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The Genetics of Brugada Syndrome

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Brugada syndrome, genetics, sodium channel, arrhythmias, polygenic risk score, sudden death

Abstract

Brugada syndrome is a heritable channelopathy characterized by a peculiar electrocardiogram (ECG) pattern and increased risk of cardiac arrhythmias and sudden death. The arrhythmias originate because of an imbalance between the repolarizing and depolarizing currents that modulate the cardiac action potential. Even if an overt structural cardiomyopathy is not typical of Brugada syndrome, fibrosis and structural changes in the right ventricle contribute to a conduction slowing, which ultimately facilitates ventricular arrhythmias. Currently, Mendelian autosomal dominant transmission is detected in less than 25% of all clinical confirmed cases. Although 23 genes have been associated with the condition, only *SCN5A*, encoding the cardiac sodium channel, is considered clinically actionable and disease causing. The limited monogenic inheritance has pointed toward new perspectives on the possible complex genetic architecture of the disease, involving polygenic inheritance and a polygenic risk score that can influence penetrance and risk stratification.

1. INTRODUCTION

1.1. Epidemiology

Brugada syndrome (BrS) is a heritable cardiac channelopathy characterized by autosomal dominant transmission. It was initially described in 1992 as a syndrome characterized by increased risk of sudden cardiac death, ventricular arrhythmias, and a peculiar surface electrocardiogram (ECG) showing 2.5–3-mm ST segment elevation with a coved morphology in the right precordial leads, in the absence of ischemia (**Figure 1**). Structural cardiomyopathy is usually absent or limited to fibrosis or scarring in specific areas (27). The predicted disease prevalence ranges from 0.017% in Europe to 0.15% in North America to 0.27% in Japan. However, since the classic ECG pattern can be intermittent and transitory, the real prevalence in the general population remains unclear (7).

BrS exhibits an autosomal pattern of transmission with incomplete penetrance when a genetic cause is detected. However, since currently only 35% of patients have an identified genetic variant, the condition's pattern of inheritance is highly debated, and new theories have recently emerged that challenge its definition as a monogenic disease (75). The vast majority of patients who actually develop symptoms are male, with an 8:1 ratio of affected men to affected women. BrS is believed to be responsible for 4–12% of all sudden cardiac deaths and at least 20% of those occurring in patients with normal hearts (21). Cardiac arrhythmias and death seem to occur largely during rest and in the presence of physiological bradycardia, such as in the morning hours during sleep (13). Approximately 20% of patients with BrS develop supraventricular arrhythmias, of which atrial fibrillation is the most common, observed in 10–20% of patients with a spontaneous BrS type 1 pattern (35).

1.2. Clinical Presentation and Diagnosis

The clinical presentation of BrS ranges from a complete absence of symptoms and incidental ECG findings to a wide spectrum of arrhythmias, including bradycardia and polymorphic ventricular tachycardia or ventricular fibrillation leading to syncope and arrest (64). In contemporary

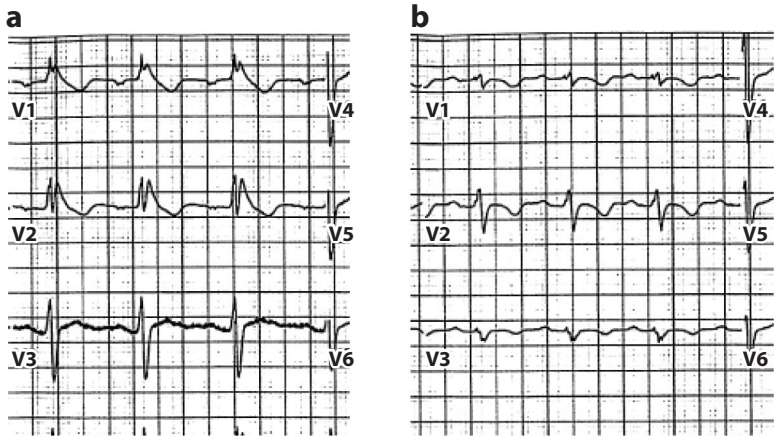

Figure 1

The transitory nature of the BrS ECG pattern. (*a*) V1–V3 leads showing a diagnostic spontaneous type 1 ECG in a patient with a pathogenic *SCN5A* variant. (*b*) V1–V3 recorded at a different date in the same patient, where only PR prolongation is visible. Abbreviations: BrS, Brugada syndrome; ECG, electrocardiogram.

practice, more than 60% of patients are asymptomatic at the time of diagnosis, and their condition is discovered upon routine evaluation, family screening, or observation of an abnormal ECG pattern during fever (7).

There are two accepted ECG repolarization patterns in the right precordial leads associated with the disease. The type 1 pattern is characterized by an ST segment elevation of at least 2 mm (0.2 mV) with a coved morphology, associated with an incomplete or complete right bundle branch block pattern and followed by a descending negative T wave, with little or no isoelectric separation (**Figure 1**). The type 2 pattern has a saddleback appearance with a high-takeoff 2-mm ST segment elevation followed by either a positive or biphasic T wave (10).

It is important to note that ECG changes associated with a BrS pattern can be intermittent and sometimes even concealed, and often only multiple ECGs over time can confirm the diagnosis (**Figure 1**). The ECG pattern can be unmasked by fever, vagal stimuli, electrolyte abnormalities, alcohol or cocaine intoxication, and certain class I antiarrhythmic drugs, such as sodium channel blockers (13).

Several acute or persistent conditions may mimic BrS on an ECG. The most common acute conditions are acute coronary syndromes, pericarditis, myocarditis, pulmonary embolism, and dissecting aortic aneurysm. However, the ECG changes typically cease to exist after the provoking event has been managed. The most common permanent conditions include left ventricular hypertrophy, athlete's heart, right bundle branch block, and even other cardiomyopathies, such as arrhythmogenic cardiomyopathy (9).

In patients with a history suggesting BrS (unexplained cardiac arrest, syncope, or family screening) but without a suggestive ECG, provocative testing with sodium channel blockers may be used to unmask the classic BrS type 1 pattern. Indeed, 30–50% of BrS patients are diagnosed through a positive drug challenge (using ajmaline, flecainide, procainamide, or pilsicainide, depending on drug availability by country) (71).

