Evaluating the Use of Genetics in Brugada Syndrome Risk Stratification

Michelle M. Monasky[1†], Emanuele Micaglio[1†], Emanuela T. Locati[1] and Carlo Pappone[1,2]*

[1] *Arrhythmology Department, IRCCS Policlinico San Donato, Milan, Italy,* [2] *Vita-Salute San Raffaele University, Milan, Italy*

The evolution of the current dogma surrounding Brugada syndrome (BrS) has led to a significant debate about the real usefulness of genetic testing in this syndrome. Since BrS is defined by a particular electrocardiogram (ECG) pattern, after ruling out certain possible causes, this disease has come to be defined more for what it is *not* than for what it *is*. Extensive research is required to understand the effects of specific individual variants, including modifiers, rather than necessarily grouping together, for example, "all *SCN5A* variants" when trying to determine genotype-phenotype relationships, because not all variants within a particular gene act similarly. Genetic testing, including whole exome or whole genome testing, and family segregation analysis should always be performed when possible, as this is necessary to advance our understanding of the genetics of this condition. All considered, BrS should no longer be considered a pure autosomal dominant disorder, but an oligogenic condition. Less common patterns of inheritance, such as recessive, X–linked, or mitochondrial may exist. Genetic testing, in our opinion, should not be used for diagnostic purposes. However, variants in *SCN5A* can have a prognostic value. Patients should be diagnosed and treated per the current guidelines, after an arrhythmologic examination, based on the presence of the specific BrS ECG pattern. The genotype characterization should come in a second stage, particularly in order to guide the familial diagnostic work-up. In families in which an *SCN5A* pathogenic variant is found, genetic testing could possibly contribute to the prognostic risk stratification.

Keywords: Brugada syndrome, sudden cardiac death, genetic testing, mutation, variant, *SCN5A*, sodium channel, arrhythmia

INTRODUCTION

The first description of Brugada syndrome (BrS) included eight unrelated patients with recurrent aborted sudden cardiac death due to ventricular fibrillation (VF) (1), in whom basal ECG showed persistent ST-segment elevation in precordial leads V1 to V2-V3. However, the genetic background was not discussed. Thus, no genotype-phenotype relationship was established. Meanwhile, Gellens and coworkers characterized *SCN5A* for the first time (2). Later, *SCN5A* was described in two unrelated families with long QT syndrome (LQTS) type-3 (LQT3) (3) (timeline, **Figure 1**).

BrS was first considered a form of idiopathic VF, resulting from abnormal electrophysiologic activity in right ventricular epicardium (4). It was described to lie on the same spectrum of cardiac

