in these IPAS lead to the gene-basis risk stratification and personalized therapeutic strategies. However, not all responsible genes could be identified in IPAS even if whole exon sequencing can be performed. Furthermore, a comprehensive genetic analysis cannot always identify the responsible genes, but rather may increase knowledge of variants of unknown significance (VUS) [3]. This review focuses on the clinical significance of genetic analysis in the LQTS, CPVT, and BrS.

Long-QT syndrome

Clinical diagnosis of LQTS

LQTS is characterized by the QT (QTc) interval prolongation of electrocardiogram (ECG) and syncope caused by a polymorphic ventricular tachycardia, named torsades de pointes (TdP), leading to SCD. LQTS is usually diagnosed by clinical findings such as the QT interval prolongation, T-wave morphology, symptoms, and family history of SCD. Schwartz et al. suggested a diagnosis and risk stratification of LQTS, in which total score $\geq$3.5 is definitely diagnosed as LQTS but 1.5–3.0 is suspected [4]. Therefore, for example, if someone whose QTc is more than 450 ms with abnormal bradycardia or history of SCD under 30 years old, they could be suspected as having the LQTS.

However, the HRS/EHRA/APHRS expert consensus statement (2013) [1] recommends that LQTS is diagnosed by (1) LQTS risk score $\geq$3.5, and/or (2) presence of an equivocally pathogenic mutation in one of the LQTS genes, or (3) QTc $\geq$500 ms in repeated ECG and in the absence of secondary cause for QT prolongation (Table 1). Thus, LQTS is now diagnosed by not only clinical findings but also genetic information.

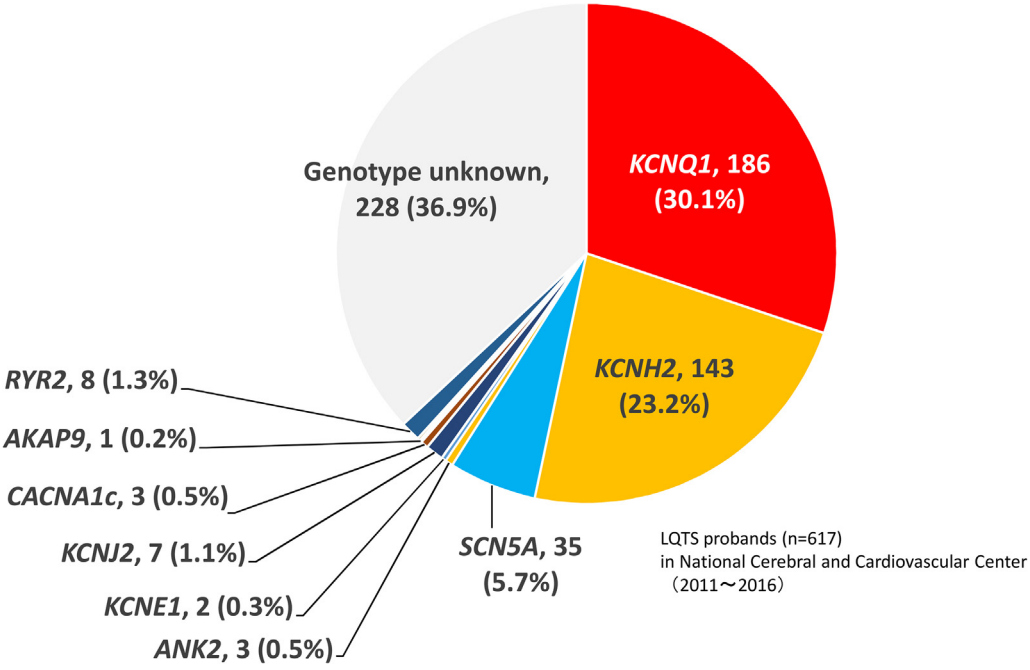

Fig. 1. Identification of long-QT syndrome (LQTS)-related genes from 617 probands with congenital LQTS. More than 90% of the genotype-positive subjects have the top three LQTS genes such as *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3). Comprehensive analysis by the next generation sequencing could identify a small number of rare LQTS genes as well as *RYR2* gene, which phenotype may be overlapped with catecholaminergic polymorphic ventricular tachycardia.
Source: Ref. [9].

Table 1
The Heart Rhythm Society(HRS), the European Heart Rhythm Association (EHRA) and the Asia Pacific Heart Rhythm Society (APHRS) Expert Consensus Recommendations on LQTS Diagnosis.