1.3. Risk Stratification and Management

The risk of arrhythmic events varies widely among patients with BrS: It is higher in survivors of sudden cardiac arrest (7.7%) and intermediate in patients with a history of cardiac syncope (1.9%). The risk of life-threatening arrhythmias in patients who are asymptomatic at the time of diagnosis is 0.5–1.5% per year (76). This relatively low risk also includes patients who are diagnosed through a sodium channel blocker challenge and do not present with a spontaneous type 1 ECG pattern. Several algorithms have been proposed by different groups to stratify patients' risk of malignant arrhythmias (especially for asymptomatic patients), but this area remains controversial.

As an example, the role of programmed electrical stimulation as a tool to identify asymptomatic patients at higher risk is still unclear. While ventricular tachycardia and ventricular fibrillation can be induced in approximately 50–70% of BrS patients, it can also be induced in 6–9% of healthy patients, and the risk of a false positive is higher when an aggressive protocol is chosen. Current guidelines state that the only available effective treatment is the implantable cardioverter defibrillator (ICD), which is recommended (class IA) in patients with a history of aborted sudden cardiac death and a type 1 ECG pattern (72). There is also a class IIA recommendation for patients with symptoms of ventricular tachyarrhythmias, such as syncope, nocturnal agonal respiration, or seizure. The indication for ICD implantation in patients with ventricular tachycardia and ventricular fibrillation inducibility on programmed stimulation remains controversial and is only a class IIA indication at the moment (72).

In terms of pharmacological treatments, the use of the class IA antiarrhythmic drug quinidine to help prevent the occurrence of severe arrhythmic events has been proposed based on its

broad-spectrum effect, blocking effect on the Ito ionic current, and demonstrated effectiveness in controlling arrhythmic storms and incessant arrhythmias in patients with ICDs. Its use as a preventive medical treatment is limited given its low long-term tolerability, especially at a high dose (2). However, a recent study by Mazzanti et al. (59) showed that in the long term, even a low dose of quinidine could significantly reduce the recurrence of life-threatening arrhythmic events in patients who had already suffered from one. Notably, 15% of patients in this study's cohort still experienced life-threatening arrhythmic events, indicating that quinidine is not a substitute for traditional ICD therapy. Finally, asymptomatic patients are usually managed through a conservative approach (74).

2. MOLECULAR MECHANISMS

BrS is defined as a channelopathy, characterized by the dysfunction of currents in one of the ion channels responsible for the generation of the cardiac action potential. The ST-T wave changes are thought to be attributable to genetically mediated alterations in the interplay between depolarizing or repolarizing cardiac currents (15). Currently, 23 genes have been identified as possibly contributing to the BrS phenotype, including genes regulating the sodium current (I_{Na}), the L-type calcium channels (I_{Ca}), or the transient outward potassium channels (I_{To}). One of the proposed theories to justify the electrophysiological changes typical of this condition is that the dysfunction of these currents, by either attenuation or enhancement, results in an outward shift in the balance of current activation during the early phases of the action potential in the right ventricular outflow tract (RVOT), which in turn creates an arrhythmogenic substrate that is responsible for the cardiac events affecting patients with BrS (6). An alternative depolarization hypothesis supports the theory that conduction slowing in the RVOT may be exacerbated in the setting of a decreased sodium current (79).

2.1. Ionic Mechanisms

At the ion channel level, a reduction of inward currents (I_{Na} or I_{Ca}) or increase in outward delayed rectifier potassium currents (I_{Kr} or $I_{K\text{-}ATP}$) gives the I_{To} current the possibility to accentuate phase 1 repolarization. I_{To} is a prominent repolarizing current: In physiological conditions, it partially repolarizes the membrane, determining the rapid (phase 1) repolarization of the action potential and setting the amplitude of the plateau (phase 2). This results in a pronounced action potential notch, which in synergy with the activation of the depolarizing L-type calcium channel gives rise to the spike-and-dome action potential morphology (4). Interestingly, the I_{To} channel presence is much more pronounced in the right ventricle (RV) than it is in the left ventricle and much more pronounced in the epicardium than it is in the endocardium, which could justify the more prominent phase 1 notch morphology of RV epicardial myocytes when compared with those of the endocardium and the left ventricle (3). On the other hand, in the pathophysiological condition of BrS, the genetically mediated reduction of inward currents (I_{Na} or I_{Ca}) or increase in outward currents (I_{Kr} or $I_{k\text{-}ATP}$) can accentuate the notch produced by I_{To}, to the point where phase 1 is repolarized beyond the voltage range in which L-type calcium channels can activate, resulting in a loss of the action potential plateau (3). Based on this theory, the typical BrS ST segment elevation or J wave in the right precordial leads is most likely due to the accentuated action potential notch in RV epicardial cells. This spike-and-dome morphology in epicardial but not endocardial cells generates a transmural voltage gradient, causing a transmural as well as epicardial dispersion, which registers as a J wave on the ECG. The ST segment elevation takes on the saddleback or coved morphology depending on the timing of the repolarization of the epicardium relative to that of the endocardium.

2.2. The Two Physiopathological Hypotheses

The cellular mechanisms underlying the typical BrS ECG changes have been extensively studied. In addition to the repolarization hypothesis described above, a depolarization hypothesis has been proposed, grounded in the reduction in the depolarizing inward current, likely in combination with a structural deficit, primarily in the RVOT subepicardium (98). Evidence supporting this hypothesis comes mainly from clinical studies, which have observed that BrS shows multiple signs of conduction slowing in ECGs. More recently, attention has shifted to the analysis of late potentials, which are highly prevalent in BrS and predictive of ventricular tachycardia and ventricular fibrillation. Interestingly, late potentials coincide with spontaneous ST elevation and late R′ in V1–V3 (62). It is now accepted that a combination of the two mechanisms most likely provides a molecular substrate for the disease. Some of the most compelling evidence in support of the depolarization hypothesis was provided by Nademanee et al. (68), who recorded late potentials and fractionated electrograms from the RVOTs of BrS patients using bipolar electrograms (**Figure 2**). This group showed how the recordings in the RVOT manifested a coved-type pattern identical to that of the signature ECG. They concluded that the unipolar recording at the anterior wall of the RVOT epicardium showed this pattern because of existing areas of slowed conduction.

