Brugada syndrome: a fatal disease with complex genetic etiologies – still a long way to go

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ABSTRACT

Brugada syndrome (BrS) is an arrhythmogenic disorder which was first described in 1992. This disease is a channelopathy characterized by ST-segment elevations in the right precordial leads and is susceptible to sudden death. BrS is a fatal disease with gender and age preferences. It occurs mainly in young male subjects with a structurally normal heart and silently progresses to sudden death with no significant symptoms. The prevalence of BrS has been reported in the ranges of 5–20 per 10 000 people. The disease is more prevalent in Asia. Nowadays, numerous variations in 23 genes have been linked to BrS since the first gene SCN5A has been associated with BrS in 1998. Not only can clinical specialists apply these discoveries in risk assessment, diagnosis and personal medicine, but also forensic pathologists can make full use of these findings as well. In this review, we discussed the prevalence, the genes associated with BrS and the application of molecular autopsy in forensic pathology. We also summarized the present obstacles, and provided a new insight into the genetic basis of BrS.

Introduction

In November 1992, Pedro Brugada and Josep Brugada identified a hereditary disorder with a cardiac abnormality characterized by a distinct electrocardiogram (ECG) pattern, and a structurally normal heart [1]. By 1996, the Japanese cardiovascular researchers named the unique ECG findings as “Brugada Syndrome” (BrS) [2]. In 1998, a publication in Nature first linked SCN5A gene to BrS, opening a new era to explore the genetic defects in BrS [3]. In 2002 and 2005, two conference reports [4,5] focused on diagnostic criteria, risk stratification schemes, and approaches to therapy of BrS were published, providing a consensus understanding about the disease.

Generally speaking, BrS is a cardiac arrhythmia, characterized by ST segment elevation in leads V1–V3 with right bundle branch block in ECG [4]. BrS is associated with a high risk of sudden death, accounting for 20% of patients with structurally normal hearts [6]. The majority of BrS patients are asymptomatic, while others may suffer from syncope or even develop sudden death due to the fast polymorphic ventricular tachycardia. These symptoms may occur mainly after a large meal or during sleep or rest [7]. There is an obvious gender difference in BrS, approximately 80% of the patients are males. The age of these patients ranges from 1 to 84 years, but it mostly occurs around the age 40–45 [8].

To date, several genes are reported to be associated with BrS. However, the etiology of BrS remains unclear. This review discussed the epidemiology, reported genes associated with BrS, the role of molecular autopsy in forensic identification and the hypothesis of possible etiology of BrS.

Epidemiology

It is difficult to determine the prevalence of BrS because the majority of these patients are asymptomatic, and in some patients, the first manifestation could be sudden death. The current estimation is based on ECG signs, which gives a rough estimation of the prevalence of the BrS [9–30]. Prevalence data of the BrS ECG pattern in different parts of the world is illustrated in Table 1. We concluded that the combined prevalence of BrS ECG is low with an incidence rate of 30 per 10 000 subjects. However, the prevalence of BrS may be lower than that due to its diagnostic strict criteria. Globally, this prevalence varies from 5 to 20 per 10 000 [8], and the syndrome appears to have a higher

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Ion channel, located on the membrane of the cardiomyocytes, is a protein which allows ions to move in and out to make an ion balance. During the process of channel opening and closing, the cardiac action potential is produced [31]. To date, studies have identified many genetic factors in cardiac sodium, potassium, and calcium channels associated with BrS. The currently reported BrS-related genes are illustrated in Table 3.

### Genetic factors of BrS

Cardiac sodium channel, consisting of a pore-forming α-subunit and several regulatory β-subunits, plays a very important role in the generation of the rapid upstroke of an action potential and the transmission of cardiac impulses.

