Chapter 2

Genetics of Brugada Syndrome

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by the influence of hormones and gender distribution of ion channels in the heart. Therefore, European series show a strong gender disequilibrium ratio of 3:1, and up to 9:1 in a South-Asian cohort [6]. The first arrhythmias normally occur around age 40 and often during rest or while sleeping, believed due to high vagal tone [7]. In addition, the phenotype is also age dependent; thus, adults show more abnormalities (0.05%-0.28%) than adolescents (0.005%-0.06%) [8]. Although there is few information on pediatric population, studies performed in children have failed to indicate a male predominance, perhaps due to low levels of testosterone in children of both genders [7].

Currently, BrS is recognized as an inherited rare cardiac entity, caused by an alteration of ionic currents that leads to ventricular arrhythmias and SCD. It is clinically characterized by ST segment elevation in leads V1-V3 of the electrocardiogram and exhibits incomplete penetrance and variable expressivity among family members [9]. Despite the identification of 24 associated genes, 65%-70% of clinically diagnosed cases remain without an identifiable genetic cause [10].

Clinical Presentation and Diagnosis

The diagnosis of BrS is based on clinical and electrocardiographic features. Despite continuous advances, incomplete penetrance and dynamic ECG manifestations confer difficulties on BrS diagnose. Some patients may suffer syncope or SCD, secondary to polymorphic ventricular tachycardia (PVT)/VF while other may remain completely asymptomatic [7]. Frequently, SCD can be the first manifestation of the disease [10]. Recent reports suggest that BrS could be responsible for 4%-12% of all sudden death (SD) and up to 20% of SD in patients with structurally normal hearts [11]. Even though the cause has not been elucidated, structural myocardial abnormalities have been reported in BrS patients [12,13].

Originally, BrS was reported as “persistent ST elevation and with right bundle branch block”, however, both criteria are no longer necessary for its diagnosis nowadays. The current clinical diagnosis of BrS requires the identification of the ST segment elevation in the right precordial leads at baseline or after the use of sodium blockers. Three types of ECG patterns were described in BrS: the type I is characterized by ST segment elevation followed by a negative T wave, with little or no isoelectric separation, with a ‘coved morphology’ [14] (Figure 1). The ECGs type II and type III are saddleback-shaped patterns, with a high initial augmentation followed by an ST elevation greater than 2 mm for type II and less than 2 mm for type III. The second Brugada consensus Report proposed that only type I is diagnostic for BrS [4] and, in 2013, it was proposed to consider both spontaneous type I pattern and a provoked type I pattern (with baseline type II or III pattern) in at least one right precordial lead (V1 or V2), for a definitive diagnosis of BrS [15]. This pattern may be spontaneous or induced by a pharmacological test using
Class I AAD –antiarrhythmic drug- (such as Flecainide, Ajmaline and Procainamide) [7]. Both type II and type III patterns are suggestive of BrS, but not diagnostic. A type I ECG pattern is observed in only 25% of tracings, and most ECG will normalize at follow-up. Therefore, the diagnosis of BrS is currently accepted in those patients with a type I ECG pattern and any of the following clinical features: documented VF; PVT; inducibility of VT with programmed electrical stimulation; family history of SCD at younger than age 45 years; covered-type ECG in family members; unexplained syncope; or nocturnal agonal respiration [4].

Figure 1: Brugada syndrome type I ECG from a 46-year-old male.

Molecular Mechanisms

Transmembrane ionic fluxes generate an action potential (AP). In the ventricular myocyte, a rapid inward Na+ current (I_{Na}) depolarizes the cell membrane, produces the phase 0 of the AP, and subsequently activates other ion currents. Activation of the transient outward K+ current, (I_{to}) will overwhelm the late phase of the inward I_{Na}, initiating the repolarization phase, which will be followed by the activation of L-type Ca2+ current, rapid and slow delayed rectifier K+ currents (I_{Kr} and I_{Ks}), and the inward rectifier K+ current (I_{Kr}) to finally reach the resting negative potential.

BrS is a channelopathy caused by an electrical dysfunction in channels involved in the generation of the cardiac AP. In general, pathogenic variants induce a loss-of-function to inward depolarizing sodium and calcium current or gain-of-function in outward repolarizing potassium current [16]. The pathophysiological mechanisms remain to be clarified and it has been proposed that several key players, including genetic predisposition, are needed to induce the BrS phenotype. Experimental and clinical data have provided insight into the cellular and molecular basis for the ECG morphology and arrhythmogenesis of BrS [17]. Despite none have been conclusively confirmed, two mechanisms have been proposed to potentially explain the ST segment elevation in the right precordial leads: the repolarization and depolarization theories [18]. The repolarization theory focuses on disequilibrium between I_{Na} and I_{to} that affects preferentially the right ventricular myocardium, generating transmural dispersion of repolarization and the substrate for arrhythmias [19]. It has been suggested that the embryologic origin of the right ventricle differs from that of the left ventricle, and this fact predisposes the right ventricle to arrhythmias in adulthood.
Depolarization theory focuses on conduction slowing in the right ventricular outflow tract (RVOT), leading to ST segment elevation in the right precordial leads [21]. These models are not mutually exclusive, and, similarly to most disease, it is likely that BrS is not fully explained by one single mechanism [22].