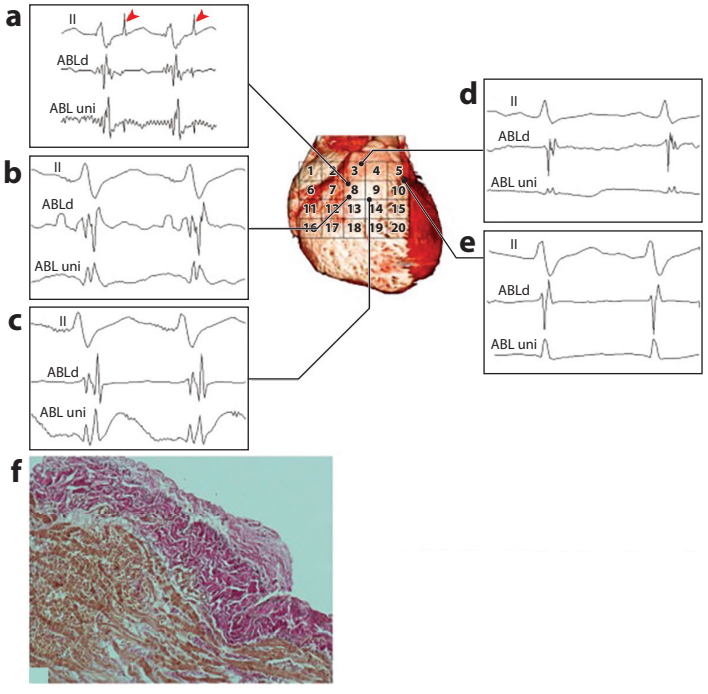

Figure 2

Fibrosis and abnormal fractionated electrograms in BrS. At the center is a computed tomography scan of the heart of one BrS patient and an anatomical grid overlaid on the anterior RVOT. (*a–c*) Abnormal fractionated electrograms. The red arrows indicate pacing stimuli. (*d*,*e*) Normal electrograms. (*f*) Epicardial biopsy and histology with picrosirius red staining at the sites of abnormal electrograms, showing epicardial fibrosis with focal finger-like projections of collagen into the myocardium. Abbreviations: ABLd, distal bipolar ablation catheter electrogram; ABL uni, unipolar ablation catheter electrogram; BrS, Brugada syndrome; II, electrogram lead II; RVOT, right ventricular outflow tract. Figure adapted from Reference 67 (CC BY 4.0).

2.3. Structural Changes in the Right Ventricular Outflow Tract: Brugada Syndrome as a Cardiomyopathy

Structural changes in the epicardium of the RV may further contribute to the typical BrS ECG and the onset of ventricular arrhythmias (**Figure 2**). Indeed, while the right precordial ST segment elevation associated with BrS usually occurs in the absence of overt structural abnormalities, it does not happen exclusively in structurally normal hearts. RV structural abnormalities have been demonstrated in a significant portion of patients with BrS ECGs. This was shown by Catalano et al. (17), who compared magnetic resonance imaging (MRI) scans of 30 BrS patients with matched healthy controls and found that, unexpectedly, the BrS group had a higher incidence of mild structural changes in the RV. Several histological and imaging studies have also demonstrated that a variety of changes are present in the myocardium that are subclinical and easily missed by a standard echocardiogram (25, 102). Frustaci et al. (32) studied 18 BrS patients and found that although the patients' hearts appeared to be normal, endomyocardial biopsies showed a high prevalence of localized RV myocarditis. Pieroni et al. (70) reported interstitial and replacement fibrosis in 15 of 20 BrS RVOT samples. Furthermore, a study by Nademanee et al. (67) on six postmortem male hearts (chosen based on sudden arrhythmic death and familial BrS) demonstrated a 32% increase in collagen proportion in the RVOT epicardium when compared with the proportions in six controls (**Figure 2**). This finding was confirmed in a recent study by Miles et al. (63), which examined 28 postmortem hearts (chosen based on the same criteria) and compared them with 29 controls, finding that the highest proportion of collagen (24%) was in the RVOT. While confirming an increasing gradient in the proportion of RV endocardial collagen to epicardial collagen, they did not demonstrate a significant difference between sampling locations. Interestingly, neither study found any significant difference in fat proportion between BrS cases and controls, which is in contrast with previous studies on an Italian population (26).

Structural changes are detected in BrS especially at the RVOT site and are responsible for localized delayed conduction. These areas are currently targeted by radiofrequency ablation as a therapeutic approach to limit arrhythmias in the disease and can reduce the appearance of the coved ST segment elevation in a resting ECG (66).

On the other hand, Hoogendijk et al. (38) showed that sodium channel blockers can exacerbate the BrS pattern in patients with arrhythmogenic cardiomyopathy and Chagas disease. This study was performed on an explanted heart from a patient with dilated cardiomyopathy and a loss-of-function mutation in *SCN5A* and found that provocation with sodium channel blockers unmasked the type 1 BrS pattern. Furthermore, the ST segment elevation coincided with the appearance of the monophasic ST segment elevation in unipolar electrograms at the basal epicardial RV, the site where fibrosis and fatty infiltration caused discontinuities in the subepicardium.

2.4. Genetics: Sodium Channel and Interacting Proteins

The first genetic variant responsible for BrS was identified in the *SCN5A* gene by Chen et al. (22) in 1998. This gene encodes the α subunit of the cardiac voltage-gated sodium channel ($Na_V1.5$), which is responsible for phase 0 of the cardiac action potential. This channel is a complex structure, composed of multiple auxiliary proteins that interact with the α subunit and assist in modulating its gating, cellular localization, intracellular transport, and degradation. These proteins interact with $Na_V1.5$ either directly by ubiquitination and internalization or by altering their gating properties through phosphorylation or dephosphorylation. Furthermore, there are adaptor proteins that anchor $Na_V1.5$ to the cytoskeleton and regulatory proteins that modulate gating properties of $Na_V1.5$ (97).