#### FGFl2

This gene, located at 3q28–q29, encodes one of the fibroblast growth factor (FGF) homologous factors (FHF). The FHFs are genes supposed to be arrhythmogenic loci due to the nature that FHFs have the ability to affect both sodium and calcium currents [32]. On the basis of the fact mentioned above, FGFl2, the major FHF expressed in the human heart ventricles, was detected in 102 unrelated patients with BrS by Hennessey et al. [32]. The study showed a missense mutation Q7R, of which the function can reduce sodium current through affecting Na channel block [32].

#### Table 1. Reported BrS incidence in different area.

| Continent   | Area     | Population | Total number of BrS | Incidence(%) | Ref. |
|-------------|----------|------------|---------------------|--------------|------|
| Asia        | Iran     | 3,895      | 100                 | 2.57         | [9]  |
| Israel      |          | 592        | 5                   | 0.84         | [10] |
| Japan       |          | 8,612      | 12                  | 0.14         | [11] |
| Korea       |          | 10,867     | 98                  | 0.90         | [15] |
| Pakistan    |          | 1,100      | 9                   | 0.82         | [16] |
| Philippines |          | 3,907      | 94                  | 2.41         | [17] |
| Taiwan      |          | 20,562     | 26                  | 0.13         | [18] |
| Turkey      |          | 1,238      | 6                   | 0.48         | [19] |
| Europe      | Austria  | 47,606     | 1                   | 0.00         | [20] |
| Denmark     |          | 4,491      | 26                  | 0.58         | [20] |
| Finland     |          | 2,479      | 15                  | 0.61         | [21] |
| Greece      |          | 542        | 3                   | 0.55         | [22] |
| Italy       |          | 35,309     | 11                  | 0.03         | [23] |
| Germany     |          | 4,149      | 0                   | 0.00         | [24] |
| North America | USA    | 12,000     | 52                  | 0.43         | [28] |
|             |          | 27,328     | 18                  | 0.07         | [29] |
| Total       |          | 415,990    | 1,252               | 0.30         | [30] |

### Table 2. BrS incidence in different continent.

| Continent | Population | Total number of BrS | Incidence(%) |
|-----------|------------|---------------------|--------------|
| Asia      | 76,022     | 540                 | 0.71         |
| Europe    | 138,050    | 196                 | 0.38         |
| North America | USA  | 120,000   | 52               | 0.43         |
| Total     | 415,990    | 1,252               | 0.30         |

### Table 3. Reported gene associated with BrS.

| Component of ionic current | Gene name | Locus | Gene product | Mutation functional effect |
|---------------------------|-----------|-------|--------------|----------------------------|
| Sodium                    | FGFl2     | 3q28–q29 | Fibroblast growth factor 12 | \[ I_{Na} \] |
| Calcium                   | CACNA1C   | 1p13.33 | Ca\textsubscript{1.2} | \[ I_{Ca} \] |
| Calcium                   | CACNB2b   | 10p12.23–p12.31 | Ca\textsubscript{1.2} | \[ I_{Ca} \] |
| Calcium                   | CACNA2D1  | 2q21.11 | Ca\textsubscript{2.3} | \[ I_{Ca} \] |
| Calcium                   | TRPM4     | 19q13.33 | TRPM4 | \[ I_{Na} \] |
| Calcium                   | KCNQ1     | 11q13.4 | MIRP2 | \[ I_{Na} \] |
| Calcium                   | KCNQ2     | 11q24.3 | MIRP4 | \[ I_{Na} \] |
| Calcium                   | SEMA3A    | 7q21.11 | Semaphorin | \[ I_{Na} \] |
| Potassium                 | ABCC9     | 12p12.1 | SUR2A | \[ I_{K} \] |
| Potassium                 | KCNQ1     | 12p12.1 | Kir6.1 | \[ I_{K} \] |
| Potassium                 | KCNQ2     | 12p12.1 | Kir6.1 | \[ I_{K} \] |
| Potassium                 | SEMA3A    | 7q21.11 | Semaphorin | \[ I_{Na} \] |

Mixed | HCN4 | 15q24.1 | HCN4 | \[ I_{Na} \] |
trafficking, suggesting that \textit{FGF12} may underline the pathogenesis of BrS.