FIGURE 1 | Timeline of Brugada syndrome discoveries.
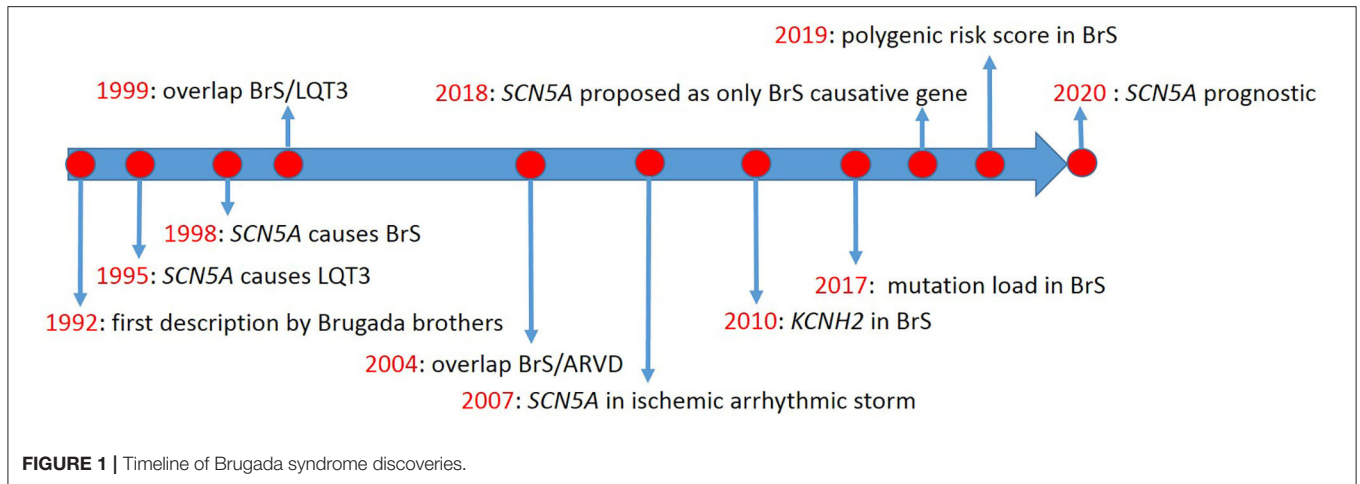
electrophysiologic pathology as LQT3, caused by the same variant in *SCN5A* (5). Today, BrS is considered a Mendelian disorder inherited in an autosomal dominant fashion, even if alternative mechanisms of inheritance have been recently proposed (6). In BrS patients, variants in *SCN5A* are found more commonly than in any other gene (7) but confirm the clinical diagnosis in only a minority of cases (8). Many other genes have been proposed to cause BrS, but their roles are hotly debated (9, 10), with some groups suggesting that only *SCN5A* should be used in BrS genetic testing (9). However, variants in *SCN5A* have long been known to not necessarily segregate with BrS (4, 11). Recently, patients harboring *SCN5A* variants were demonstrated to have a worse prognosis (12).

These challenges have resulted in two important consequences: an overestimation of *SCN5A* diagnostic value and a contemporary underestimation of the clinical significance of genes different from *SCN5A*. All considered, the goals herein are to reevaluate the clinical significance of genetic data found in patients with BrS and to provide new insights about the complex genetics of BrS.

Clinical Definition of BrS

The difficulty in understanding BrS genetics may lie in the definition of BrS, based on the electrocardiogram (ECG), specifically the type-1 BrS pattern, an ST-segment elevation with coved morphology, ≥2 mm, often associated with a sharp transition from elevated ST-segment to negative T-wave, among right precordial leads V1-V2, positioned in the 2nd, 3rd, or 4th intercostal space (13). This type-1 BrS pattern can occur either spontaneously or be unmasked with intravenous administration of Class 1c antiarrhythmic drugs, such as ajmaline or flecainide (13). Recently, it was hypothesized that BrS might actually be a heterogeneous disease with a common ECG phenotype (14). While this phenotype has been commonly attributed to loss-of-function of the Na$_V$1.5 cardiac sodium channel, such phenotype could result from a number of molecular origins, not only *SCN5A* variants, but also alterations in proteins that modify the channel, or even environmental influences. Regarding the environmental

influences, "true BrS" is diagnosed by ruling out such causes as electrolyte disturbances or myocardial ischemia. BrS patterns in these cases are said to be "BrS phenocopies" (15, 16). We disagree with the definition of "phenocopy," because it is based upon what BrS is *not* rather than providing a clear picture of what BrS *is*. This is especially concerning since environmental influences can have a pivotal role in BrS (17). Perhaps a better view would be to consider the "BrS pattern" as a warning of risk for sudden cardiac death, regardless of the underlying cause (18). We are aware that this concept challenges the autosomal dominant model of BrS, largely based on the accepted etiologic role of *SCN5A*.

BrS has also been attributed to an increase in potassium current (19, 20). Furthermore, several studies have suggested BrS may be similar to a cardiomyopathy (21–26). Thus, it is likely that the ECG pattern used to define "BrS" is actually a common clinical manifestation, resulting from a multitude of different molecular causes. Further development of this concept may lead to a new paradigm for BrS, which may be considered not only as a Mendelian disorder, but as a complex condition, which might be caused by a huge variety of genetic variants, interacting with environmental factors (14, 27). In any case, since our current understanding of BrS genetics is still elementary, today BrS should be diagnosed by the type-1 ECG pattern (see **Figure 2**), not by genetic findings, especially additional findings during screening for other diseases.