Variants in *SCN5A* linked to the BrS phenotype cause a loss of function of the protein, resulting in a reduction of I_{Na} (5). These variants account for the vast majority of genotype-positive patients (75%) and approximately 11–28% of all BrS probands. Interestingly, different BrS *SCN5A* mutations can cause different degrees of I_{Na} reduction, and it is therefore possible that they modulate the severity of clinical manifestations (47). One multicenter study of more than 2,000 affected individuals discovered 293 different *SCN5A* variants, the majority of which were present in only one individual, emphasizing how heterogeneous the disease is and the complexity of determining whether all of the variants are definitely pathogenic (46). Assessment of the decrease in I_{Na} by transient transfection in HEK293 cells or similar heterologous systems is usually considered a valid evaluation of the potential effect (and hence pathogenicity) of a given *SCN5A* variant. Indeed, a more recent analysis by Kroncke et al. (49) of all *SCN5A* variants reported either in the literature in at least one patient with BrS or in the Genome Aggregation Database (gnomAD) identified at least 1,712 distinct variants and confirmed that loss of peak current is the most reliable predictor of a deleterious effect.

Rare variants in genes coding for the auxiliary proteins interacting with $Na_V1.5$ have also been linked to the BrS phenotype, although their role has been recently disputed because of their extremely low prevalence among clinically affected patients (69). Three of these genes encode the β subunits of the cardiac sodium channel. Interestingly, most mutations causing arrhythmic phenotypes are located at the extracellular amino terminus of the β subunits. Some of these genes, as well as variations of the resulting phenotype, are noted below.

The *SCN1B* gene encodes the β1 and β1b subunits of $Na_V1.5$; when mutated, it is responsible for a reduction of I_{Na}. Variants in this gene were discovered by Watanabe et al. (94) in three BrS patients who did not have *SCN5A* mutations; the authors noted that I_{Na} was lower when $Na_V1.5$ was coexpressed with mutant β1 or β1b subunits than when it was coexpressed with wild-type subunits, implicating *SCN1B* as a potential disease gene. Subsequent coimmunoprecipitation experiments showed that a structural association among Na_Vβ1b, $Na_V1.5$, and $K_V4.3$ involving an elevated level of I_{To} is a possible mechanism (41).

The *SCN2B* gene, which encodes the β2 subunit of $Na_V1.5$, coimmunoprecipitates with $Na_V1.5$ and colocalizes at the intercalated discs in cardiac myocytes (57). Rare variants in this gene could cause a BrS phenotype by leading to a significant reduction in I_{Na} density due to a decreased $Na_V1.5$ cell surface expression (81).

The β3 subunit, encoded by *SCN3B*, coimmunoprecipitates with $Na_V1.5$. A study by Hu et al. (40) revealed that missense variants in the *SCN3B* gene, which encodes the β3 subunit of $Na_V1.5$, result in decreased I_{Na} density, accelerated inactivation, and delayed channel reactivation by impairing intracellular transport and cell surface expression of $Na_V1.5$.

The *SCN10A* gene, which encodes the neuronal sodium channel ($Na_V1.8$), has also been associated with BrS by recent genome-wide association studies (GWASs); the strongest association appeared to be when considering the combined signals at *SCN5A–SCN10A* (11). Furthermore Hu et al. (42) showed that coexpression of wild-type *SCN5A* with wild-type *SCN10A* resulted in a gain of function of I_{Na}, while coexpression of wild-type *SCN5A* with a mutant *SCN10A* resulted in a significant loss of function of I_{Na}, thus causing the BrS phenotype.

The *GPD1L* gene encodes the glycerol-3-phosphate dehydrogenase 1–like protein. A few extremely rare variants in this gene have been detected in BrS patients. The mechanism by which these variants cause a BrS phenotype is considered to be a reduction of I_{Na} through *GPD1L*-dependent phosphorylation of $Na_V1.5$. This results in a decreased *SCN5A* surface membrane expression and a reduced depolarizing current. Progressive conduction disease is seen in these patients together with a BrS ECG (54).

2.5. Genetics: Calcium Channel–Associated Genes

Approximately 13% of BrS cases have been associated with loss-of-function mutations in the cardiac calcium channel, which result in a reduction of the depolarizing $I_{Ca,L}$ (7). Rare loss-of-function variants in the *CACNA1c* gene, which encodes the α subunit of the human L-type voltage-gated calcium channel ($Ca_V1.2$), have been reported. Rare variants in the *CACNB2b* gene, which encodes the β subunit of $Ca_V1.2$, and in the *CACNA2D1* gene, which encodes the α2δ subunit of $Ca_V1.2$, have also been described, although together they account for only few sporadic case reports. Interestingly, these patients not only presented with a BrS ECG but also had additional signs of abbreviated repolarization, such as an early-repolarization ECG and/or a shorter QT interval. Rare variants of these genes have been described not only in BrS but also in individuals showing short QT syndrome or idiopathic ventricular fibrillation (14, 24).

2.6. Genetics: Potassium Channels

Variants leading to BrS in one of the potassium channels generally cause a gain-of-function change. Few genes that regulate potassium currents have been involved in BrS, all in single or extremely rare cases, altogether accounting for less than 1% of described patients and often without a strong cosegregation within families. One of them is *KCNE3*, which encodes the auxiliary β subunit (MiRP2) of the transient outward potassium channel ($K_V4.3$) and regulates several potassium currents, including I_{To} and I_{Ks}. Coexpression of this mutated gene with wild-type *KCNDR*, which encodes the α subunit of the I_{To} channel, is associated with accelerated kinetics of I_{To} (29). In addition, rare variants in *KCNJ8*, which encodes Kir6.1, have been shown to augment $I_{K\text{-}ATP}$, thus leading to an accentuation of the action potential notch as well as depression of the plateau, causing not only the ECG changes seen in BrS but also short QT syndrome (8). Rare variants in *ABCC9*, which encodes SUR2A, the ATP-binding cassette transporter of the $I_{K\text{-}ATP}$ channel, have been possibly associated with the BrS phenotype, and it is believed that the ECG is provoked by an increase in the $I_{K\text{-}ATP}$ current (43).