1. LQTS is diagnosed:
a. In the presence of a LQTS risk score ≥3.5 in the absence of a secondary cause for QT prolongation and/or
b. In the presence of an unequivocally pathogenic mutation in one of the LQTS genes or
c. In the presence of a QT interval corrected for heart rate using Bazett's formula (QTc) ≥500 ms in repeated 12-lead electrocardiogram (ECG) and in the absence of a secondary cause for QT prolongation.
2. LQTS can be diagnosed in the presence of a QTc between 480 and 499 ms in repeated 12-lead ECGs in a patient with unexplained syncope in the absence of a secondary cause for QT prolongation and in the absence of a pathogenic mutation.
Source: Priori et ?al. [1].

Major LQTS genotypes

Congenital LQTS had first been classified into two types with its familial preponderance, the Romano-Ward syndrome accompanied with the autosomal dominant inheritance and the Jurvell and Lange-Nielsen syndrome with deafness and autosomal recessive inheritance, however, many of them are the former. In the 1990s the linkage analysis of LQTS families classified LQTS into subtypes based on the genetic loci. In 1995, subsequently, *KCNH2* and *SCN5A* were identified as the responsible genes for LQT2 and LQT3, respectively [5,6]. In 1996, *KCNQ1* was identified as that for LQT1 [7]. To date, a total of 15 genes have been reported as the disease-causing genes, however, most of the identified genes in patients with LQTS are *KCNQ1* (30–35%), *KCNH2* (25–30%), and *SCN5A* (5–10%); in contrast, identification of the other genes (LQT4–15) is still rare [8] (Table 2) (Fig. 1).

Table 2
Culprit gene and its functions in inherited primary arrhythmic syndrome.

Gene	Ion channel	LQTS	CPVT	SQTS	BrS	PCCD	ERS	SSS
*KCNQ1**	I_{Ks}	●		○				
*KCNH2**	I_{Kr}	●		○				
KCNE1	I_{Ks}	●						
KCNE2	I_{Kr}	●						
KCNE3	I_{to}				○			
KCND3	I_{to}				○			
KCNJ2	I_{K1}	●	●	○				
KCNJ5	$I_{K\text{-}ACh}$	●						
KCNJ8	$I_{K\text{-}ATP}$				○		○	
ABCC9	$I_{K\text{-}ATP}$				○			
AKAP-9	I_{Ks}	●						
ANK2	Ankyrin-B	●	(●)					●
HCN4	I_f							●
*SCN5A**	I_{Na}	○			●	●	●	●
SCN1B	I_{Na}				●	●		
SCN3B	I_{Na}				●			
SCN4B	I_{Na}	○						
CAV3	I_{Na}	○						
CACNA1C	$I_{Ca\text{-}L}$	○		●	●		●	
CACNB2	$I_{Ca\text{-}L}$			●	●		●	
CACNA2D1	$I_{Ca\text{-}L}$						●	
RYR2	RyR		○					●
CASQ2	RyR		○					●
CALM1	Calmodulin	○	○					
CALM2	Calmodulin	○						
TRDN	Triadin		○					
TRPM4	Non-selective cation					(●)		
GJA5	Connexin-40					●		

●: loss of function.
○: gain of function.
*: major LQTS genes. LQTS:long-QT syndrome, CPVT: Catecholaminergic Polymorphic Ventricular Tachycardia, SQTS: Short-QT syndrome, BrS: Brugada syndrome, PCCD: Progressive Cardiac Conduction Defect, ERS: Early Repolarization Syndrome.

The *KCNQ1* (LQT1) gene encodes the alpha-subunit of the voltage-gated potassium channel, which is responsible for the slow component of the delayed rectifier current (I_{Ks}). Pathogenic variants in *KCNQ1* impair the function of I_{Ks} (loss-of-function), resulting in prolongation of action potential duration (APD) of myocardium and increase the QT interval of ECG. While, the *KCNH2* (LQT2) gene encodes the rapidly activating component of the delayed rectifying potassium current (I_{Kr}) and loss-of-function variants of *KCNH2* reduce I_{Kr} and prolong APD and QT interval. On the other hand, *SCN5A* encodes alpha-subunit of the voltage-gated sodium channel (Nav1.5), and the gain-of-function mutations of *SCN5A* increase late inward Na^+ current (late-I_{Na}) resulting in prolongation of APD and QT interval.

Genotype-phenotype correlations in LQT1–3

In patients with LQT1–3, not only the genetic diagnosis but also the genotype-specific difference of prognosis, risk of TdP, and therapeutic strategies could be demonstrated (Fig. 2). In general, the QT (QTc) interval is longer in females than in males after puberty. Therefore, patients with the QTc interval ≥500 ms are the highest risk in spite of genotypes. In addition, even if the QTc is <500 ms, male LQT1 <13 years old, or female LQT1 ≥13 years old, and female LQT2 at any age have a considerable risk for events [10,11]. On the other hand, there is no evidence of the risk of lethal arrhythmic events (LAEs) in patients with LQT4–15 or those without identifying the genotype.