\textbf{GPD1-L}

In 2002, Weiss et al. \cite{33} described a novel BrS-susceptibility gene locus in a large multi-generational family of Italian descent. This gene is located on 3p22.3, of which the position is nearby the \textit{SCN5A} gene. After 5 years, London et al. \cite{34} identified the gene as \textit{GPD1-L} gene, which encodes the glycerol-3-phosphate dehydrogenase 1-like protein. Using direct sequencing, London et al. found a missense mutation (A280V) in the gene. Compared with WT \textit{GPD1-L} and WT \textit{SCN5A}, the co-expression of A280V \textit{GPD1-L} with WT \textit{SCN5A} led to a reduced inward Na\textsuperscript{+} current approximately by 50%, and a decreased \textit{SCN5A} surface membrane expression, indicating that \textit{GPD1-L} gene causes BrS through affecting the expression of \textit{SCN5A}. As for the mechanism of how a mutation in \textit{GPD1-L} gene influences \textit{Na\textsubscript{v}1.5}, based on the homology between \textit{GPD1-L} and \textit{GPD}, Liu et al. \cite{35} put forward a hypothesis that \textit{GPD1-L} may serve a similar function as \textit{GPD}, which involves in NAD-dependent energy metabolism. They also demonstrated that A280V \textit{GPD1-L} can induce elevation of [NADH]i, which can downregulate \textit{I\textsubscript{Na}} acutely through a PKC activation and increased superoxide. The study suggests metabolism plays a role in \textit{I\textsubscript{Na}}. Another study \cite{36} showed that the \textit{GPD1-L} mutation itself causes a loss function of enzymatic activity, and decreased \textit{GPD1-L} activity would have then increased the substrate G3P, and fed the PKC-mediated phosphorylation of \textit{SCN5A} at S1503 where such phosphorylation was known to decrease \textit{I\textsubscript{Na}}. A study showed that \textit{GPD1-L} gene accounted for 11%–12% BrS probands \cite{37}, but other studies showed no identification on any missense \textit{GPD1-L} gene mutation, suggesting that \textit{GPD1-L} may not be a major cause of BrS \cite{38,39}. Further studies are needed to obtain the accurate prevalence of \textit{GPD1-L} variants in BrS.

\textbf{HEY2}

Located at 6q22.31, the gene encodes Hey2 – a basic helix–loop–helix transcriptional repressor which is expressed in the cardiovascular system \cite{40}. Bezzina et al. \cite{41} identified a mutation rs9388541, located downstream of the gene, in 312 individuals with BrS through a genome-wide association study. The gene, supported by the evidence that \textit{HEY2} regulates cardiac electrical activity, is associated with the pathogenesis of BrS.

\textbf{PKP2}

\textit{PKP2} gene is located at 12p11.21, and encodes the desmosomal protein plakophilin-2, which is the major genetic cause of arrhythmogenic right ventricular cardiomyopathy (ARVC) \cite{42}. It is reported that plakophilin-2 is associated with sodium channel, of which absence can decrease \textit{I\textsubscript{Na}} \cite{43}. Cerrone et al. \cite{44} analyzed \textit{PKP2} variants in 200 BrS patients, and found five amino acid substitutions (Q62K, S183N, T526A, R635Q, and M365V) which can result in loss of sodium channel function, indicating that \textit{PKP2} may be a molecule substrate of BrS. Cerrone et al. \cite{44} proposed that reduced \textit{PKP2} expression could modify the interaction of \textit{Na\textsubscript{v}1.5} with other partners that impact on gating properties.