2.7. Genetics: Additional Genes

Throughout the years, a handful of additional genes have been described in small series of patients and families presenting clinical features of BrS. The *PKP2* gene, which encodes the desmosomal protein plakophilin 2, a known susceptibility gene for arrhythmogenic cardiomyopathy (20), has recently also been described in a few patients who present features of BrS in the absence of overt structural cardiomyopathy. In vitro functional expression in HL-1 cells, as well as in human cardiomyocytes derived from induced pluripotent stem cells, showed that these *PKP2* mutants could decrease the I_{Na} current by disrupting the interaction between *PKP2* and $Na_V1.5$ at the cardiac intercalated disc (19). These findings prompted the hypothesis that arrhythmogenic cardiomyopathy and BrS are not entirely different conditions but could be seen as being at the ends of a spectrum of structural myocardial abnormalities and sodium current deficiency that share a common origin as diseases of the cardiac connexome (7) (**Figure 3**).

A handful of sporadic variants in three other genes have also been detected on rare occasions: *FGF12*, encoding fibroblast growth factor homologous factor 1, which is responsible for modulating cardiac sodium and calcium channels (37); *RANGRF*, encoding MOG1, which is responsible for modulating $Na_V1.5$, (48); and *SLMAP*, encoding the sarcolemmal membrane-associated protein (SLMAP), which is a component of transverse tubules and the sarcoplasmic reticulum (14).

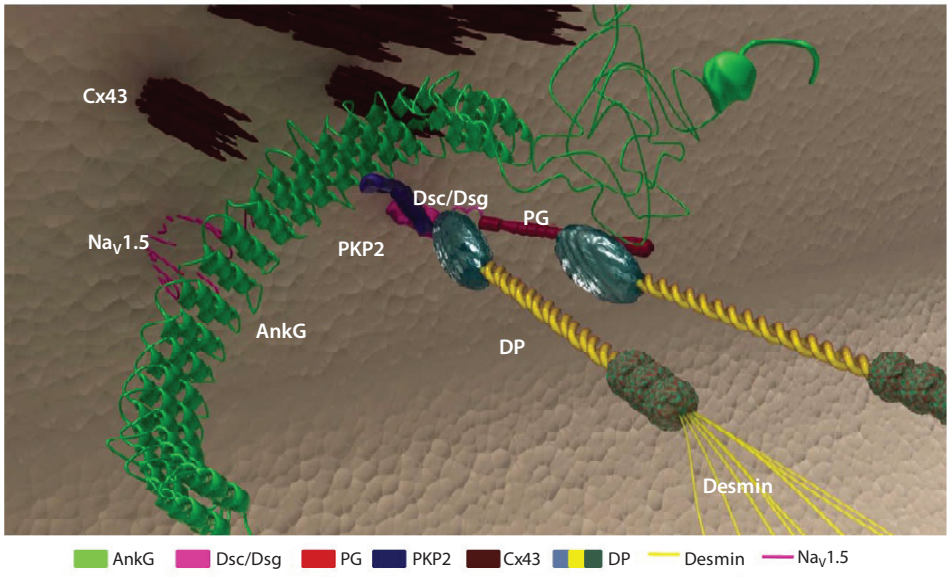

Figure 3

The cardiac connexome, showing a representation of the potential interactions among the cardiac sodium channel $Na_V1.5$, gap junctions, and desmosomal proteins. Abbreviations: AnkG, ankyrin G; Cx43, connexin 43; DP, desmoplakin; Dsc, desmocollin; Dsg, desmoglein; PG, plakoglobin; PKP2, plakophilin 2. Figure adapted with permission from Reference 83.

3. THE GENETICS OF BRUGADA SYNDROME: A CONTROVERSIAL MATTER

3.1. Indications for Genetic Testing and the Role of Minor Prevalence Genes

To date, 23 genes have been reported to be associated with BrS (39). However, they are detected in less than 30% of clinically affected individuals, and the progressive addition of novel gene–disease associations in the past two decades has not been able to increase the yield of genotype-positive cases. Genetic testing is recommended for diagnostic purposes and the detection of potential at-risk relatives, given the transitory nature of ECGs and the incomplete penetrance in familial cases. According to current recommendations (75), there is a class I indication for relatives of an index case with an identified BrS causative mutation and a class II indication "for any patient in whom a cardiologist has established a clinical index of suspicion for BrS based on examination of the patient's clinical history, family history, and expressed electrocardiographic phenotype" (1, p. 1311). On the other hand, the joint position paper from the Canadian Cardiovascular Society and Canadian Heart Rhythm Society suggested that a type 1 BrS ECG alone should be enough (class I indication) for genetic testing (36).

Considering that variants in *SCN5A* cover approximately 15–20% of clinically recognized cases and that the only genotype–phenotype correlations that could be established were in either *SCN5A*-positive or *SCN5A*-negative cases as a common group (44, 61, 65), the role of all of the so-called minor genes has been highly debated (53). An analysis of a population database of ostensibly healthy controls showed that several variants in minor BrS genes occur with a relatively high prevalence in the general population, raising further questions about their causative role in the disease (39). A study by Ghouse et al. (34) showed that up to 6% of variants previously linked