Genetics

BrS is a channelopathy with an autosomal dominant pattern of inheritance. The first gene associated with BrS was SCN5A, which encodes the α-subunit of the cardiac sodium channel [23]. Since then, more than 400 pathogenic variants have been identified in 24 genes, which primarily encode for sodium, potassium, and calcium channels or proteins associated with these channels [10] (Figure 2). Pathogenic variations in genes encoding desmosomal proteins have also been associated with BrS [24,25]. Recently, an individual diagnosed with BrS and concomitant conduction system disease carried a large-scale deletion of the SCN5A gene [26]. To date, this copy number variation (CNV) is the only rearrangement identified as a cause of the disease. Despite the ongoing developments in understanding the genetic causes of BrS, only 30%-35% of clinically diagnosed cases are genetically diagnosed, and most of these (25%-30%) result from pathogenic alterations in SCN5A [27].

Figure 2: Genetic contribution to BrS. BrS related genes can be divided according to whether they affect the sodium current $I_{Na}$, the potassium current $I_K$, or the calcium current $I_{Ca}$. To date, only those variants in the SCN5A gene have been significantly associated with BrS. Although several
rare variants in genes other than SCN5A have been reported associated with BrS, their overall contribution to disease prevalence and role is still unclear. In 2015, Le Scouarnec S et al [28] showed that, in a population with European ancestry, except for SCN5A, variants in previously reported arrhythmia-susceptibility genes do not contribute significantly to the occurrence of BrS. Indeed, for every tested gene except SCN5A, rare variants were found in the same proportion in cases than in controls. Therefore, genetic variants in BrS candidate genes should be interpreted with caution and further investigation needs to be performed in order to avoid false-positive reports of causality in the context of genetic counseling.

**SCN5A**

SCN5A (ID: 6331, OMIM: 600163) encodes the alpha subunit of the cardiac voltage-gated sodium channel, Na$_{1.5}$ (Q14524), which is responsible for the sodium inward current (I$_{Na}$). SCN5A is located at coordinates 38,548,057 – 38,649,672 of the reverse strand in chromosome 3p21. The protein has a size of 101,617 bases (2,016 amino acids) with a weight of 226,940 Da. The α-subunit protein Na$_{1.5}$ is made up of a cytoplasmic N terminus, four internal homologous domains (DI-DIV, each with 5 hydrophobic segments (S1, S2, S3, S5 and S6) and one positively charged segment (S4)), interconnected by cytoplasmic linkers, and a cytoplasmic C terminal domain. Segments S4 act as the voltage sensors responsible for increased channel permeability (channel activation) during membrane depolarization and are characterized by a series of positively charged amino acids at every third position [29]. The Na$_{1.5}$ protein mediates the voltage-dependent sodium ion permeability of excitable membranes. Na$_{1.5}$ is found primarily in cardiac muscle and is responsible for the initial upstroke of the action potential, and its inactivation is regulated by intracellular calcium levels [30]. The first SCN5A pathogenic variant associated with BrS was identified in 1998 [23]. Pathogenic variants in the SCN5A gene have been associated with multiple diseases, such as Long QT Syndrome (LQTS) [31], Atrial Fibrillation (AF) [32], Sick Sinus Syndrome (SSS) [33], Progressive Conduction Cardiac Disease (PCCD) [34] and Sudden Infant Death Syndrome (SIDS) [35,36]. Despite most of these diseases belong to the group of channelopathies without structural heart alterations, SCN5A pathogenic variants have also been reported with dilated cardiomyopathy (DCM) [37]. Last compendium of SCN5A genetic variations was published in 2010 [27]. It is suggested that SCN5A account for 25%-30% of total BrS. Nowadays, nearly 390 pathogenic variants have been reported (293 missense/nonsense, 21 splicing, 50 small deletions, 20 small insertions, 3 small indels –insertion/deletion-, and 1 gross deletion), resulting in loss of function of the sodium channel. Variants are homogeneously distributed across the gene, without clear associated hot-spot zones [27]. All these pathogenic variants are point mutations or changes
of small number of nucleotides in the genomic sequence, with the exception of a large SCN5A deletion, involving exon 9 and 10, that was identified for the first time in BrS (CG1111456) in 2011 [26].

**GPD1L**

*GPD1L* (ID: 23171, OMIM: 611778) encodes the glycerol-3-phosphate dehydrogenase 1-like, G3PD1L (Q8N335). *GPD1L* is located between nucleotides 32,105,689 and 32,168,713 in chromosome 3p22.3 forward strand. The protein has a size of 63,027 bases (351 amino acids) with a weight of 38,419Da. The encoded protein is found in the cytoplasm, associated with the plasma membrane, where it binds the Na\(^{+}\) 1.5. *GPD1L* may affect trafficking of the cardiac sodium channel to the cell surface and regulate cardiac sodium current [30]. It catalyzes the conversion of sn-glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate (DHAP) (G3P + NAD\(^{+}\) = DHAP + NADH) [38]. Decreased enzymatic activity induces increased levels of glycerol 3-phosphate which activate the *GPD1L*-dependent Na\(^{+}\) 1.5 phosphorylation pathway, leading to a decrease of sodium current. In addition, cardiac sodium current may also be reduced due to alterations of NAD(H) balance, mainly induced by DPD1L [39]. Pathogenic variants in *GPD1L* have been associated with SIDS [40] and BrS. This gene is suggested as responsible only for a minimum number of BrS cases because, so far, only one missense pathogenic mutation has been reported (p.A280V → p.Ala280Val, c.839C>T) (CM074892). This pathogenic variant causes abnormal trafficking of the cardiac sodium channel to the surface membrane and a reduction of nearly 50% of the inward sodium current [41].

