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SCN5A Mutation Increases the Risk of Major Arrhythmic Events in Asian Population of Brugada Syndrome: Systematic Review and Meta-analysis

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Abstract

Background: Brugada syndrome (BrS) is an inherited arrhythmic disease linked to *SCN5A* mutations. It is controversial whether *SCN5A* mutation carriers possess a greater risk of major arrhythmic events (MAE). We examined the association of *SCN5A* mutations and MAE in BrS patients.

Methods: We comprehensively searched the databases of MEDLINE and EMBASE from inception to September 2017. Included studies were published cohort and case-control studies that compared MAE in BrS patients with and without *SCN5A* mutations. Data from each study were combined using the random-effects model. Generic inverse variance method of DerSimonian and Laird was employed to calculate the risk ratios (RR) and 95% confidence

intervals (CI).

Results: Seven studies from March 2002 to October 2017 were included (1,049 BrS subjects). *SCN5A* mutations were associated with MAE in Asian populations (RR=2.03, 95% CI: 1.37-3.00, p = 0.0004, I^2=0.0%), patients who were symptomatic (RR=2.66, 95% CI: 1.62-4.36, p=0.0001, I^2=23.0%), and individuals with spontaneous Type-1 Brugada pattern (RR=1.84, 95% CI: 1.05-3.23, p=0.03, I^2=0.0%).

Conclusions: *SCN5A* mutations in BrS increase the risk of MAE in Asian populations, symptomatic BrS patients, and individuals with spontaneous Type-1 Brugada pattern. Our study suggests that *SCN5A* mutation status should be an important tool for risk assessment in BrS patients.

Keyword : *SCN5A*, Brugada syndrome, major arrhythmic events, sudden cardiac death, genetic

Abbreviations

BrS	Brugada syndrome
ICD	implantable cardioverter defibrillator
MAE	major arrhythmic events
CI	confidence intervals
RR	risk ratio
CNVs	copy-number variants

Introduction

Brugada syndrome (BrS), first described by Brugada et al. in 1992 (Brugada & Brugada, 1992), is an autosomal dominant inherited arrhythmia syndrome characterized by ST-segment elevation in the right precordial leads without obvious evidence of ischemia, electrolyte disturbances, or structural heart disease. It predisposes patients to major arrhythmic events (MAE) including sustained ventricular tachycardia, ventricular fibrillation, appropriate implantable cardioverter defibrillator (ICD) shocks, aborted cardiac arrest, and sudden cardiac death (Brugada & Brugada, 1992; Priori et al., 2013). There are geographic differences in the prevalence of BrS: it varies from 0.5 to 4 per 1,000 in Asian countries such as Thailand, the Philippines, Japan, and Singapore (Rattanawong, Ngarmukos, et al., 2017; Rattanawong et al., 2016), whereas the prevalence is less than 0.2 per 1,000 in the western hemisphere (Kamakura, 2013). It is more common in men and can be induced by fever, with the prevalence of fever-induced BrS of approximately 2-4% (Kamakura, 2013; Rattanawong et al., 2016).

The most common identifiable genetic defect in BrS lies in the *SCN5A* gene, which encodes the α-subunit of the NaV1.5 cardiac sodium channel and accounts for 14-26% of the cases (Chen et al., 1998; Yamagata et al., 2017). More than 300 mutations in the *SCN5A* gene have been linked to the syndrome (Juang & Horie, 2016). Until now, the only preventive measure for sudden cardiac death in BrS is ICD implantation; thus, risk stratification to select the patient in whom ICD is appropriate is crucial (Probst et al., 2010). Yet, the use of *SCN5A* mutation status to prognosticate the risk of MAE in BrS patients has been controversial (Adler et al., 2016): some studies showed positive results (Makarawate et al., 2017; Nishii et al., 2010; Yamagata et al., 2017), while others failed to correlate the mutation to subsequent

MAE (Andorin et al., 2016; Gehi, Duong, Metz, Gomes, & Mehta, 2006; Priori et al., 2002; Probst et al., 2010). The goal of this systematic review and meta-analysis was to examine the association of *SCN5A* mutations and MAE in BrS patients.

Method

Search strategy

Two investigators (WV and PC) independently searched for published studies indexed in MEDLINE and EMBASE databases from inception to September 2017 using a search strategy that included the terms for "*SCN5A*", "mutation", and "Brugada". Only English language publications were included. A manual search for additional pertinent studies and review articles using references from retrieved articles was also completed.