\textbf{RANGRF}

The \textit{RANGRF} gene, also known as \textit{MOG1} gene, is located at 17p13.1, which encodes MOG1 protein (a co-factor of \textit{Na\textsubscript{v}1.5}). Expression of \textit{MOG1} in \textit{Na\textsubscript{v}1.5}-expressing cells increased \textit{I\textsubscript{Na}} without changes in biophysical properties of the channel \cite{45}. Kattygnarath et al. \cite{46} identified a missense mutation E83D in \textit{RANGRF} gene. Expression of this mutation failed to increase \textit{I\textsubscript{Na}}, demonstrating the mutant exerts a dominant negative effect on WT-\textit{MOG1}. The studies mentioned above suggest that the mutation in \textit{RANGRF} gene may cause BrS by reducing \textit{Na\textsubscript{v}1.5} channel trafficking to the cell surface, and \textit{RANGRF} is a new susceptibility gene for BrS. In two subsequent studies, both Olesen et al. \cite{47} and Campuzano et al. \cite{48} uncovered another \textit{RANGRF} variant E61X in 220 Danish patients, including 197 AF patients, 23 BrS patients and a Spanish family. This variant caused a complete loss function of \textit{MOG1}, thus eliminating the \textit{I\textsubscript{Na}} and conferring a loss of function of \textit{I\textsubscript{Na1.5}}. Based on the mechanism of \textit{MOG1} interaction with \textit{Na\textsubscript{v}1.5}, Chakrabarti et al. \cite{49} supposed that use of \textit{MOG1} to enhance \textit{Na\textsubscript{v}1.5} trafficking to plasma membrane may be a potential personalized therapeutic approach for some patients with BrS in the future.

\textbf{SCN5A}

The first gene associated with BrS is \textit{SCN5A}, located at chromosome 3p22.2, which encodes the \textit{\alpha}-subunit of the voltage-dependent cardiac sodium channel, \textit{Na\textsubscript{v}1.5}. In 1998, Chen et al. \cite{3} described the mutations in \textit{SCN5A} which is relevant to BrS, since then more than 300 BrS-related mutations in \textit{SCN5A} have been found. It is accepted that \textit{SCN5A} represents the major gene in BrS pathogenesis, and mutations in the \textit{SCN5A} gene account for approximately 18%–30% of BrS cases \cite{50}. Many different \textit{SCN5A} mutations have been studied in a various expression systems, and the results lead to a reduction in the cardiac sodium current. Actually, several studies have been performed to demonstrate that mutations in \textit{SCN5A} such as R1432G \cite{51}, G1743R \cite{52} and T353I \cite{53} give rise to defect trafficking of the channel. Hsu et al. \cite{54} and Callo et al. \cite{55} characterized mutations in \textit{SCN5A}, and concluded that the mutations can reduce the current via a shift in the sodium channel current activation, inactivation or a closed state. There are also
several manuscripts that focused on the intermediate state of inactivation, from which it recovers slower than normal [56,57]. In other words, the defects of sodium channel caused by mutations in SCN5A are diverse, and mutations in SCN5A gene play a key role in the pathogenesis of BrS.

**SCN10A**

This gene, located at 3p22.2, encodes a neuronal sodium channel NaV1.8 which is principally involved in nociception [58]. NaV1.8 was reported to modulate SCN5A expression and cardiac electrophysiology in heart [59], and genome-wide association study (GWAS) showed that single nucleotide polymorphisms in SCN10A were associated with cardiac conduction defects, including rs6795970, rs6798015, rs6800541 and rs7430477 [60], suggesting that SCN10A plays an important role in cardiac arrhythmia. Based on its role in arrhythmogenesis, Hu et al. [61] screened SCN10A variants in 150 BrS patients, and identified 17 mutations in 25 probands. Functional study showed that co-expression of SCN5A/WT and SCN10A/WT led to a doubling gain of Ina, in contrast to co-expression of SCN5A/WT and SCN10A/(R14L, and R1268Q). The obviously decreased Ina, together with the identification of SCN10A in 16.7% of BrS probands, indicates SCN10A as a major susceptibility gene for BrS. Recently, Fukuyama et al. [62] identified 5 SCN10A variants (W189R, R844H, N1328K, R1380Q and R1863Q) in 6 out of 240 BrS probands, although the functional significance of these variants remains unclear, stressing an important role of SCN10A in BrS.