The mutation site-specific differences in risk of LAEs were also reported in LQT1 and LQT2 [12,13]. In those studies, patients with a mutation in the transmembrane domain in *KCNQ1* gene, or in the pore-region (S5-loop-S6) in *KCNH2* gene are known to be at higher risk of LAE than those with C-terminus or other mutations, respectively. Those international LQT registries also demonstrated β-blocker therapy reduced the risk of LAEs in 74% (LQT1) or 63% (LQT2). On the other hand, another international registry for LQT3 showed that the prolonged QTc and syncope may predispose patients to LAEs. However, in LQT3, β-blocker therapy may reduce this risk in females but not in males, although it was not determined conclusively because of the smaller number of events [14].

The minor allele frequency (MAF) of variants is different among the races. For example, the *SCN5A*-R1193Q (rs41261344) was firstly reported as a pathogenic variant in the BrS but now it has been considered as benign or likely benign because it is rare in Caucasians or Africans but relatively common in East Asians,

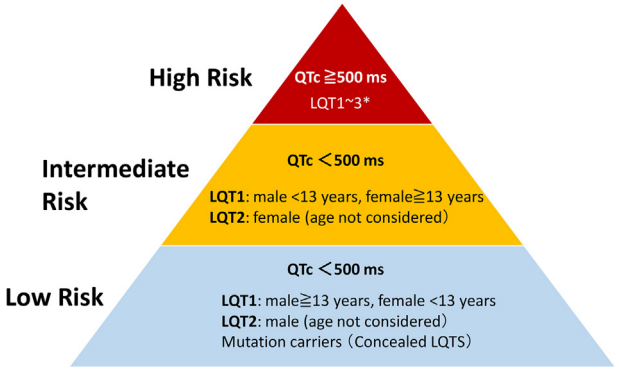

Fig. 2. Proposed scheme for risk stratification among patients with long-QT syndrome according to genotype, age, and sex. *LQT1 in the transmembrane or C-loop mutations, and LQT2 and 3 in the S5-pore-S6 region missense mutations are extremely high risk.
Source: Aonuma [11].

including the Han Chinese and Japanese [15]. Therefore, pathogenicity of variants needs to be checked.

A recent multi-center LQTS registry demonstrated genotype-phenotype correlations in 1124 genotype-positive Japanese LQTS (616 probands, 508 family members) including LQT1 (n = 521), LQT2 (n = 487), and LQT3 (n = 116) [16]. Kaplan–Meier cumulative analysis of the first events revealed that syncope or LAEs occurred at between 5 and 15 years of age in LQT1, whereas, they occurred after the teen years and gradually increased in frequency with age in LQT2 (Fig. 3A). After 25 years of age, the cumulative probability of syncope or LAEs was higher in LQT2 than in LQT1. Those events were significantly less common in LQT3 than in LQT1 or 2, yet they could occur in LQT3 patients at any age, even in infancy. On the other hand, rates and timing of LAEs were similar between the genotypes (Fig. 3B). Thus, the ratio of LAEs was higher in LQT3 and LQT2 than in LQT1. These findings were similar to those of the international LQTS registry previously reported [17]. Furthermore, a recent European LQTS registry also demonstrated higher risk of LAEs in LQT2 or LQT3 compared to LQT1 [18].

Female gender at risk for LQT2

Pathogenic variants in the pore-regions of the channels in each genotype were associated with higher arrhythmic risk than others, while gender-associated differences were observed in LQT1 and LQT2 but not in LQT3 [16]. Particularly in LQT2, the Japanese LQT registry also showed that female gender itself was significantly associated with a higher arrhythmic risk regardless of the QTc interval and mutation site. In another study, the risk of cardiac events in LQT2 carriers with normal QTc was associated with abnormal T-wave morphology in *women* but only pore location of mutation in *men* [19].

Furthermore, patients who are younger and female are at risk for TdP in LQTS, thus pregnancy and postpartum periods are one of the biggest concerns for women with LQTS, and β-blocker therapy continuation is recommended during pregnancy especially for high-risk patients with LQTS to decrease the risk of LAEs. However, pregnant women with LQTS taking β-blockers sometimes worry about side effects for babies. A previous study showed that many of the LQTS patients who had cardiac events during pregnancy or postpartum were LQT2 with no medication. Thus, early diagnosis and β-blocker therapy for high-risk patients with LQTS are effective for the prevention of cardiac events during pregnancy and postpartum period. Moreover, maternal β-blocker therapy seems to be well tolerated by the fetus. Thus, continuation of β-blocker is recommended for pregnant women with LQTS [20].