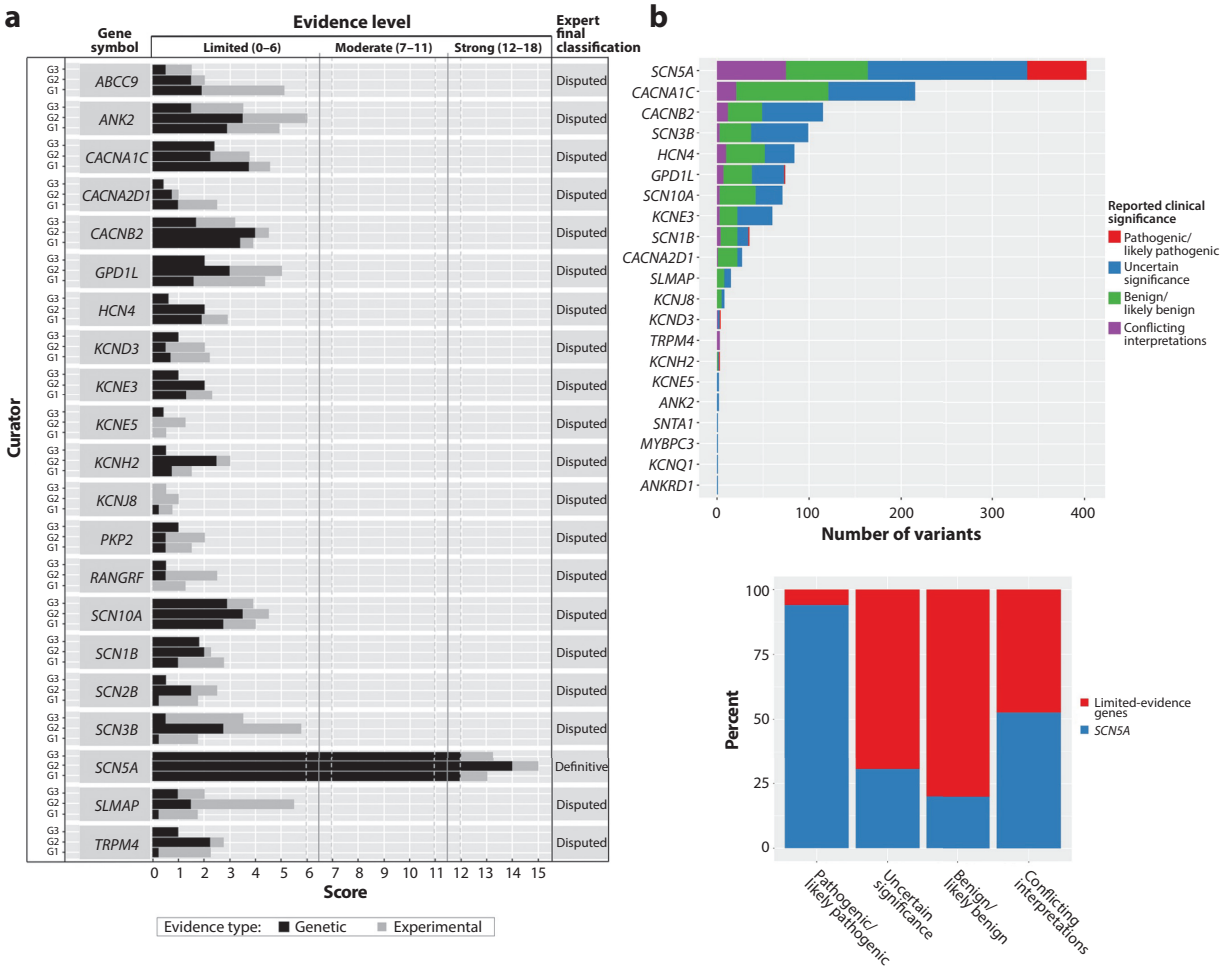

Figure 4

(*a*) Summary of the ClinGen consortium's expert recommendations, showing that among BrS-linked genes, only *SCN5A* has reached a definite final classification. (*b*, *top*) Plot of all 1,223 BrS-related variants reported in ClinVar (**http://clinvar.com**) for different genes. (*Bottom*) Relative proportions of submitted variant interpretation classifications by American College of Medical Genetics and Genomics criteria for *SCN5A* and the limited-evidence genes. Abbreviation: BrS, Brugada syndrome. Figure adapted from Reference 39 (CC BY 4.0).

to BrS were found in healthy controls who had no clinical or electrocardiographic features of the disease.

All BrS genes have been recently reassessed as part of the effort of the ClinGen consortium (39, 78) to establish evidence-based gene curation that indicates which ones could be considered clinically actionable. The consortium effort concluded that only *SCN5A* has definite evidence that it has a causative role in BrS (39) (**Figure 4**). All other genes were considered disputed at best, based on the lack of sufficient familial cosegregation data and the number of rare benign genetic variants identified in the population database (**Figure 4**). The expert panel concluded that even if the majority of mutants found in minor prevalence genes have in vitro functional characterization data, in the absence of in vivo reproducibility of the phenotype, this information is not conclusive evidence of disease causation (39).

3.2. Mendelian Transmission Versus Polygenic Inheritance: The Role of Genetic Modifiers

The understanding of the complex genetic architecture of inherited cardiac disorders has markedly increased in the past years. While discoveries were initially limited to the identification of genes underlying disorders with a Mendelian (i.e., monogenic) inheritance, in recent years it has become clear that the majority of cardiac phenotypes have a much more complex genetic structure, strongly supporting the possibility of polygenic inheritance and of a polygenic risk score for disease. Furthermore, genetic modifiers, such as common genetic variants inherited together with pathogenic ones, may modulate the expression of the phenotype, thus influencing penetrance and risk stratification (21).

BrS has been commonly referred to as an autosomal dominant Mendelian disease, but several recent studies have challenged this concept and proposed a role for a cumulative effect of common variants in the expression of the phenotype (18). The first report of a compound heterozygous inheritance of BrS dates back to 2006, when Cordeiro et al. (23) described a family carrying two mutations in *SCN5A* (P336L and I1660V): Only the patient carrying both mutations exhibited the BrS phenotype, and neither mutation alone produced this phenotype. In 2009, Probst et al. (77) questioned the purely Mendelian inheritance of the disease when they found eight mutation-negative members of five families who presented with a diagnostic type 1 ECG pattern without carrying any additional mutations in *SCN5A*. Bezzina et al. (11) further explored this matter in a GWAS of 312 BrS patients and 1,115 controls and showed that some single-nucleotide polymorphisms (SNPs) in *SCN10A* and *SCN5A* seemed to have a cumulative effect in associating with the likelihood of carrying a BrS phenotype. These results are in line with those of a GWAS by Ritchie et al. (80) that demonstrated that specific SNPs in *SCN10A* (rs6795970) and *SCN5A* (rs1805126) that are associated with QRS variability in subjects without cardiac disease are also associated with a subsequent diagnosis of cardiac arrhythmias.

Bezzina et al. (11) additionally identified a locus at 6q22.31 associated with BrS and demonstrated that the *HEY2* gene, which encodes a transcription factor belonging to the helix-loop-helix class, is the causal gene at this locus and is involved in RVOT conduction and action potential morphology. More recently, Veerman et al. (92) discovered that a SNP in the *HEY2* gene (rs9388451) is also associated with the likelihood of disease and demonstrated that the expression of *HEY2* correlates with the expression of *KNIP2*, which encodes the β subunit of the channel for the I_{To} current. Using transcriptomic studies in human hearts and electrophysiological studies in *HEY2* heterozygous knockout mice, they demonstrated that the knockout mice showed decreased transmural depolarization and repolarization gradients across the ventricular wall, suggesting an association between *HEY2* mutations and BrS through a *HEY2*-dependent alteration of ion channel expression across the ventricular wall.