**SCN1B**

The SCN1B gene encodes two isoforms of β3-subunit, β1 and β1b, the former arises from splicing of exons 1–5 of the gene, and the latter arises from splicing of exons 1–3 with retention of a segment of intron 3, both of which are expressed through the heart [63]. In 2001, Isom reported the function of β-subunit, including an increase in Nav1.5 expression on the cell surface, modulation of channel gating, and voltage dependence, and playing a role in cell adhesion, and recruitment of cytosolic proteins such as Ankyrin-G [64]. In 2008, Watanabe et al. [65] investigated SCN1B variants in 282 probands with BrS, and identified one mutation (W179X) in a patient with BrS, which can cause a reduced Nav1.5 sodium current as a result of loss or altered β-subunit modulation of Nav1.5 current. Hu et al. [66] detected an R214Q variant in SCN1Bb. When co-expressed with SCN5A/WT, and KCND3/WT separately, the variant induced a decrease in the peak sodium current, and a greater Ito, providing another mechanism that a combined loss of function of INa and a gain function of Ito are responsible for BrS pathogenesis. Lin et al. [67] proposed that life-threatening arrhythmias in patients with mutations in SCN1B gene can be partly consequent to be disrupted intracellular Ca2+ homeostasis. There were several studies reporting the SCN1B gene mutation variants among BrS patients, including W15Y, R124G, A197D and H162P, suggesting that the occurrence of SCN1B gene variants in BrS is not rare [68,69].

**SCN2B**

This gene encodes the β2-subunit of the cardiac sodium channel, and was linked to BrS by Riuro et al. [70] in 2013. They found a novel missense mutation D211G in a woman diagnosed with BrS. Compared with cells co-expressing SCN5A/WT+SCN2B/WT, a 39.4% reduction of Ina was observed within the cells co-expressing SCN5A/WT+SCN2B/D211G without any changes on unitary channel conductance, suggesting that D211G reduced Ina by decreasing Nav1.5 cell surface expression. As for the incidence of BrS in this gene, Koopmann et al. [71] reported no mutation in SCN2B, and concluded that SCN2B is rare in BrS.

**SCN3B**

Morgan et al. [72] identified this gene in 2000. SCN3B is located at 11q24.1, which encodes the β3-subunit, Navβ3. The β3-subunit plays a role in making subtle changes to the sodium channel gating. The gene was first linked to BrS by Hu et al. [73], who identified a novel mutation (L101P) of SCN3B in a male with BrS. Compared to SCN5A/WT+SCN1B/WT+SCN3B/WT, the missense mutation co-expressed with SCN5A/WT +SCN1B/WT induced a decrease in peak sodium current density, accelerated inactivation, and slowed reactivation, causing a loss function of Ina. Another mutation V110I was identified in 3 of 178 (1.7%) Japanese BrS patients [74], and the functional study showed this mutation led to a reduced sodium current by impairing the cytoplasmic trafficking of Nav1.5. The two studies above suggest the SCN3B gene plays a role in the pathogenesis of BrS.

**SLMAP**

SLMAP, located at 3p14.3, encodes the sarcolemmal membrane-associated protein. This protein is a component of T-tubules and sarcoplasmic reticulum, of which the functional association can regulate the excitation of cardiomyocytes [64]. Ishikawa et al. [75] identified two missense mutations (V269I and Q710A) in 190 unrelated BrS patients. The functional study showed that the mutations reduced expression of Nav1.5 on the cell surface by impairing the trafficking process of Nav1.5, thus resulting in a decreased sodium current.
**Gene encoding calcium channels in BrS**

Human L-type voltage-gated calcium channel (L-TCC), also known as CaV1.2, consists of a complex of α1, αδ, β and γ subunits in a 1:1:1:1 ratio, which mediates the influx of calcium ions into the cell upon membrane polarization. Each protein mentioned above has multiple isoforms as a result of alternative splicing.