Minor LQTS genotypes

LQT4 is caused by a mutation/rare variant within *ANK2*, the protein product of which is ankyrin-B responsible for the coordinated assembly of the sodium/calcium exchanger, the sodium/potassium ATPase, and inositol triphosphate receptor [21]. While patients with LQT4 are rare, the *ANK2* have not only LQT but also other arrhythmias such as SSS or CPVT (Table 2) [22], thus arrhythmic syndrome caused by *ANK2* variants is sometimes called 'Ankyrin syndrome'. In Japan, we screened 535 probands with IPAS and analyzed 46 genes including *ANK2* using next-generation sequencing (NGS). As a result, 12 of 535 probands (2.2%, aged 0–61 years, 5 males) were found to carry 7 different heterozygous *ANK2* variants [23].

LQT5 or 6 are caused by a mutation/rare variant in *KCNE1* or *KCNE2*, which is a β-subunit of I_{Ks} or I_{Kr}. Both are also rare and the phenotypes are similar to LQT1 or LQT2. *KCNE1*-D85N, which has been shown as loss-of-function in I_{Ks} [24], is now determined as a polymorphism. However, a recent study revealed that *KCNE1*-D85N was more prevalent in subjects with LQTS compared to the Genome Aggregation Database [33/1248 (2.6%) vs 1552/126,652 (1.2%); p = 0.0001] [25], suggesting this common variant as a 'modifier' of LQTS. Similar to *KCNE1*, many *KCNE2* variants have also been erroneously designated as LQTS-causative mutations [26]. Instead, *KCNE2* variants may confer proarrhythmic susceptibility when provoked by additional environmental/acquired or genetic factors, or both [27].

LQT7 is caused by mutations/rare variants in *KCNJ2* (Kir2.1), which is relatively common in the minor LQTS (Fig. 1) but also results in neurologic musculoskeletal disorders, such as periodic paralyzes, and dysmorphic features, thus named the Andersen-Tawil syndrome (ATS) [28]. Some patients with ATS show longer QT (or rather QT-U) interval, and often have a variety of VAs such as

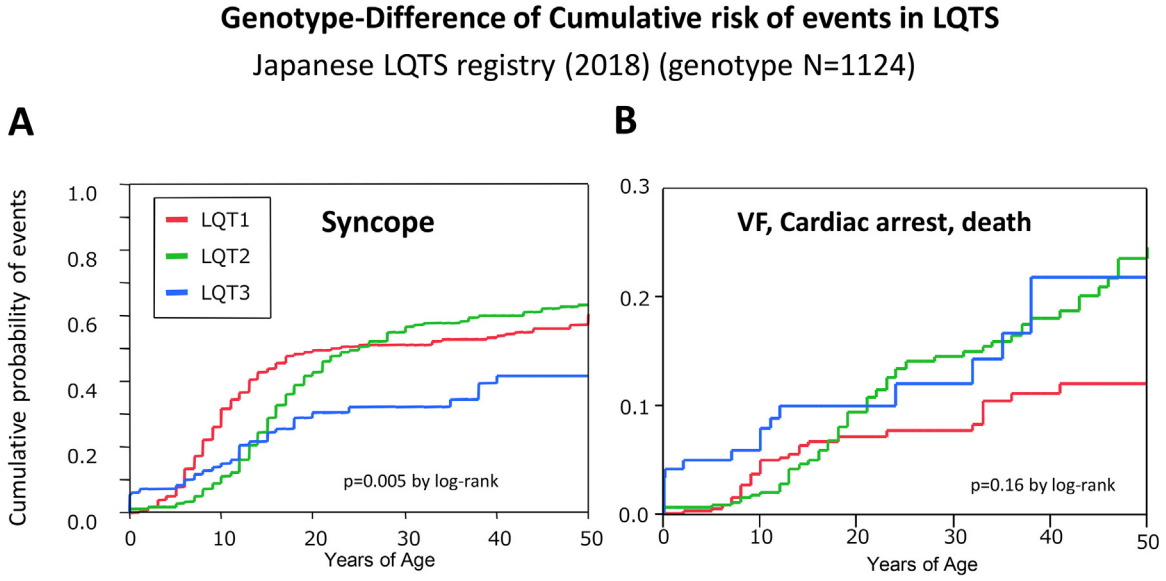

Fig. 3. Kaplan–Meier analysis for the cumulative probability of events (A: syncope; B: VF, cardiac arrest, and death) in LQT1, 2, and 3 from the Japanese LQTS registry. LQTS, long-QT syndrome; VF, ventricular fibrillation.
Source: Shimizu et al. [16].

premature ventricular complex, polymorphic ventricular tachycardia (VT), and bi-directional VT, thus ATS needs to be differentially diagnosed with CPVT [29].