Inclusion criteria

The eligibility criteria included the following:

(1) Cohort study (prospective or retrospective) or case control study reporting the incidence of MAE in BrS patients with and without *SCN5A* mutations

(2) Calculation of relative risk, hazard ratio, odds ratio, incidence ratio, or standardized incidence ratio with 95% confidence intervals (CI) or provision of sufficient raw data for these calculations were provided.

(3) Use of BrS participants without *SCN5A* mutations were used as controls.

Study eligibility was independently determined by two investigators (JC and PM) and any discrepancies were resolved by mutual consensus. Newcastle-Ottawa quality assessment scale was used to evaluate each study's quality. The scale uses a star system (0 to 9) to evaluate three domains: recruitment and selection of the participants, similarity and comparability between the groups, and ascertainment of the outcome of interest among cohort studies. Higher scores represent higher study quality (Stang, 2010).

Data extraction

A standardized data collection form was used to obtain the following information from each study: title of study, name of first author, year of study, year of publication, country of origin, number of participants, demographic data of participants, method used to identify cases and controls, methods used to diagnose the outcomes of interest (*SCN5A* mutation and MAE), methods to verify if the variants were disease-causing, and average

duration of follow-up with confounders that were adjusted effect estimates with 95% CI and covariates that were adjusted in the multivariable analysis.

To ascertain the accuracy, all investigators independently performed this data extraction process. Any data discrepancy was resolved by referring back to the original articles.

Statistical analysis

We performed a meta-analysis of the included cohort studies using a random-effects model. The extracted studies were excluded from the analysis if they did not present an outcome in each intervention group or did not have enough information required for continuous data comparison. We pooled the point estimates from each study using the generic inverse-variance method of Der Simonian and Laird (DerSimonian & Laird, 1986). The heterogeneity of effect size estimates across these studies was quantified using the I^2 statistic and Q statistic. For the Q statistic, substantial heterogeneity was defined as $p<0.10$. The I^2 statistic ranges in value from 0 to 100% ($I^2<25\%$, low heterogeneity; $I^2=25\%–50\%$, moderate heterogeneity; and $I^2>50\%$, substantial heterogeneity) (Higgins, Thompson, Deeks, & Altman, 2003). A sensitivity analysis was performed to assess the influence of the individual studies on the overall results by omitting one study at a time. We used a sequential omitting strategy, as described by Patsopoulos and colleagues, to examine whether overall estimates were influenced by the substantial heterogeneity observed (Patsopoulos, Evangelou, & Ioannidis, 2008). Publication bias was assessed using a funnel plot and Egger's regression test ($p<0.05$ was considered significant) (Sterne & Egger, 2001). Potential sources of heterogeneity from clinical characteristics were analyzed with subgroup analysis and were compared with meta-regression. Pooled risk ratio (RR), sensitivity analysis, funnel plot, and forest plot were performed using Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. Egger test was performed using the Stata SE 14.1 software from StataCorp LP.

Results

Description of included studies

Our search strategy yielded 382 potentially relevant articles (227 articles from EMBASE and 155 articles from MEDLINE). After exclusion of one duplicate, 253 articles underwent title and abstract review. Two hundreds and eleven articles were excluded at this stage since they were not cohort studies and they were not conducted in patients with BrS, leaving 42 articles for full-length article review. Thirty-five articles were excluded because

they did not report the outcome of interest or they did not have control. Therefore, seven prospective cohort studies of BrS patients were included in this meta-analysis. Figure 1 outlines the search and literature review process. The clinical characteristics and summary of included studies are provided in Table 1. The Newcastle–Ottawa scales of the included studies are described in the Table 1.

Meta-analysis results

Seven studies from March 2002 to October 2017 were included in this meta-analysis involving 1,049 subjects with BrS (302 patients with *SCN5A* mutations and 747 patients without *SCN5A* mutations). Five studies revealed an increased MAE among BrS patients with *SCN5A* mutations (Andorin et al., 2016; Conte et al., 2015; Makarawate et al., 2017; Nishii et al., 2010; Yamagata et al., 2017) with one of the five studies (Makarawate et al., 2017) achieving statistical significance. The pooled analysis demonstrated a non-significant increased risk of MAE in BrS patients with *SCN5A* mutations compared to those without the mutation, with a pooled RR of 1.50 (95% CI: 0.93-2.41, p= 0.10, I^2=38.0%). A forest plot of this meta-analysis is shown in Figure 2.