**CACNA1C**

CACNA1C is located at 12p13.33 and encodes the α1 subunit of CaV1.2 [76]. In 2007, Antzelevitch et al. [77] identified two missense mutations in two out of 82 BrS probands, including G490R and A39V. Functional studies revealed that both mutations led to a loss-of-function in calcium channel activity, and the displayed loss-of-function effect induced by A39V was found to be caused by a trafficking defect. Since then, the gene has been associated with BrS. In 2014, Beziau et al. [78] found a missense mutation N300D in four out of a family with five BrS patients, and expression of N300D in COS-7 cells led to a loss-of-function in CaV1.2. Besides, a global expression defect of the mutation was present in all six phenotype-positive, but absent in all four phenotype-negative family members. Genetic testing was performed on the family members of this proband, and the S481L mutation was found in three BrS patients out of 205 patients with BrS, short QT, idiopathic ventricular fibrillation and early repolarization syndrome (ERS). However, further functional studies are needed to certify whether this gene is associated with BrS, and to understand more about the role of these mutations in BrS.

**CACNB2b**

This gene, located at 10p12.33-p12.31, encodes the β-subunit of CaV1.2, also known as CaVβ2, which is involved in regulation and intracellular trafficking of L-type calcium channel current (I_{Ca-L}) [80]. Antzelevitch et al. [77] found a heterozygous C1442T transition in exon 13, causing a substitution of leucine for serine at position 481 (S481L) of CaV1.2. Besides, a global expression defect of the mutant CaV1.2 and an increased mobility of the mutant CaV1.2 were observed, suggesting an acceleration of CaV1.2 turnover by destabilization which may lead to a quicker degradation of the protein. In another study [79], a novel mechanism emphasized that the role of splicing mutation in CACNA1C and the nonsense-mediated mRNA decay (NMD) can lead to a decrease in mutant mRNA, suggesting it to be associated with the pathogenesis of BrS.

**TRPM4**

The transient receptor potential melastatin protein 4 gene, also known as TRPM4, encodes a calcium-activated nonselective cation channel (NSC_{Ca}), which has been implied with progressive cardiac conduction blocks [86]. Since BrS is frequently associated with cardiac conduction abnormalities, Liu et al. [87] screened TRPM4 in 248 BrS cases without SCN5A mutation, and found 11 mutations, of which five were absent from control alleles, and four were statistically more prevalent than in control alleles. They further studied four selected mutations, and revealed these mutations can result in both a gain-of-function (P779R and K914X) and a loss-of-function (T873I and L1075P) of NSC_{Ca}. However, further studies are needed to demonstrate its role in the pathophysiology of BrS.

**Gene encoding potassium channels in BrS**

The potassium channel represents the most complex class of ion channels from both functional and structural standpoints [88]. The ion-conducting pore of a K⁺ channel is formed by four α-subunits that co-assemble as homo- or heterotetramers with different biophysical properties. Their gating characteristics are further modulated by ancillary subunits. The potassium channels can be divided into different types, for instance, voltage-gated potassium (Kᵥ) channels (I_{T_K}, I_{K_r}) and inward-rectifier type potassium channel (I_{K_Ab}, I_{K_ATP}, I_{K_G}) [89].