LQT8 is caused by a pathogenic variant in *CACNA1C*, alpha-subunit of L-type Ca^{2+} channel ($I_{Ca,L}$). A gain-of-function variant enhances delay of inactivation of $I_{Ca,L}$ and increased late-$I_{Ca,L}$, resulting in prolongation of the QT interval. LQT8 is also named Timothy syndrome (TS), which is characterized by dysmorphic features, syndactyly, congenital heart disease, immune deficiency, and developmental delay along with QT prolongation and an increased risk of sudden cardiac death [30]. However, TS results from specific mutations, p.G402S and p.G406R, in *CACNA1C* exon 8. After using the NGS-based sequencing, several *CACNA1C* mutations without typical TS have been identified in LQTS patients [31,32].

LQT9 caused by a mutation in *CAV3* [33], coding for caveolin-3, the major constituent scaffolding protein of cardiac caveolae, LQT10 caused by *SCN4B* [34], encoding a sodium channel β-subunit 4, and LQT12 caused by alpha-1 syntrophin (*SNTA1*) have been associated with increased late-I_{Na} thus showing a similar phenotype with LQT3 as well as skeletal muscle disease, cardiomyopathy, and sudden infant death syndrome. However, those are rare and the role of Cav3 in LQTS has been disputed [35].

LQT11 is caused by a mutation within *AKAP9*, which encodes an A kinase-anchoring protein, also known as Yotiao, responsible for facilitating phosphorylation of *KCNQ1* by protein kinase A, impairs I_{Ks} augmentation, leading to a clinical phenotype similar to that for LQT1 [36]. LQT13 is caused by a mutation in *KCNJ5*, resulting in reducing the inwardly rectifying potassium channel protein.

In 617 Japanese LQTS probands who had been introduced for genetic testing to the National Cerebral and Cardiovascular Center between 2011 and 2016, almost 60% of patients were identified as LQT1–3, and in the remaining 253 subjects, comprehensive genetic testing could identify only 16 (6%) minor candidates such as *ANK2*, *KCNE1*, *KCNJ2*, *CACNA1c*, *AKAP9* (Fig. 1) but no mutation in *CAV3*, *SCN4B*, *SNTA1*, and *KCNJ5* could be identified. Thus, there are some minor genotypes identified in LQTS but these genes are not a major part of the responsible genes in the LQTS.

Multiple LQTS-associated mutations

The LQTS are not only caused by a single mutation but 5–10% of these have compound heterozygous mutations in the LQTS-related genes, which are associated with a more severe phenotype [8]. In the Japanese LQTS registry, 8.4% of genotyped LQTS had two variants in LQTS-genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, or *KCNJ2*) [37]. Interestingly, single mutation carriers of their families remained asymptomatic or 'latent' LQTS. Therefore, we need to consider patients as having 'double-hit' mutations if it looks a much severer phenotype for a single mutation in the LQTS-gene.

Overlap between LQTS and CPVT

With the use of whole exome sequencing, specific mutations in *CALM1* (LQT14) [38] and *CALM2* (LQT15) [39] have been identified. Calmodulin (CaM) is a primary sensor of intracellular calcium levels in eukaryotic cells, playing a key role in the proper mediation of Ca^{2+} signaling, and interacts with several known LQTS genes (*SCN5A*, *SNTA1*, and *CACNA1C*), suggesting that these candidate genes also play a pathogenic role in LQTS. In particular, *RYR2* has previously been reported as a gene associated with several arrhythmic diseases, including LQTS, CPVT, arrhythmogenic right ventricular dysplasia, and sudden infant death syndrome.

Among patients with *RYR2* mutations, not only CPVT but also gene-negative LQTS was a major part of the phenotype [40]. Whole exon sequencing in the previous study also demonstrated that *RyR2* pathogenic variants were identified in 9 of 186 probands clinically diagnosed with LQTS, giving a possibility of the importance of *RYR2* in LQTS pathogenesis [41]. Thus, some of the patients clinically diagnosed or suspected as having LQTS (probably as LQT1) were able to be genetically found a *RyR2* mutation, and diagnosed as CPVT, or an overlap between LQT and CPVT. As much as LQTS, CPVT patients develop syncope during exercise or emotional stress due to polymorphic VT, and sometimes leading to SCD, thus undiagnosed patients have more poor prognosis in CPVT than in LQTS.