**SCN1B**

*SCN1B* (ID: 6324, OMIM: 612838) encodes the beta subunit type I of the cardiac voltage-gated sodium channel (Q07699). It is located at coordinates 35,030,684 – 35,040,448 of chromosome 19q13.1 forward strand. The protein has a size of 9,762 bases (218 amino acids) with a weight of 24,707Da. Voltage-gated sodium channels are heteromeric proteins that function in the generation and propagation of action potentials in excitable cells, including nerve, muscle, and neuroendocrine cell types. Structurally, Na\(^{+}\) channels are composed of one pore-forming alpha-subunit, which may be associated with either one or more beta-subunits, where the alpha subunit provides channel activity and the beta-1 subunit modulates the kinetics of channel inactivation. Hence, the *SCN1B* gene is crucial in the heterotrimeric complex of the sodium channel [30]. *SCN1B* pathogenic variants were originally identified in patients with genetic epilepsy with febrile seizures plus spectrum disorders [42,43]. However, pathogenic variants in this gene have also been associated to AF [44,45], cardiac conduction disease [46], SIDS [47], LQTS [48] and BrS [46]. So far, only one pathogenic variant has been associated with BrS, the p.Glu87Gln (c.259G>C, p.E87Q) (CM081777). Sodium current decreased when Na\(^{+}\) 1.5 was co-expressed with mutant beta1/beta1B sub-
units due to sodium channel trafficking alterations [46]. This gene is responsible for a low number of cases (<1%) of total BrS patients.

**SCN2B**

**SCN2B** (ID: 6327, OMIM: 601327) encodes the beta subunit type 2 of the cardiac voltage-gated sodium channel (Q060939). SCN2B maps to 118,161,951 – 118,176,673 in chromosome 11q23.3 reverse strand. The protein has a size of 14,723 bases (215 amino acids) with a weight of 24,326Da. The sodium channel consists of a pore-forming alpha subunit, beta-1 and beta-2 subunits. Beta-1 is non-covalently associated with alpha, while beta-2 is covalently linked by disulfide bonds. The subunit beta-2 causes an increase in the plasma membrane surface area and in its folding into microvilli [30]. Pathogenic variants in SCN2B have been associated with AF [44], SIDS [49] and BrS. This gene has been recently associated with BrS by our group [50]. Nav1.5 cell surface expression is decreased by the identified pathogenic *missense* mutation, p.Asp211Gly (p.D211G, c.632A>G). So far, no more pathogenic variations have been associated with BrS in this gene. Therefore, this gene is responsible for a minor number of cases (<1%) of total BrS patients.

**SCN3B**

**SCN3B** (ID: 20665, OMIM: 608214) encodes the beta subunit type 3 of the cardiac voltage-gated sodium channel (Q9NY72). It is located between nucleotides 123,629,187 and 123,655,244 in chromosome 11q24.1 reverse strand. The protein has a size of 26,058 bases (215 amino acids) with a weight of 24,702Da. The voltage-sensitive sodium channel consists of an ion conducting pore forming alpha-subunit regulated by one or more beta-1, beta-2 and/or beta-3 subunits. Beta-1 and beta-3 are non-covalently associated with alpha, while beta-2 is covalently linked by disulfide bonds [29]. Pathogenic variants in SCN3B have been associated with SIDS [49], AF [51,52] and BrS. There are two pathogenic variants associated with BrS, both *missense* mutations: p.Leu10Pro (c.29T>C, p.L10P) (CM093686) [53] and p.Val110Ile (c.328G>A, p.V110I) (CM1212171) [54]. Pathogenic mutations in this gene lead to a loss of function of the sodium cardiac channel. SCN3B is suggested as responsible only for a reduced number of BrS cases (<1%).

**SCN10A**

**SCN10A** (ID: 6336, OMIM: 604427) encodes the neuronal sodium channel Na1.8, a tetrodotoxin-resistant voltage-gated sodium channel (Q9Y5Y9, CCDS: 33736.1). It is located at coordinates 38,696,891 – 38,794,010 of chromosome 3p22.2 reverse strand. The protein has a size of 6,416 bases (1,956 amino acids) with a weight of 220,625Da. The interaction with different beta subunits can alter the properties of the channel formed by the encoded transmembrane protein. Na1.8 was principally considered a neuronal sodium channel involved in
nociception, however, recent evidences have implicated SCN10A in the modulating of SCN5A and in the electrical function of the heart [55]. Pathogenic variants in the SCN10A gene have been associated with the onset of pain associated with peripheral neuropathy [56], AF [57], alterations on QRS duration [58] and BrS [55]. So far, 26 missense pathogenic mutations and 1 small indel have been reported associated with BrS in SCN10A. This gene is also responsible for a low number of cases (<1%) of total BrS patients.

HEY2

**HEY2** (ID: 23493, OMIM: 604674) encodes the hairy/ enhancer-of-split related with YRPW motif protein 2 (Q9UBP5). **HEY2** maps to 125,747,664 – 125,761,269 coordinates in chromosome 6q22.31 forward strand. The encoded protein forms homo- or hetero-dimers that localize to the nucleus and interact with a histone deacetylase complex to repress transcription. The **HEY2** gene is a homologue of the zebrafish “gridlock gene”, which contributes to the atrial-venous cell fate decision. **HEY2** is a suspected repressor of transcription downstream of Notch signaling in cardiac development. It is expressed in the embryonic heart, mainly in developing ventricles and arteries. Variants in **HEY2** have been associated with congenital heart defects with cognitive impairment [59] and in 2013, Bezzina CR et al identified **HEY2** as a transcriptional regulator of cardiac electrical function involved in the pathogenesis of BrS [60]. This gene is also suggested as responsible for a reduced number of cases (<1%) of total BrS patients.