Genotype-Phenotype Relationships

Genotype-phenotype relationships are difficult to establish in BrS patients, because the clinical manifestations can be very subtle, and because the differential diagnosis can be extremely complex (28). Additionally, *SCN5A* variants have since been associated with a variety of pathologies (29, 30). Other works (31, 32) demonstrated both rare mutations and common variants in *SCN5A* can be considered phenotype modulators in myocardial infarction (33), arrhythmic storm (34), epilepsy (35), and even colon (36) and breast cancer (37). Thus, although *SCN5A* is
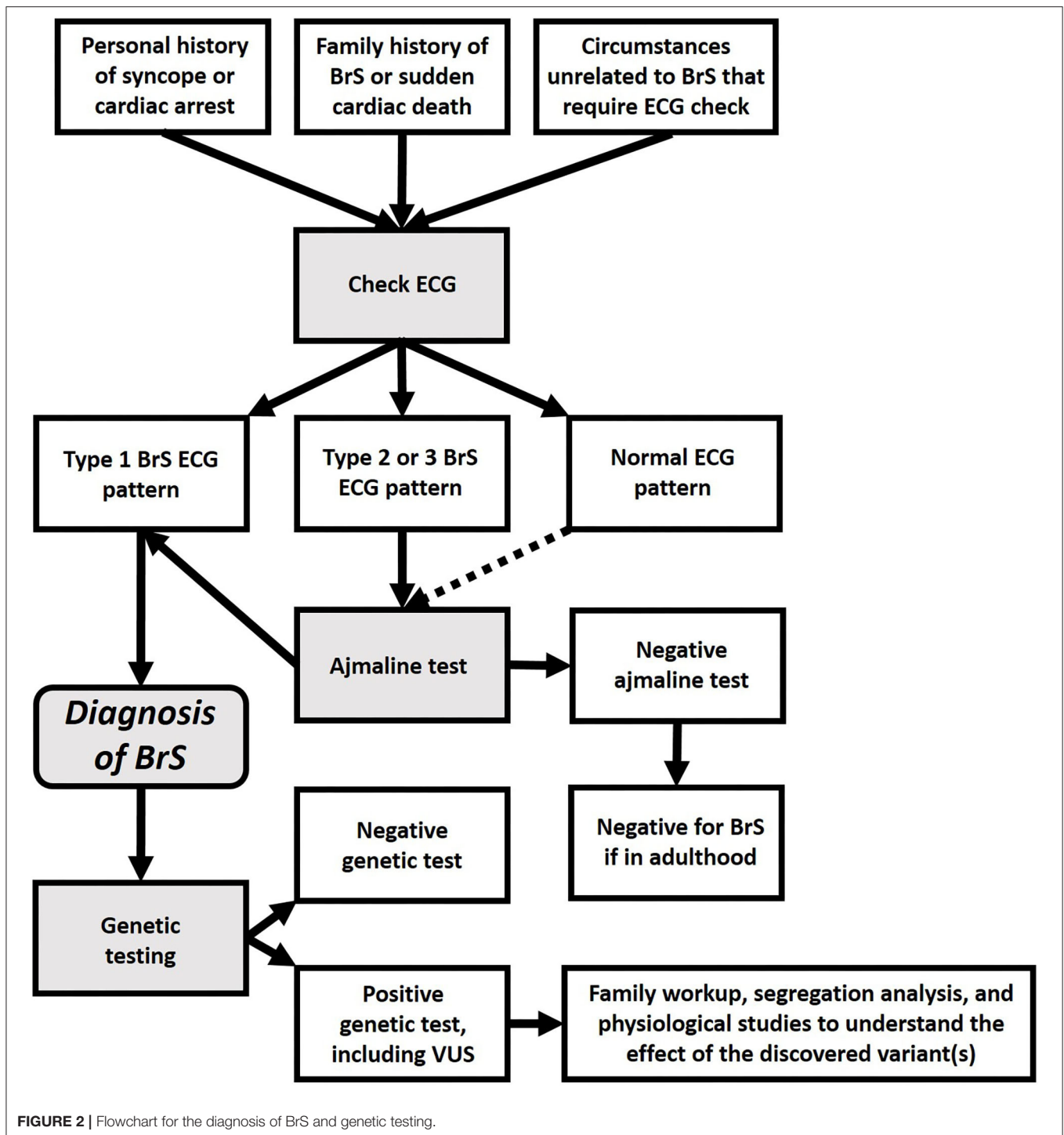
FIGURE 2 | Flowchart for the diagnosis of BrS and genetic testing.