Furthermore, a genotype-specific therapeutic strategy, not only β-blockers but flecainide selectively affects Ca^{2+} release from ryanodine receptor, resulting in suppression of the bi-directional polymorphic VT in CPVT. A similar bi-directional VT occurs in LQT7 (ATS), that needs to be differentially diagnosed with CPVT, is also suppressed by flecainide therapy [29]. Therefore, genetic screening for *RYR2* gene in genotype-unknown LQTS is clinically relevant.

Genetic background for acquired LQTS

LQTS can be manifested by bradycardia, hypokalemia, and several drugs, which is called acquired (secondary) LQTS. However, in recent studies, nearly 30% of patients diagnosed with acquired LQTS are believed to have genetic abnormalities, and their congenital and acquired boundaries are becoming ambiguous [41]. From this study, clinical findings based on control QTc >440 ms, age <40 years old, and symptoms allowed identification of patients more likely to carry some LQTS mutations. These findings suggest that secondary LQTS is more likely to be called 'aymptomatic' or 'concealed' LQTS rather than 'acquired'.

In whom the genetic test should be performed in LQTS?

HRS/EHRA/APHRS expert consensus report (2013) [1] and Japanese Circulation Society guideline (2017) [11] recommended the genetic test to those whom doctors strongly suspect to have LQTS or to someone with QTc interval ≥500 ms (adult) or ≥480 ms (children) even though they were asymptomatic. For family members who had also been suspected as having LQTS, genetic testing to check the same pathogenic variant is also recommended after informed consent was given.

Most of the pathogenic variants are in the major LQTS genes such as *KCNQ1*, *KCNH2*, and *SCN5A*, thus standard genetic screening for LQTS is usually performed within the LQT1–3 genes. However, when patients with definite LQTS of QTc interval ≥500 ms, history of LAEs, or familial LQTS but none of the mutations in the three genes, more comprehensive screening for other IPAS genes should be performed.

Brugada syndrome

Clinical diagnosis of BrS

BrS is characterized by ECG findings of the J point and ST segment elevation and a history of cardiac arrest due to VF, which was first described by Pedro and Josep Brugada in 1992 [43]. BrS shows a marked male predominance and significant population variance according to ethnicity, thus the prevalence is much higher in East Asians including Japanese (0.7–1.0%) than in Caucasians (0.012–0.26%) [44].

Diagnosis of BrS is only based on the clinical history and ECG findings, which consists of a 0.2 mV ST segment elevation followed by a negative T wave in more than one right precordial lead of ECG, called the coved-type or type 1 Brugada ECG. A type 1 ECG pattern may be manifested by provocation testing with sodium channel-blocking agents (flecainide, pilsicainide, ajmaline, etc.) or during

febrile illness. Other ECG findings, specifically the type 2 or 3 Brugada ECG, may suggest a Brugada pattern, however, either a symptomatic or asymptomatic subject, now only type 1 ECG is diagnosed as the BrS [1].

Genetics of BrS

In 1998, mutations of the *SCN5A* gene were first identified in a small number of families and individuals with idiopathic VF and an ECG characteristic for the BrS [45]. To date more than 300 mutations or rare variants were reported in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) as pathogenic or likely pathogenic changes for the BrS as well as its related IPAS (PCCD, ERS, and SSS). Not only missense or non-missense variants, copy number variations of *SCN5A* are also found [46] only in a few percentage of the BrS. Furthermore, variants in the core promoter region and the transcription regulatory region of *SCN5A* were also identified in multiple arrhythmia phenotypes (atrial fibrillation, SSS, CCD, BrS, idiopathic VF), consistent with the idea that altered *SCN5A* transcription levels modulate susceptibility to arrhythmias [47].

Not only *SCN5A*, several mutations or variants such as *GPD1L* (BrS2), *CACNA1C* (BrS3), *CACNB2* (BrS4), *SCN1B* (BrS5), *KCNE3* (BrS6), and *SCN3B* (BrS7) have been identified as a pathogenic candidate or causing-gene for the BrS previously. There is limited evidence that BrS is caused by these minor genes. *SCN1B* variants (BrS5) are recently reported as lack of phenotype-genotype correlation in family members, suggested that *SCN1B* is not a monogenic cause but just a modifier gene of BrS [48]. Therefore, now only the *SCN5A* is considered as a significant responsible gene for BrS.