**RANGRF**

**RANGRF** (ID: 29098, OMIM: 607954) encodes the RAN guanine nucleotide release factor (Q9HD47). It is located between 8,288,497 and 8,290,092 in chromosome 17p13 forward strand. The protein has a size of 1,596 bases (186 amino acids) with a weight of 20,448Da. This gene encodes a protein that regulates the expression, function and cell surface localization of the NaV1.5 cardiac sodium channel in human cardiac cells. It interacts with the cytoplasmic loop 2 of the NaV1.5, playing a key role in intracellular trafficking of RAN between the nucleus and cytoplasm [30]. Recently, Kattygnarath et al [61] published a study supporting that **RANGRF** can impair the trafficking of Nav1.5 to the membrane, leading to I$_{Na}$ reduction and clinical manifestation of BrS. This was the first BrS-related pathogenic variant identified in this gene, p.Glu83Asp (p.E83D, c.249G>C) (CM114693). The second genetic variation identified in this gene was the nonsense p.E61X (p.Glu61STOP, c.181G>T) [62]. However, there is controversy about the deleterious effect of this last variant as 2/488 healthy individuals (0.4%) carry the same variation. This fact suggests p.E61X as a genetic variation of unknown significance (GVUS). Thus, **RANGRF** is responsible for a minor number of cases (<1%) of total BrS patients.
SLMAP

SLMAP (ID: 7871, OMIM: 602701) encodes the sarcolemmal associated protein (Q14BN4). It is located at 57,755,450 – 57,929,168 in chromosome 3p21.2-p14.3 forward strand. The protein has a size of 173,719 bases (828 amino acids) with a weight of 95,198Da. This gene encodes a homodimer that interacts with myosin protein, playing a role during myoblast fusion. Sarcolemmal membrane-associated protein is a protein of unknown function localizing at T-tubules and near the junctional sarcoplasmic reticulum membrane-associated (along the Z- and M-lines). This gene was associated with BrS in 2012 [63], via modulating the intracellular trafficking of Na_{v}1.5 but no direct interaction between SLMAP and hNa_{v}1.5 was observed. So far, only two pathogenic missense mutations have been associated with BrS, the p.Val269Ile (c.805G>A, p.V269I) (CM129586), and p.Glu710Ala (c.2129A>C, p.E710A) (CM129587). Therefore, this gene is also suggested as responsible for a reduced number of cases (<1%) of total BrS patients.

FGF12

FGF12 (ID: 2257, OMIM: 601513) encodes the fibroblast growth factor 12 (P61328). FGF12 maps to 192,139,395 – 192,767,764 in chromosome 3q28 reverse strand. The protein has a size of 628,521 bases (243 amino acids) with a weight of 27,399Da. This growth factor lacks the N-terminal signal sequence present in most of the FGF family members, but it contains clusters of basic residues that have been demonstrated to act as a nuclear localization signal. When transfected into mammalian cells, this protein was accumulated in the nucleus, but was not secreted. The specific function of this gene has not yet been determined. FGF12 is the major fibroblast growth factor homologous factor expressed in the human ventricle. In 2012, Hennessey JA et al [64] suggested that p.Q7R in FGF12 leads to a Na^{+} channel loss-of-function phenotype consistent with the BrS diagnosis. Thus, FGF12 is suggested as responsible for a minor number of BrS cases (<1%).

PKP2

PKP2 (ID: 5318, OMIM: 602861) encodes the plakophilin 2 protein (Q99959). It is located at coordinates 32,790,745 – 32,896,840 of chromosome 12p11 reverse strand. The protein has a size of 106,102 bases (881 amino acids) with a weight of 97,415Da. Plakophilin 2 is an armadillo repeat-containing protein localized in the desmosomal plaque and cell nucleus. Desmosomal plakophilins, like plakophilin 2, form part of the link between the cytoplasmic tail of cadherins and the intermediate filament cytoskeleton [65]. This gene product may regulate the signaling activity of beta-catenin. PKP2 is the primary gene responsible for arrhythmogenic cardiomyopathy [66], a desmosomal disease characterized by fibro-fatty replacement of myocardium leading to SCD in young men, mainly during exercise. However, correlation between the loss of expression of PKP2 and reduced I_{Na} has been identified in BrS patients [67]. To date, six missense path-
ogenic variants have been reported in association with BrS (CM1511405, CM142721, CM142723, CM142722, CM1310619 and CM1511404). So, this gene is responsible for a minor number of cases (<1%) of total BrS patients.