**ABCC9**

ATP-sensitive potassium cardiac channels (I_{K_ATP}) consist of inward rectifying channel subunit Kir6.1 or Kir6.2, and the sulfonylurea receptor subunits SUR2A. ABCC9, located at 12p12.1, encodes the ATP-binding cassette transporter of I_{K_ATP} (SUR2A). Hu et al. [90] identified several mutations in 150 BrS or ERS
proband. However, it remains unclear whether these mutations can be considered a causality for BrS. Further studies are needed to confirm the role of ABCC9 in BrS.

**KCND3**

I_{To,f} (fast transient outward potassium current), a voltage-gated potassium channel expressed in heart, is a complex that consists of four α-subunits (encoded by KCND3, Kv4.3) and two β-subunits (K+ channel interacting protein 2, KChIP2). In 2011, Giudicessi et al. [91] identified two novel mutations (L450F and G600R) in two out of 86 unrelated BrS patients. Compared with KCND3/WT+KChIP2/WT, co-expression of KCND3/L450F or G600R)+KChIP2/WT revealed a significant increase in I_{To,f}. Subsequently, the mutations (L450F and G600R) caused a gain-of-function of I_{To,f} through increasing membrane protein expression, and slowing channel inactivation is demonstrated [92].

**KCNE3**

I_{Kr} (slowly activated delayed rectifying potassium current) is composed of four α-subunits (encoded by KCNQ1, Kv7.1) and two β-subunits (encoded by KCNE1, Mink). KCNE3, located at 11q13.4, is a member of five KCNE genes, of which the encoding product MiRP2 is involved in modulating the function of cardiac potassium currents, for example, I_{To} and I_{Kr} [89,93]. Delpon et al. [93] first identified the association between KCNE3 and BrS. They found an R99H missense mutation in one male out of 105 probands with BrS. The results of gene testing on the family of this individual showed that 4/4 phenotype-positive and 0/3 phenotype-negative family members had the mutation. They also demonstrated that Kv4.3 and KCNE3 can be co-immunoprecipitated. Compared with KCND3/WT+KCNE3/WT, co-expression of KCNE3/R99H and KCND3/WT led to a significant increase in peak current and an accelerated inactivation of I_{Kr}. Not only did the study show that KCNE3 had a modulation of I_{Kr} but also suggested that the mutation in KCNE3 underlies the development of BrS. Nakajima et al. [94] also identified a mutation T4A in one out of 40 BrS ECG-pattern patients with an increased I_{To}.

**KCNJ8**

This gene, located at 12p12.1, encodes Kir6.1, an inward rectifying channel subunit involved in both ventricular and atrial repolarization [100]. Medeiros-Domingo et al. [101] found a KCNJ8 missense mutation S422L in one out of 87 BrS patients, and functional investigation revealed that this mutation led to a gain-of-function of I_{Kr,ATP}. The same missense mutation was also found in three other BrS patients by Barajas-Martinez et al. [102]. The whole cell patch clamp studies showed a two-fold gain of function of glibenclamide-sensitive I_{Kr,ATP} when KCNJ8/S422L was co-expressed with SUR2A/WT. Besides, it has been also demonstrated that S422L-induced gain-of-function in I_{Kr,ATP} is due to reduced sensitivity to intracellular ATP.

**SEMA3A**

This gene, located at 7q21.11, encodes semaphorin 3A, a chemorepellent which involved in both neural
and cardiac innervation patterning. It has been demonstrated that semaphorin 3A is a naturally occurring protein inhibitor of KV4.3 [92]. When co-expressed with KV4.3, SEMA3A altered KV4.3 by significantly reducing peak current density. Besides, co-immunoprecipitations of SEMA3A and KV4.3 revealed a potential direct binding interaction between these proteins. Nicole et al. [103] identified two rare missense SEMA3A mutations in 198 unrelated BrS patients with non-SCN5A mutation; on the cellular basis, these mutations disrupted the ability of semaphorin 3A to inhibit KV4.3, and resulted in a significant gain of I\textsubscript{Na} compared with SEMA3A/WT, indicating a novel susceptibility of the SEMA3A gene for the pathogenesis of BrS.