Clinical relevance for SCN5A

Most of the *SCN5A* variants associated with the BrS or SSS, PCCD, and ERS were reported as loss-of-function in cardiac Na^+ channel, thus subjects with *SCN5A*-mediated BrS have a higher incidence of conduction abnormalities, longer P-R, QRS, and fragmentation on ECG. However, even though *SCN5A* is the most well-observed causing gene for BrS, pathogenic variants were identified in almost 20% of the BrS. Furthermore, *SCN5A* variants are not only related to the BrS but also other IPAS including LQTS, PCCD, ERS, and SSS (Table 1). The *SCN5A*-E1784K mutation is well known to be associated with mixed clinical phenotype of LQT3 and BrS or CCD, SSS [49]. Probst et al. reported 13 *SCN5A*-related BrS family pedigrees, in which 8 individuals affected by BrS but with a negative genotype were found [50]. Thus, *SCN5A* mutations may not be directly causal to the occurrence of a Brugada ECG pattern. Furthermore, in the past two decades, there was no evidence in clinical prognosis of BrS with or without pathogenic *SCN5A* variants.

Although genetic testing and the clinical relevance of *SCN5A* in BrS has been controversial, some pathogenic variants particularly non-missense ones are more likely associated with arrhythmias than the missense ones [51]. Recently, from the Japanese BrS registry, we have firstly shown the BrS proband with *SCN5A*-positive has higher arrhythmic events compared with those with

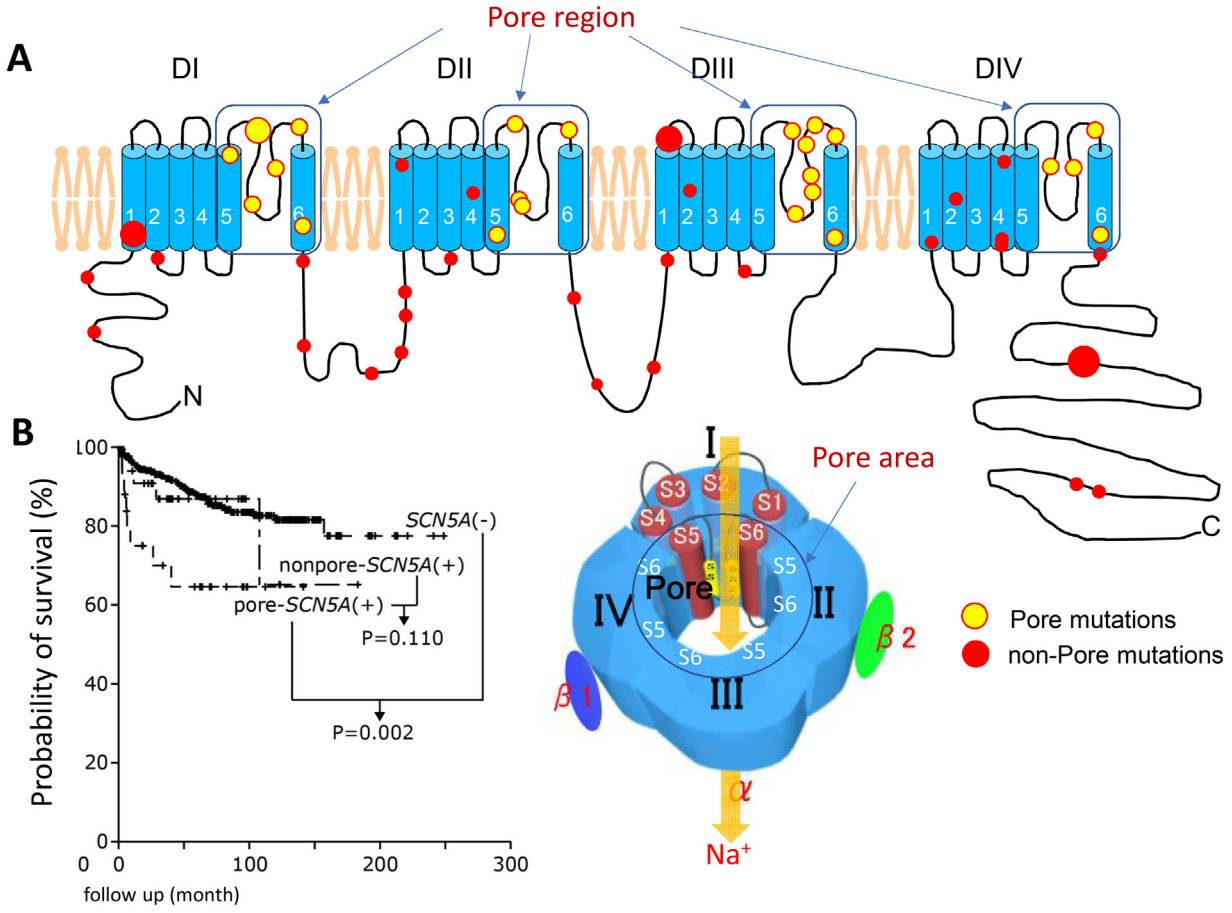

Fig. 4. (A) Topology of *SCN5A* with pore-site mutations (yellow circle) and non-pore mutations (red circle) from the Japanese Brugada syndrome registry. (B) Kaplan–Meier analysis for probability of event-free survival in the Brugada syndrome probands with pore- or non-pore *SCN5A* mutation and without *SCN5A* mutation. Pore-*SCN5A* subjects had higher event risk compared with *SCN5A*-negative subjects.
Source: Yamagata et al. [2].