Common variants in *SCN5A* may play a regulatory role similar to that of rare deleterious variants in the same gene and thus modulate the expression of the BrS phenotype. Wijeyeratne et al. (95) analyzed the *SCN5A* E1784K mutation, which is identified in 3% of unrelated BrS cases, and derived a BrS genetic risk score based on common genetic variation, showing that common variants have an important cumulative role in the expression of the BrS phenotype independent of the presence of an *SCN5A* mutation. A small study by Makarawate et al. (55) analyzed a Thai cohort of BrS patients and showed that the *SCN5A* R1193Q polymorphism was independently associated with cardiac conduction disturbance, leading to appropriate ICD therapy. Similarly, the common *SCN5A* H558R polymorphism has been reported to be a modulator, as studies have shown that H558R improves sodium channel activity in mutated channels by repairing abnormal channel kinetics and membrane trafficking (85, 86). Matsumura et al. (58) genotyped the *SCN5A* H558R polymorphism in 100 BrS patients and 1,875 controls, finding that the frequency

of H558R was lower in BrS patients than in normal controls. Furthermore, the BrS patients who had H558R but did not carry an *SCN5A* mutation showed improved ECG findings and did not experience ventricular fibrillation events. Finally, the authors demonstrated that the *SCN5A* expression level was significantly higher and the methylation rate significantly lower in patients with H558R than in those without it and concluded that this polymorphism may be a modifier that protects against ventricular fibrillation occurrence in BrS. Indeed, an expression imbalance in BrS patients with a heterozygous H558R may also contribute to the protective effects in heterozygous mutations.

Tadros et al. (89) sought to identify predictors of ajmaline-induced PR and QRS changes and type 1 BrS ECGs. The group analyzed the ECGs of 1,368 patients who underwent ajmaline infusion for suspected BrS and calculated a polygenic risk score for PR interval, QRS duration, and BrS using three SNPs that they derived from the abovementioned GWAS by Bezzina et al. (11). They demonstrated that genetic factors underlie the variable cardiac electrical response to sodium channel blockers, showing that polygenic risk scores combining 44 common variants associated with PR interval duration and 26 common variants associated with QRS interval duration in the general population are associated with ajmaline-induced PR and QRS prolongation, respectively. Furthermore, a family history of BrS, baseline QRS, the presence of a type 2 BrS pattern on a surface ECG, and the presence of polygenic risk scores derived from the three SNPs are independently associated with an ajmaline-induced type 1 BrS ECG. These findings are in line with the current understanding that genetic modifiers play a substantial role in modulating disease phenotype.

The presence of copy number variations in genes associated with BrS are additional rare alterations that potentially have a causative effect in BrS (16). There are currently fewer than 10 copy number variations potentially associated with BrS, all located on the *SCN5A* gene, and they appear to underlie no more than 1% of BrS cases (88). However, while further studies are necessary to clarify its potential role in BrS, a recent GWAS by Juang et al. (45) on 335 BrS patients and 997 controls identified a diallelic copy number variation deletion of *GSTM3* that was present in 23.9% of the BrS patients but only 0.1% of the controls. The group also showed that patients carrying the gross deletion of the *GSTM3* gene had higher rates of syncope and sudden cardiac death compared with those who did not carry this deletion. This result was further confirmed in an in vivo model of *GSTM3* knockout and wild-type zebrafish, suggesting a modulatory effect of this deletion on arrhythmic risk.

Alterations in the core promoter and transcriptional regulatory regions of *SCN5A* were recently described in BrS, although they account for only 1% of cases. Indeed, since reduced sodium channel expression is a major mechanism by which mutations in sodium channel genes alter sodium currents and thus create susceptibility to arrhythmias, genetic variants in regulatory regions of *SCN5A* may be arrhythmogenic. Yagihara et al. (99) tested the hypothesis that variants in the *SCN5A* core promoter region influence susceptibility to electrical cardiac disease and identified rare variants in this region in patients with multiple arrhythmia syndromes. Similarly, Tarradas et al. (90) surveyed the promoter and first intronic regions of the *SCN5A* gene. In an earlier study, Yang et al. (100) had found that intron 1 of *SCN5A* contains a predicted GATA1 binding site, whose mutation reduces the promoter activity of *SCN5A* in neonatal mouse cardiomyocytes, thus suggesting a role of the GATA transcription factors in the regulation of basal *SCN5A* transcription. While the human genome encodes six GATA transcription factors, *GATA4* is considered the regulator of cardiac transcriptional networks and is involved in the development of the atrioventricular cardiac conduction system. Accordingly, mutations in *GATA4* have been linked to heart dysfunction (33). In their study, Tarradas et al. (90) identified a potential role of mainly *GATA4* and *GATA5* in the regulation of the expression of *SCN5A* via a synergistic mechanism. Indeed, they

showed that in fresh-frozen human heart samples, *GATA4* transcript levels correlate with *SCN5A* transcript levels, and thus mutations in it could cause arrhythmogenicity.

These findings highlight the fact that although noncoding regions of DNA do not cause arrhythmic syndrome at the monogenic level, they may indeed increase susceptibility to arrhythmias (16). MicroRNA expression may also be a genetic modifier of *SCN5A* variants that are causative for BrS and subsequent high risk of arrhythmias; indeed, microRNAs are emerging as pivotal regulators of gene expression and protein translation and are thought to be involved in cardiac remodeling, growth, and arrhythmias (31). As such, the loss or gain of function of a specific microRNA may influence the phenotype of inherited diseases such as BrS. Daimi et al. (28) reported a family with BrS carrying the *SCN5A* Q1000K mutation as well as two common genetic polymorphisms, H558R and D1819D. They showed that the presence of two polymorphisms (rs4073797 and rs4073796) in the *SCN5A* 3′ untranslated region created a new binding site for miR-1270 in a genetically conserved region. Interestingly, H558R was revealed in a heterozygous allele in all of the family members except one. Furthermore, the combination of the H558R polymorphism with the new Q1000K mutation was present in a healthy subject, showing that there was no cosegregation. This adds to the genetic complexity of the disease and supports the hypothesis of an additive contribution to the phenotype, rather than a single variant with a clear causative link.