the only undisputed gene in which mutations are thought to cause BrS, genetic testing alone is insufficient to diagnose BrS, as mutations in this gene could result in a number of different phenotypes. Instead, BrS must be diagnosed only in the presence of a diagnostic type 1 BrS ECG pattern (spontaneous or drug-induced), not due to secondary causes, such as electrolyte disturbances or myocardial ischemia.

Other Candidate Genes

A recent study (9) concluded that only the *SCN5A* gene should be analyzed in BrS patients. We agree that mutations in *SCN5A* could be the cause of BrS in some patients. However, the study did not address what should be done in the majority of BrS patients, who test negative for any *SCN5A* mutations, nor provide clarity of the disease mechanism in those patients negative

for *SCN5A* mutations, especially regarding the role of copy number variations and mitochondrial DNA. London expressed his disagreement, arguing that eliminating other genes from testing panels could stifle scientific advancement (10). Wilde and Gollob (38), however, countered by arguing that undue harm from incorrect interpretation could result in a life-changing diagnosis, require intervention, create life-long anxiety, and impact asymptomatic family members. We believe that suspected candidate genes should be tested and studied so that we can better understand their effects. However, all suspected cases should be confirmed by the presence of the BrS pattern, including patients found to have mutations in *SCN5A*, as single mutations in this gene are responsible for a variety of phenotypes, not only BrS (39, 40), and may not even cause BrS on their own (41).

Many other genes such as *SCN10A* (42, 43), *SCN4A* (44), *SCN1B* (45), *KCNH2*(46), *RANGRF* (47), *PKP2*(48), *TPM1*(49), and several calcium channels genes (50–53) have been described in patients clinically affected by BrS. Whole exome sequencing with a high coverage was performed in a family with both hypertrophic cardiomyopathy and type-1 BrS, apparently caused by the same heterozygous *TPM1* mutation (49). Thus, several candidate genes exist and should be further studied. Physiologic studies should follow the discovery of candidate mutations in the clinic, as abnormal effects in the physiology laboratory can provide useful insights to understanding particular new mutations.

Modes of Inheritance

In spite of recent developments in the field of genetics, BrS is often still considered a monogenic Mendelian disease (54) inherited in an autosomal dominant fashion with incomplete penetrance (55–58). This is mainly due to the description of BrS in a family in which the genetics were consistent with this kind of transmission (59), making *SCN5A* the only accepted BrS gene (9). Another reason why *SCN5A* is so "popular" is because the segregation of variants in this gene show incomplete penetrance and marked variability in a significant percentage of patients (60). However, increasing evidence suggests that BrS in some patients might be actually caused by a digenic inheritance (61) or a combined effect of multiple variants (62), including polymorphisms (63). In this subset of patients, it is difficult to identify the real molecular cause of BrS, making it difficult to understand, using only genetic testing, which family members have inherited the syndrome and which have not. Additionally, since BrS may be due to a combined effect of multiple variants, the severity can often be different between family members (40). Furthermore, there might be other cases in which some family members have the syndrome but others do not, despite sharing certain variants, because of differences in modifier genes.

Although autosomal dominant inheritance with incomplete penetrance is the most commonly accepted mode of transmission of BrS, other forms of transmission have been suggested, such as recessive (64) and X-linked (19, 20). It is also possible that yet-undiscovered somatic mutations could have an effect on the heart. Furthermore, an autosomal dominant inheritance pattern could imply that the disease is Mendelian in nature, caused by a single mutation in a single gene. However, several studies have demonstrated an oligogenic mode of inheritance (7).