**ABCC9**

*ABCC9* (ID: 10060, OMIM: 601439) encodes the ATP binding cassette subfamily C, member 9 (Q60706). *ABCC9* maps to 21,797,401 – 21,941,402 coordinates of chromosome 12p12.1 reverse strand. The protein has a size of 144,475 bases (1,549 amino acids) with a weight of 174,223Da. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MRP subfamily which is involved in multi-drug resistance. This protein is thought to form ATP-sensitive potassium channels in cardiac, skeletal, and vascular and non-vascular smooth muscle. Protein structure suggests a role as the drug-binding channel-modulating subunit of the extra-pancreatic ATP-sensitive potassium channels. Pathogenic variants in *ABCC9* have been associated with Cantu syndrome [68], DCM [69], AF [70], early repolarization syndrome (ERS) and BrS [71]. Gain-of-function pathogenic variants in *ABCC9* induce changes in ATP-sensitive potassium (K-ATP) channels, and, when coupled with loss-of-function pathogenic variants in SCN5A, these pathogenic variants may underlie a severe arrhythmic phenotype of BrS [71]. So far, only three pathogenic *missense* variants have been associated with BrS: CM141608, CM141610, and CM141611. Thus, *ABCC9* is suggested as responsible for a minor number of cases (<1%) of total BrS patients.

**KCNE3**

*KCNE3* (ID: 10008, OMIM: 613119) encodes the β-subunit that regulates the potassium channel I_to, MIRP2 (Q9Y6H6). It is located between nucleotides 74,454,841 and 74,467,729 in chromosome 11q13.4 reverse strand. The protein has a size of 12,889 bases (103 amino acids) with a weight of 11,710Da. Voltage-gated potassium (Kv) channels represent the most complex class of voltage-gated ion channels from both functional and structural standpoints. Their diverse functions include regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume. The MIRP2 assembles with a potassium channel alpha-subunit to modulate the gating kinetics and enhance stability of the multimeric channel complex [72]. Pathogenic mutations in this gene have been associated with LQTS [73], AF [74,75] and BrS. Currently, two pathogenic *missense* variants have been reported in BrS; the first one was p.Arg99His (c.296G>A, p.R99H) (CM086422), resulting in an increase of I_to magnitude and density [76]. In addition, in 2012 [77] the pathogenic missense mutation, the p.T4A (c.10A>G), was identified in a Japanese patient presenting Brugada-pattern ECG and neurally mediated syncope. Its functional consequence was the gain of function of I_to, which could underlie the pathogenesis of BrS-pattern ECG. So, *KCNE3*
is responsible for a minor number of cases (<1%) of total BrS patients.

**KCNJ8**

*KCNJ8* (ID: 3764, OMIM: 600935) encodes the ATP-sensitive inward rectifier potassium channel eight – Kir6.1- (Q15842). It is located at coordinates 21,764,955 – 21,775,581 of chromosome 12p12.1 reverse strand. The protein has a size of 10,627 bases (424 amino acids) with a weight of 47,968Da. This potassium channel is controlled by G proteins. Inward rectifier potassium channels are characterized by a greater tendency to allow potassium to flow into the cell rather than out of it. Their voltage dependence is regulated by the concentration of extracellular potassium; as external potassium is raised, the voltage range of the channel opening shifts to more positive voltages [78]. Pathogenic mutations in this gene have been associated to SIDS [79], J-wave syndromes and BrS. The first BrS genetic variation was published in 2010 (p.Ser422Leu, p.S422L, c.1265C>T), conferring predisposition to dramatic repolarization changes and ventricular vulnerability [80]. However, it was not until 2012 when p.S422L (CM091549) was associated with a pathogenic role. This deleterious role is due to gain of function in ATP-sensitive potassium channel current, induced by a sensitivity reduction to intracellular ATP [81]. Despite this fact, in 2014, compared with other population, a significantly higher frequency of p.S422L allele was found in Ashkenazi Jews population (>4%), suggesting that either previous studies implicating p.S422L as pathogenic for J-wave syndromes failed to appropriately account for European population structure and the variant is likely benign, or that Ashkenazi Jews may be at significantly increased risk of J-wave syndromes and ultimately SCD [82]. Thus, *KCNJ8* is suggested as responsible for a minor number of cases (<1%) of total BrS patients.

**KCNH2**

*KCNH2* (ID: 3757, OMIM: 152427), also known as human ether-a-go-go related gene (*hERG1*) encodes the potassium channel, voltage gated ether-a-go-go related sub-family H, member 2 (Q12809) (Kv11.1 or HERG). *KCNH2* is located at coordinates 150,944,961 – 150,978,315 in chromosome 7q36.1 reverse strand. The protein has a size of 33,360 bases (1,159 amino acids) with a weight of 126,665Da. This gene encodes the pore-forming alpha-subunit of a rapidly activating I_{kr} expressed in the heart and in nervous tissue and that plays an essential role in the final repolarization of the ventricular action potential [83]. Pathogenic variants in this gene have mainly been associated to LQTS [84,85]. However, *KCNH2* has also been associated to SQT [86,87], AF [88], and BrS. In 2005, 2 novel *KCNH2* mutations, p.Gly873Ser (c.2617G>A, p.G873S) (CM056970) and p.Asn985Ser (c.2954A>G, p.N985S) (CM056969), were identified in patients with BrS who had no SNC5A mutation. Functional analyses of the 2 mutant channels and the computer stimulation
revealed that they caused gain-of-function of the $K_v 11.1$ channel [89]. In 2010, Wilders R et al [90] suggested that the novel $KCNH2$ mutation p.Arg1135His (c.3404G>A, p.R1135H) (CM086664), identified by Itoh et al [91] in a patient showing a short-QT interval and Brugada ECG, could itself be involved in the observed Brugada-type ECG of the patient. In 2014, Wang Q et al [92] reported 4 $KCNH2$ mutations in BrS patients. Three of these mutation carriers showed QTc intervals shorter than 360 milliseconds, except for p.Thr152Ile carrier (c.455C>T, p.T152I) (CM141127) who exhibited normal QTc interval. All mutations functionally increased $I_{Kr}$, suggesting their correlation with the phenotypes.