**Gene encoding mixed ion channel in BrS**

**HCN4**

HCN4, located at 15q24.1, of which the encoding product is hyperpolarization-activated cyclic nucleotide-gated channel 4 protein. This protein forms the tetrameric complex of the human cardiac pacemaker channel of which the ion current is the funny current. The funny current is made up of two ion component: sodium and potassium. In 2012, Croitti et al. [84] identified an HCN4 mutation S841L in one out of 129 unrelated BrS possible patients, which was absent in 1 400 Caucasian reference allele. It has been reported that HCN4 expresses at a very low level in the ventricular myocardium, indicating an indirect role in causing BrS. However, further studies are needed to assess the functional effect of the mutation and to investigate whether this gene plays a role in the BrS pathogenesis.

**The role of post-mortem genetic testing in the forensic identification**

In forensic pathology, the whole process of an autopsy is a naturally occurring protein inhibitor of KV4.3 [92]. When co-expressed with KV4.3, SEMA3A altered KV4.3 by significantly reducing peak current density. Besides, co-immunoprecipitations of SEMA3A and KV4.3 revealed a potential direct binding interaction between these proteins. Nicole et al. [103] identified two rare missense SEMA3A mutations in 198 unrelated BrS patients with non-SCN5A mutation; on the cellular basis, these mutations disrupted the ability of semaphorin 3A to inhibit KV4.3, and resulted in a significant gain of I\textsubscript{Na} compared with SEMA3A/WT, indicating a novel susceptibility of the SEMA3A gene for the pathogenesis of BrS.

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translation, such as DNA methylation, post-translational modifications and RNA mechanism [79]; (4) another issue should be taken into consideration is the complex inheritance of BrS. The disease has been regarded as a monogenic disease with autosomal dominant mode of inheritance, and many studies were mainly focused on seeking for new genes or genetic variants in individuals or families affected by BrS. However, the inheritance of BrS does not meet the criteria of Mendelian inheritance after analyzing genetic variants in large family pedigrees with BrS, and this phenomenon was described as low-penetrance or phenocopies. A recent study [108] showed that common polymorphic alleles strongly associated with disease risk, and stressed the importance of defining a proper genetic model for BrS.

**Perspective**

BrS is a channelopathy characterized by a structurally normal heart leading to arrhythmogenesis, syncope and sudden death. The disease has an age and gender preference, and, as aforementioned, most death events occur in males in their forties. The syndrome is more prevalent in Asia, while most BrS deaths occur at rest or during sleep. Progress in genetics has helped both to unravel the origin of BrS and to understand the mechanistic pathways. Compared to nearly 20 years ago, nowadays mutations in 23 genes have been associated with BrS or BrS ECG phenotype. In most cases, the gene or the rare genetic variants (named as candidate genes) were speculated to play a pathogenic role when compared with in-house or public disease variants database, and then the putative-causing genes or variants were identified on the basis of the pathophysiological experiment from candidate genes [108]. However, the study design, the number of studied population and the functional data are different, and the strength of disease association needs further assessments [108]. Regarding this, the previously published “pathogenic” variants have been shown to be at a high minor allele frequency in general population [109], and thus its role in BrS needs to be re-assessed. At present, direct sequencing and functional studies still play an important role in the identification of pathogenic genes. Despite the complicated etiology of BrS, new genomic technologies, for example, GWAS and whole-genome next-generation sequencing, can be used to identify new candidate genes responsible for BrS. Besides, induced pluripotent stem cells (iPS) can be differentiated into cardiomyocytes, which provides a new insight for the electrophysiological and molecular study of BrS. In conclusion, the progress in genetics has provided a better understanding and facilitated the study of many diseases including BrS, and also contributed to the development of both forensic pathology and clinical cardiology. Continuing efforts in researching the etiology of disease is needed to help us understand the pathogenesis of BrS by new technologies.

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