Therefore, likely, in some families, a particular variant causes BrS in a Mendelian fashion, while in other families, the pattern of inheritance is more complicated to understand, because the disease is caused by a combination of factors, resulting in different phenotypes even between family members (65). Tadros et al. calculating polygenic risk scores (PRSs) for PR interval, QRS duration, and BrS, reported that 44 common variants associated with PR, and 26 common variants associated with QRS, in the general population, were associated with ajmaline-induced PR and QRS prolongation, respectively. Also, a 3-single-nucleotide-polymorphism PRS derived from a case-control BrS GWAS was independently associated with ajmaline-induced type-1 BrS ECG (66). This demonstrates the importance of polymorphisms that might predispose to arrhythmias and create a pathological effect, especially in the presence of other variants in the same patient.

Overlap Syndromes

Since variants in *SCN5A* can be found in several cardiogenetic disorders, it is not surprising to observe an overlap between BrS and other pathologies. For example, BrS can be diagnosed in the proband while LQTS, epilepsy, febrile seizures, or complete bundle branch block can be present in the family members (67–70).

Overlap between arrhythmogenic right ventricular (RV) dysplasia/cardiomyopathy (ARVD/C) and BrS has been described by many groups (71), the mechanism of which may involve cell-cell junctions (24). Both ARVC and BrS can originate from mutations in the connexome, and the phenotype that emerges depends on the type of connexome mutation (72, 73). *PKP2* may be an important gene in this regard, as mutations in *PKP2* can result in loss of desmosomal integrity, cause sodium current deficit, and be found in patients with BrS (74, 75). The presence of ARVC in BrS patients has been associated with higher arrhythmic risk (76). The genetics of families with overlap syndromes should be carefully considered, as these genetic causes may be different than other families in which BrS is the only phenotype observed. This is yet another example of the need for personalized medicine and to consider the genetics of BrS on a family-by-family basis.

Mitochondrial Considerations

Many recent studies have related cardiac arrhythmias, and particularly BrS, to mitochondrial function, or the effect of mitochondrial products on the sodium channel. Heart arrhythmias can originate from pathophysiology of the mitochondria, which produce adenosine triphosphate, a compound required for normal ion channel function (77). Aiba et al. described a family with BrS and the *SCN5A* mutation R526H, which is a PKA consensus phosphorylation site and associated with reduced basal I_{Na} due to the inability of PKA to act on the sodium channel to increase the sodium current (78). A mutation in the GPD1-L protein reduces I_{Na} by raising intracellular NADH levels and inducing reactive oxygen species (ROS) (79). This process of ROS production, its release from mitochondria, and thus its detrimental effect on the sodium current can be reversed in several ways, namely by NAD+, inhibition of mitochondrial electron transport, a mitochondrial targeted antioxidant, and an inner membrane

anion channel modulator (80). A specific mitochondrial DNA (mtDNA) allelic combination and a high number of mtDNA single nucleotide polymorphisms (SNPs) have been reported in association with more severe cases of BrS, suggesting that these are important cofactors in the expression of the clinical phenotype (81, 82). Tafti et al. suggested that BrS may be caused by mutations in mitochondrial transfer RNA (tRNA) genes, leading to deficiencies in the translational process of critical proteins of the respiratory chain (83). Reports have demonstrated that tRNAMet, tRNAIle, tRNATrp and tRNAGln genes are hot spots for cardiovascular diseases (83, 84). Thus, mitochondrial function, or malfunction, contributes to sodium channel function and to cardiac rhythm.