**HCN4**

$HCN4$ (ID: 10021, OMIM: 613123) encodes the hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 or $I_p$ channel (Q9Y3Q4). $HCN4$ maps to 73,319,859 – 73,369,264 in chromosome 15q24.1 reverse strand. The protein has a size of 49,406 bases (1,203 amino acids) with a weight of 129,042Da. The hyperpolarization-activated ion channel with very slow activation and inactivation exhibits weak selectivity for potassium over sodium. It contributes to the native pacemaker currents in heart ($I_p$), and activated by cAMP mediates responses to sour stimuli [93]. Pathogenic variants in this gene have been associated to SSS [94], LQTS associated with bradycardia [95], AF [96] and BrS. It was also associated with hypertrophic cardiomyopathy (HCM) [97]. In 2009, $HCN4$ was associated for the first time to BrS with the identification of a heterozygous $HCN4$ pathogenic variant in a symptomatic BrS patient [98]. This mutation (IVS-2DS) was an insertion of four bases (GTGA) at a splicing junction donor site of exon 2 and intron 2, predicted to cause a frameshift, the addition of 44 no relevant C-terminal residues and a premature truncation. Recently, two other BrS pathogenic variant have been reported in $HCN4$, p.Ser841Leu (c.2522C>T, p.S841L) (CM127063) [99] and p.Glu1193Gln (c.3577G>C, p.E1193Q) (CM1511396) [100]. Thus, this gene is responsible for a small number of cases (<1%) of all BrS cases.

**KCNE5**

$KCNE5$ (ID: 23630-, OMIM: 300328) encodes the potassium voltage-gated channel, Isk-related family, member 1-like –$KCNE1L$– (Q5JWV7). It is located at coordinates 109,623,700 – 109,625,172 in chromosome Xq22.3 reverse strand. The protein has a size of 1,465 bases (142 amino acids) with a weight of 14,993Da. This gene encodes a membrane protein which has sequence similarity to the $KCNE1$ gene product, a member of the potassium channel, voltage-gated, isk-related subfamily. Pathogenic variants in this gene have been associated with AF [101] and BrS. Concerning to BrS, only two pathogenic variants have been published: a novel missense variant causing gain-of-function effects on $I_{to}$ -p.Tyr81His (c.241T>C, p.Y81H) (CM113001)-, and an insertion c.276_277delCGinsAT
Thus, KCNE5 (KCNE1L) is suggested as responsible for a minor number of BrS cases (<1%).

**KCND2**

*KCND2* (ID: 3751, OMIM: 605410) encodes the potassium voltage-gated channel, Shal-related subfamily, member 2 –Kv4.2- (Q9NZV8). *KCND2* is located between nucleotides 120,273,668 and 120,750,331 in chromosome 7q31 forward strand. The protein has a size of 476,693 bases (630 amino acids) with a weight of 70,537Da. Kv4.2 contributes to the cardiac I\textsubscript{to}, the main contributing current to the repolarizing phase 1 of the cardiac action potential. This rapidly inactivating, A-type outward potassium current is not under the control of the N terminus as it is in Shaker channels. Pathogenic mutations in *KCND2* have been associated with epilepsy [103], autism with seizures [104] and J-wave syndromes associated with SCD [105]. In 2014, Perrin MJ et al [105] were the first to observe the novel association of a rare, gain-of-function mutation in the *KCND2* gene, p.Asp612Asn (c.1834G>A, p.D612N) (CM1413104), in a patient with sudden cardiac arrest and an anterior J-wave ECG pattern. Currently, this has been the only pathogenic mutation in *KCND2* associated with J-wave syndrome. *KCND2* is suggested as responsible only for a reduced number of BrS cases (<1%).

**KCND3**

*KCND3* (ID: 3752, OMIM: 605411) encodes the potassium voltage-gated channel, Shal-related subfamily, member 3 –Kv4.3- (Q9UK17). *KCND3* maps to 112,313,284 – 112,531,777 coordinates in chromosome 1p13.2 reverse strand. The protein has a size of 218,494 bases (655 amino acids) with a weight of 73,451Da. It is a pore-forming (alpha) subunit of voltage-gated rapidly inactivating A-type potassium channels that contribute to I\textsubscript{to} current. Channel properties are modulated by interactions with other alpha subunits and with regulatory subunits. Pathogenic mutations in this gene have been associated with SIDS [106], AF [107] and BrS. In 2011, Giudicessi et al [108] provided the first molecular and functional evidence implicating novel *KCND3* gain-of-function mutations (Kir4.3 protein) in the pathogenesis and phenotypic expression of BrS, with enhanced I\textsubscript{to} current gradient within the right ventricle where the *KCND3* gene expression is the highest. Currently, two pathogenic *missense* mutations have been associated with BrS, p.Leu450Phe (c.1348C>T, p.L450F) (CM111334), and p.Gly600Arg (c.1798G>C, p.G600R) (CM1110945) [106] although this last variation was reported in a SUDS case with no confirmed BrS, so far. This gene is also responsible for a minor number of all BrS cases (<1%).