3.3. Genetic Pleiotropy and Brugada Syndrome: Overlap and Comparison with Other Cardiac Diseases

The conventional knowledge that a gene product is responsible for one function has been challenged in recent years, giving way to the notion that most genes are pleiotropic—that is, they can be involved in multiple functions and multiple phenotypic expressions. Often, different mutations in one gene can lead to multiple, even seemingly unrelated phenotypes (93). This observation applies to the *SCN5A* gene, which is linked to a wide spectrum of diseases (**Figure 5**), ranging from channelopathies, such as BrS and familial progressive conduction disease (loss-of-function variants), to long QT syndrome type 3 (gain-of-function variants), to structural phenotypes, such as dilated cardiomyopathy, arrhythmogenic cardiomyopathy, and multifocal ectopic Purkinje premature contractions (21, 50, 51, 101).

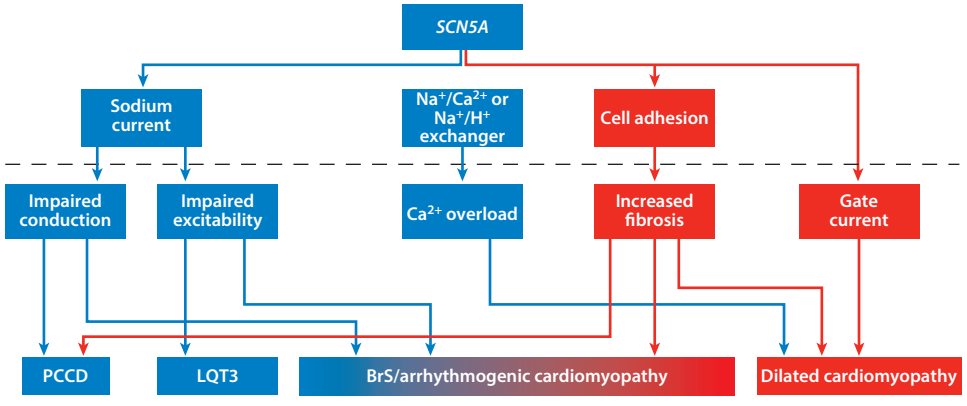

Figure 5

SCN5A as a pleiotropic gene. Seemingly unrelated functions of *SCN5A* can, if impaired, cause a clinical phenotype that can be purely electrical (*blue*), purely structural (*red*), or a combination of both. Abbreviations: BrS, Brugada syndrome; LQT3, long QT syndrome type 3; PCCD, progressive cardiac conduction defect. Figure adapted with permission from Reference 21.

Long QT syndrome type 3 is caused by gain-of-function mutations that lead to increased sodium influx into cardiac myocytes through aberrant channel gating (96). Interestingly, this disease is characterized by increased arrhythmic risk during rest conditions, which is a common feature of many inherited disorders associated with *SCN5A* (73).

Some *SCN5A* variants can affect the channel properties in such a fashion that a combined BrS and long QT syndrome type 3 phenotype may manifest. The most frequent among these variants are 1795insD (12) and E1784K (56). Carriers of these mutations usually present with loss-of-function features of $Na_V1.5$, such as sinus node dysfunction, bradycardia, conduction disease, and BrS, as well as gain-of-function features, such as a prolonged QT interval. Notably, the most frequent combination of phenotypes is BrS and conduction defects, in line with the fact that *SCN5A*-related BrS is characterized by prolonged conduction intervals throughout the heart (87).

Interestingly, studies have recently shown that all of these electrical disorders are accompanied by increased fibrosis in different areas of the conduction system, showing that a defective $Na_V1.5$ could affect not only the action potential but also the structural myocardium (**Figure 5**). This has been confirmed by the fact that *SCN5A* variants have been linked to two inherited structural heart diseases: dilated cardiomyopathy, which is characterized by dilation and impaired contractility of the left or both ventricles (60), and arrhythmogenic cardiomyopathy, which is characterized by progressive fibro-fatty infiltration and a high incidence of ventricular arrhythmias (91) (**Figure 5**). In the case of *SCN5A*-related dilated cardiomyopathy, it is important to note that most cases exhibit severe conduction defects and arrhythmias in addition to biventricular dilation and dysfunction (79).

The link between *SCN5A* variants and structural changes could be explained by taking into account the interaction between $Na_V1.5$ and additional proteins located at the cardiac intercalated disc, such as PKP2 and connexin 43, which together with others form a functional unit called the cardiac connexome (52, 83) (**Figure 3**). PKP2 is a structural component of the desmosome that is responsible for the maintenance of cell-to-cell adhesion. However, Sato et al. (84) showed that loss of *PKP2* expression in rat ventricular myocytes leads to a decrease in I_{Na} density and a shift in voltage-dependent inactivation properties of $Na_V1.5$. This result has been confirmed by Cerrone et al. (19), who detected some *PKP2* variants in patients with the BrS phenotype and an *SCN5A*-negative genotype. Expression of these mutants in HL-1 cells and in human cardiomyocytes derived from induced pluripotent stem cells showed a decreased sodium current mediated by defective sodium channel trafficking at the cell membrane (19). Furthermore, an analysis by Di Resta et al. (30) of 158 genes in *SCN5A*-negative BrS patients identified *DSG2*, the gene coding for desmoglein 2 and linked to arrhythmogenic cardiomyopathy, as a possible candidate gene in BrS. Indeed, a reduction in I_{Na} density has been reported in *DSG2* mutant mice, leading to the hypothesis that cell junction alterations and arrhythmia susceptibility could share a common substrate (82).

These genetic findings are currently deemed less relevant and not actionable in terms of clinical care management (72), but they provide important insights on how action potential and structural cardiomyopathy can be mediated by genetic alterations that reside on different proteins and interact at the connexome level (**Figure 3**).

4. CONCLUSIONS

While the genetics of BrS does not yet have an impact on the clinical care of affected patients, it provides insight into potential affected family members and is also starting to be integrated into potential risk stratification strategies. This makes the correct interpretation of the genetics of the patient all the more important. While the biggest challenge remains the correct assessment

of genetic mutations associated with BrS, it is now clear that this assessment cannot be based on Mendelian transmission of the disease and needs to take into account the presence of genetic modifiers in the phenotypic expression of BrS.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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