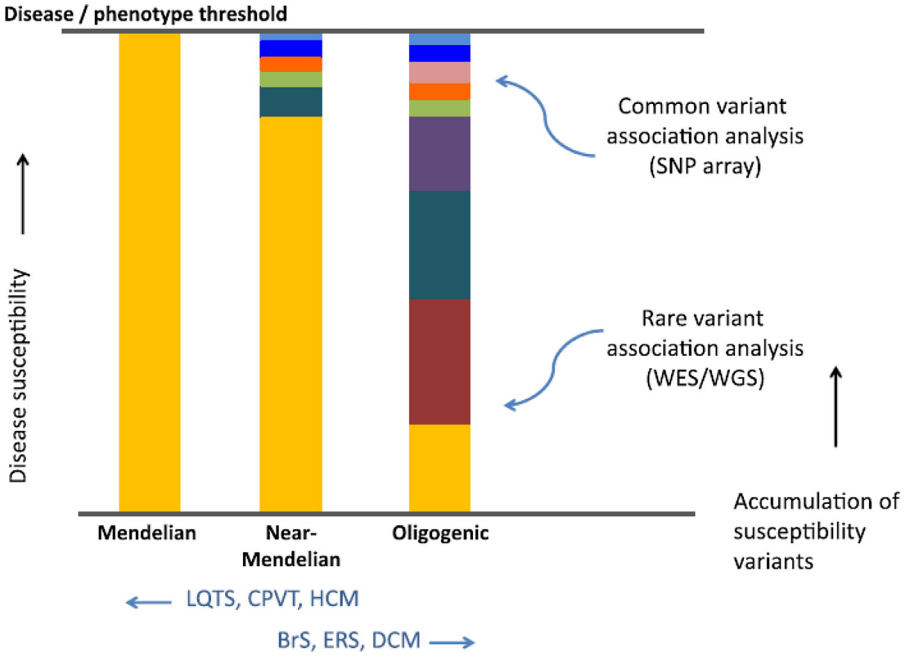

Fig. 5. The likely continuum of complexity of genetic architecture in the rare inherited cardiac disorders. Although some disorders (e.g. LQTS or CPVT) are Mendelian or near-Mendelian, where a strong monogenic component contributes substantially to disease susceptibility, genetic susceptibility for other disorders (e.g. BrS, ERS, etc.) may be determined by the cumulative effect of multiple genetic variants. BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; ERS, early repolarization syndrome; HCM, hypertrophic cardiomyopathy; LQTS, long-QT syndrome; SNP, single-nucleotide polymorphism; WES, whole exome sequencing; WGS, whole genome sequencing.
Source: Bezzina et al. [53].

SCN5A-negative, furthermore, in the *SCN5A*-positive probands, only the pore-region variants are associated with the LAEs (Fig. 4A and B) [2].

Recent understanding of genetic background in IPAS

BrS is genetically more complex than LQTS or CPVT, and most of the BrS are not likely a monogenic disorder but rather an oligogenic model, since the pathophysiology of BrS includes various elements beyond the *SCN5A* mutation. To investigate these hypotheses, a genome-wide association study (GWAS), comparing 312 BrS with 1115 controls has been performed, and two significant association signals at the *SCN10A* (rs10428132) and near the *HEY2* gene (rs9388451) could be detected in addition to *SCN5A* (rs11708996) [52]. Therefore, common genetic variations in *SCN5A-SCN10A* or *HEY2*, modulating cardiac conduction or regulating altered transcriptional programming during cardiac development can influence susceptibility to cardiac arrhythmia in BrS.

In IPAS, LQTS is most likely based on monogenic, caused by the Mendelian disorder, however, there have still been some modifiers such as D85N-*KCNE1* [24], or *NOS1AP* associated with the QT interval. On the other hand, in the other IPAS or cardiomyopathies, a somewhat more complex genetic inheritance (oligogenic model) is now suspected (Fig. 5), in contrast to the monogenic paradigm, the coinheritance of many genetic risk variants are thought to conspire to cause the disease [53].

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Conflict of interest

Dr. Aiba reported being affiliated with the endowed department by Japan Medtronic Inc.

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