**SEMA3A**

*SEMA3A* (ID: 10371, OMIM: 603961) encodes the semaphorin-3A protein (Q14563) with an Ig-like C2-type domain, a PSI domain and a Sema domain. It is located at coordinates 83,955,777 – 84,492,724 of chromosome 7p12.1 reverse strand. The protein has a size of 536,948
bases (771 amino acids) with a weight of 88,889Da. The secreted protein can function as either a chemorepulsive agent, inhibiting axonal outgrowth, or as a chemoattractive agent, stimulating the growth of apical dendrites. In both cases, the protein is essential for normal neuronal pattern development. Although initial studies focused on \textit{SEMA3A}'s role in neurodevelopment, \textit{SEMA3A} is also involved in cardiac innervation patterning. Pathogenic mutations in \textit{SEMA3A} have been mainly associated with Kallmann syndrome [109]. In 2014, Boczek et al [110] identified that pathogenic variants in the \textit{SEMA3A} gene disrupted \textit{SEMA3A}'s ability to inhibit Kv4.3 channels, resulting in a significant gain of Kv4.3 current. This is the first study to demonstrate \textit{SEMA3A} as a naturally occurring protein that selectively inhibits Kv4.3 and as a possible BrS susceptibility gene through a Kv4.3 gain-of-function mechanism. So far, only two \textit{missense} mutations have been associated with BrS, p.Arg552Cys (c.1654C>T, p.R552C) (CM147487) and p.Arg734Trp (c.2200C>T, p.R734W) (CM147488). \textit{SEMA3A} is suggested as responsible only for a reduced number of BrS cases (<1%).

\textbf{CACNA1C}

\textit{CACNA1C} (ID: 775, OMIM: 611875) encodes the voltage-dependent L-type calcium channel subunit alpha-1C (Q13936). It is located at coordinates 1,970,786 – 2,697,950 of chromosome 12p13.33 forward strand. The protein has a size of 727,164 bases (2,221 amino acids) with a weight of 248,977Da. Voltage-sensitive calcium channels mediate the entry of calcium ions into excitable cells upon membrane polarization and are also involved in a variety of calcium-dependent processes, including muscle contraction. The calcium channel is a multisubunit complex consisting of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio [111]. Pathogenic mutations in the \textit{CACNA1C} gene are responsible for a defective alpha unit of the type-L calcium channel (Ca_{1.2}), inducing a loss of channel function. It is responsible for LQTS [112] and a syndrome overlapping BrS ECG pattern with a shorter than normal QT interval [113]. So far, 7 pathogenic mutations have been reported: 8 \textit{missense} (CM070048, CM070047, CM133491, CM109282, CM133493, CM109283, CM109284, and CM109285), one splice mutation (c.1896G>A) (CS133492) [114], and duplication (c.5487_5501dup15) (CI109286) [115]. Despite number of pathogenic mutations reported, this gene is suggested as responsible for a number of cases nearly 1%.

\textbf{CACNB2}

\textit{CACNB2} (ID: 783, OMIM: 611876) encodes the calcium channel, voltage-dependent, beta 2 subunit (Q08289. It is located between nucleotides 18,140,677 and 18,541,869 in chromosome 10p12.3 forward strand. The protein has a size of 401,193 bases (660 amino acids) with a weight of 73,581Da. \textit{CACNB2} protein is a member of the voltage-gated calcium channel superfamily. It contributes to the function of the calcium channel by increasing
peak calcium current, shifting the voltage dependencies of activation and inactivation, modulating G protein inhibition and controlling the alpha-1 subunit membrane targeting [111]. Pathogenic mutations in the \textit{CACNB2B} gene are responsible for a defective β-subunit (Ca b2b) inducing a loss of channel function. It is responsible for a syndrome overlapping BrS ECG pattern with a short QT interval [113,116]. So far, 7 \textit{missense} pathogenic mutations have been reported (CM095258, CM109288, CM127056, CM109291, CM109289, CM127057, and CM109290). Despite number of pathogenic mutations reported, this gene is suggested as responsible for a number of cases nearing 1%.

\textbf{CACNA2D1}

\textit{CACNA2D1} (ID: 781, OMIM: 114204) encodes the calcium channel, voltage-dependent, alpha 2/delta subunit 1 (P54289, CCDS: 5598.1). \textit{CACNA2D1} maps to 81,946,444 – 82,443,798 coordinates in chromosome 7q21-q22 reverse strand. The protein has a size of 497,355 bases (1,103 amino acids) with a weight of 124,568Da. The protein is a dimer formed of alpha-2-1 and delta-1 chains disulfide-linked. The alpha-2/delta subunit of voltage-dependent calcium channels regulates calcium current density and activation/inactivation kinetics of the calcium channel. It plays an important role in excitation-contraction coupling [111]. Pathogenic mutations in this gene have been associated with epilepsy [117,118], SQTS [119] and BrS [115]. So far, 4 \textit{missense} pathogenic mutations have been reported (CM109296, CM109295, CM109297, and CM109434). Despite number of pathogenic mutations reported, this gene is suggested as responsible for a number of cases nearing 1%.