Risk Stratification

Risk stratification in BrS has previously relied on clinical scores (85), including familial history of sudden cardiac death, personal history of syncope, aborted cardiac arrest, spontaneous type-1 BrS pattern, or male gender. It was also reported that proband status, inducibility toward ventricular arrhythmias (86), arrhythmogenic substrate area, and late potentials (87) were predictors of higher risk. Our group recently proposed the *SCN5A* genetic status as a prognostic factor for BrS patients (12, 88). In particular, *SCN5A* mutation carriers exhibited more pronounced epicardial electrical abnormalities and a more aggressive clinical presentation. In at least a subgroup of patients, the mutated *SCN5A* gene acts more like a phenotype modulator than a real Mendelian dominant cause of the displayed phenotype, possibly calling into question the autosomal dominant inheritance of BrS. This is true also for variants of "unknown significance" (VUS), which are generally treated as "benign." However, in our experience, several of these VUS are later reclassified as pathogenic. We believe that, in time, many other VUS, especially in the *SCN5A* gene, will be determined to be pathogenic, considering also that the oligogenic model is likely to be accepted in the near future.

DISCUSSION

The genetics of BrS have likely remained elusive because of how the disease has been considered only an autosomal dominant Mendelian disorder. However, when BrS is considered an oligogenic disorder, it may be possible to use genetics to predict the BrS phenotype. Besides direct modifications in the $Na_V1.5$ protein, its function can be altered by many regulatory proteins like Hey2, Mog1, Gpd1-L, and others. According to us, studying the genes encoding those proteins is very important for the clinical management of BrS patients. Additionally, environmental factors might influence channel function through post-translational modifications. Even in families where *SCN5A* variants have been found, segregation analysis is not always consistent with autosomal dominant inheritance, demanding caution be used when interpreting genetic test results. Currently, it is necessary that all suspected cases of BrS are confirmed with ECG, using, when necessary, drug challenge to elicit the type-1 pattern. In other words, genetic testing alone should not be used for diagnostic purposes at this time, but rather, the

patients should each fulfill the diagnostic criteria for BrS at an arrhythmologic examination, as per the current guidelines (89). However, in families in which a *SCN5A* pathogenic variant is found, genetic testing could possibly contribute to the prognostic risk stratification.

Ideally, whole exome or whole genome testing should be performed to both confirm candidate genes and identify new ones. Collecting family segregation is mandatory to understand whether a particular variant might be clinically relevant. Ideally, such data should then be deposited into international databases. The specific effects of distinct variants should be studied, rather than necessarily grouping together, for example, "all *SCN5A* variants" when trying to determine genotype-phenotype relationships, because not all variants within a particular gene act similarly.

Identifying variants involved in oligogenic cases of BrS is extremely complicated. For this, the effect of polymorphisms, which, on their own, are considered benign, should be considered, as they may act as modifiers in the presence of other variants. For example, two variants in a particular gene may exist, which, individually, result in a benign phenotype, as neither variant, on their own, significantly modifies the ultimate function of the resulting protein. However, if those two (or three, or more) variants occur together in the same person, together they could ultimately impair the function of the protein, altering the clinical picture. This "mutational load" is an important concept in BrS, explaining why the genetics of this disease have been so difficult to elucidate. However, to understand the effect of mutational load, or compound heterozygosity (i.e., two or more heterogeneous recessive alleles at a particular locus), extensive research studies should be performed, also identifying other genes responsible for BrS, besides *SCN5A*. Only then it will be possible to study these concepts of oligogenic inheritance in the majority of patients. Probably, whole genome or whole exome studies would be useful in determining the genes involved, along with family segregation analysis.

Finally, non-genomic DNA considerations should be mentioned, as post-translational modifications of the sodium channel could affect its function without any variants in the *SCN5A* gene. Studies should be expanded to better understand any possible role for mitochondrial involvement, including the analysis of mitochondrial genes, their products, and their functional effects on the cells. Environmental factors should also be studied, including anything to which families may be exposed, resulting in post-translational effects, especially when probands test negative for variants in all BrS candidate genes. Environmental factors could be mistaken as a genetic condition when several family members living in the same environment are affected.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MM and EM drafted the paper. EL and CP provided revisions and useful feedback. CP secured funding for the project. All authors approved the final version of the manuscript.

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