\textbf{TRPM4}

\textit{TRPM4} (ID: 54795, OMIM: 604559) encodes the transient receptor potential cation channel, subfamily M, member 4 (Q8TD43, CCDS: 843.1). It is located at coordinates 49,157,741 – 49,211,836 in chromosome 19q13.33 forward strand. The protein has a size of 54,042 bases (1,214 amino acids) with a weight of 134,301Da. The protein encoded by this gene is a homomultimer that mediates transport of monovalent cations (Na+>K+>Cs+>Li+) across membranes, thereby depolarizing the membrane of cardiomyocytes. The activity of the encoded protein increases with increasing intracellular calcium concentration, but this channel does not transport calcium [120]. Pathogenic mutations in this gene have been associated with Progressive Familial Heart Block type 1B [121] and BrS [122]. So far, 9 \textit{missense} pathogenic mutations have been reported (CM120320, CM105352, CM120323, CM105353, CM120321, CM120324, CM105354, CM120325 and CM120322). Consequences of these mutations alters the resting membrane potential, and a reduction or increase of \textit{TRPM4} channel function may both reduce the availability of sodium channel and thus, lead to BrS. Despite number of pathogenic variants, this gene is suggested as responsible for a reduced number of BrS cases.
Genetic Testing in Brugada Syndrome

Recent advances in the identification of the genes involved in BrS have enabled the use of genetic testing in the clinical practice. These advances have focused on identifying the genetic cause of the disease, which will help the detection of asymptomatic genetic carriers at risk for SCD. Despite all reported genes associated with BrS, current guidelines only recommend genetic testing of the SCN5A gene in clinical diagnosed cases, but do not recommend in those cases without a diagnostic ECG [15]. These current guidelines that took into consideration technological and economic variables are facing the advent of genetic technologies, like next-generation sequencing (NGS), which have the capacity to analyze many genes, even exomes or genomes, in a shorter time and at reduced cost [123]. The identification of a genetic defect provides several benefits: confirmation of the disease; identification of non-genetic carriers; implementation of preventive strategies in genetic carriers; reproductive decisions, including preimplantational analyses; and others. All these benefits have to be taken into account when deciding whether to perform limited genetic testing, complex testing, or no genetic testing.

Despite the use of NGS technology, performing a comprehensive genetic testing test is expensive and the probability of pathogenic variant identification is low. In public systems, the main reason not to perform the test is the economic issue. However, this reason will not be an acceptable explanation to the family, especially if there is a history of sudden death. We believe that all families with a BrS diagnosis should be further genetically tested in order to potentially identify the genetic cause of BrS in the family. Consequently, when a positive result is identified in an index case, genetic screening of family members should be performed, whatever their ages, to search for the pathogenic variants and to implement recommendations regarding fever control and avoidances of listed drugs (http://www.brugadadrugs.org). It is essential that families with a BrS diagnosis receive genetic counseling, not only during the genetic testing process but also to assess other factors related with BrS and its inheritance like transmission probability and reproductive decisions and to manage the psychosocial aspects that could arise from a clinical or genetic diagnosis in order to improve the adaptation to the disease or to the risk of developing the disease. It is also very important to address the concept of the incomplete penetrance reported in BrS families, as being a carrier of the pathogenic variant identified in the family does not confirm either the presence of the disease or the mandatory development of the disease. However, carriers of pathogenic variants should go through a close follow-up, and risk stratification should be assessed using clinical parameters. In contrast, when the relative is not carrying the pathogenic variant identified in the family, it means that person will not suffer from the disease associated with the pathogenic variant identified in the family but we cannot discard the disease due to other genetic variants.
To date, neither whole exome nor genome analysis has been reported for BrS patients. However, in 2011, exome data of healthy populations from the NHLBI GO Exome Sequencing Project were published (http://snp.gs.washington.edu/EVS/), identifying nearly 10% of rare variants previously associated with BrS as highly pathogenic. These data have helped to clarify the potential pathogenic role of the variants in BrS [124]. The large amount of data provided and the insufficient experience to translate this information into clinical practice are the main problems concerning the use of NGS technologies. Genotype-phenotype correlation is an essential element for the correct interpretation of pathogenicity. Therefore, each family needs to be assessed separately, analyzing the variants in each relative, and correlating clinical and genetic information. One of the crucial elements for the correct interpretation of pathogenicity is the genotype-phenotype correlation in families. This leads to the need for each family to be studied separately, analyzing the variations in each relative, and correlating clinical-genetic information. Cardiologists should work together with genetic counselors in order to establish a final pathogenic interpretation of variants in each family.

**Conclusion**

BrS is an important hereditary cardiac disease leading to ventricular arrhythmias and SCD. More than 20 years after the first description of BrS, and despite continuous improvement both in clinical and molecular basis, several key features still remain to be clarified. The etiology of BrS is multifactorial, with several modulators of the disease progression and outcome. Therefore, epigenetic mechanisms should be considered in families showing incomplete penetrance and variable expressivity. In addition, familial cosegregation, *in vitro* studies, and *in silico* predictions are crucial to clarify the role of rare genetic variants in BrS phenotype. Genetic background is essential to understand both the cause of the disease and the variable phenotype. Elucidating the exact mechanisms underlying BrS and the precise genetic characteristics of each patient are essential to personalizing therapies. Despite the identification of several genes associated with BrS, nearly 65% of total cases remain without an identified genetic cause. So far, given our limited knowledge on the genetic of BrS, management and risk stratification should be performed always under a clinical context. Nonetheless, genetic testing allows confirmation of the diagnosis in borderline cases and identification of asymptomatic genetic carriers that may be at risk of SCD, enabling the undertaking of preventive strategies. New genetic technologies allow the performance of wide-range genetic analysis in BrS patients, increasing the identification of pathogenic variants and consequently, the complexity of their clinical interpretation. Genetic counseling should be incorporated into the evaluation and management of patients and families with BrS, as well as into the genetic testing process.
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