In subgroup analysis among ethnicities, three studies (two studies from Japan and one study from Thailand) were included in Asian populations (Makarawate et al., 2017; Nishii et al., 2010; Yamagata et al., 2017) involving 486 subjects with BrS (80 patients with *SCN5A* mutations and 406 patients without *SCN5A* mutations). All three studies revealed increased MAE among BrS patients with *SCN5A* mutations (Makarawate et al., 2017; Nishii et al., 2010; Yamagata et al., 2017) with one study (Makarawate et al., 2017) achieving statistical significance. The pooled analysis demonstrated a significant increased risk of MAE in Asian BrS patients with *SCN5A* mutations compared to those without the mutation (RR=1.78, 95% CI: 1.23-2.58, p = 0.002, I^2=0%). A forest plot of this meta-analysis is shown in Figure 2.

In Caucasian cohorts, four studies were included in the analysis (Andorin et al., 2016; Conte et al., 2015; Eckardt et al., 2005; Priori et al., 2002) involving 563 subjects with BrS (222 patients with *SCN5A* mutations and 341 patients without *SCN5A* mutations). One study revealed a non-significant increase in MAE among BrS patients with *SCN5A* mutations (Andorin et al., 2016). The pooled analysis did not demonstrate an increased risk of MAE in Caucasian BrS patients with *SCN5A* mutations compared to those without the mutation (RR=0.78, 95% CI: 0.34-1.80, p=0.57, I^2=15%). A forest plot of this meta-analysis is shown in Figure 2.

In symptomatic BrS patients, there were four studies (Andorin et al., 2016; Makarawate et al., 2017; Nishii et al., 2010; Yamagata et al., 2017) involving 271 subjects with BrS (60 patients with *SCN5A* mutations and 211 patients without *SCN5A* mutations). Every study revealed an increased MAE among symptomatic BrS patients with *SCN5A* mutations with two studies (Makarawate et al., 2017) achieving statistical significance. The pooled analysis demonstrated a significant increased risk of MAE in Asian BrS patients with *SCN5A* mutations compared to those without the mutation (RR=1.78, 95% CI: 1.23-2.58, p = 0.0001, I^2=23%). A forest plot of this meta-analysis is shown in Figure 3.1. The pooled analysis of asymptomatic BrS patient showed an increased but non-significant risk of MAE (RR=1.85, 95% CI: 0.60-5.68, p = 0.28, I^2=0%). However, only two studies involving 290 subjects with asymptomatic BrS (78 patients with *SCN5A* mutations and 212 patients without *SCN5A* mutations) reported data suitable for meta-analysis (Figure 3.2).

Two studies reported on spontaneous Type-1 Brugada pattern (Andorin et al., 2016; Yamagata et al., 2017) involving 327 subjects with BrS and *SCN5A* status (68 patients with *SCN5A* mutations and 259 patients without *SCN5A* mutations). The pooled analysis demonstrated a significant increased risk of MAE in BrS patients who presented with spontaneous Type-1 Brugada pattern with *SCN5A* mutations compared to those without the mutation (RR=1.84, 95% CI: 1.05-3.23, p = 0.03, I^2=0.0%). A forest plot of this meta-analysis is shown in Figure 3.3.

Sensitivity analysis

To assess the stability of the result, we conducted a sensitivity analysis by omitting one study at a time. We used a sequential omitting strategy, as described by Patsopoulos and colleagues, to examine whether overall estimates were influenced by the substantial heterogeneity observed (Patsopoulos et al., 2008). In the overall analysis, when omitting the study reported by Eckardt et al., the pooled analysis demonstrated a significantly increased risk of MAE in BrS patients with *SCN5A* mutations compared to those without the mutation, with a pooled RR of 1.65 (95% CI: 1.09-2.51, p= 0.019, I^2=24.3%) as well as the study reported by Priori et al. 1.89 (95% CI: 1.31-2.74, p= 0.001, I^2=0.0%) (Figure 5). In the subgroup analysis, none of the results was significantly altered.

Publication bias

To investigate potential publication bias, we examined the contour-enhanced funnel plot of the included studies in assessing change in the log odd ratio of death or composite outcome (Figure 4). The vertical axis represents study size (standard error) while the horizontal axis represents effect size (log odds ratio). The distribution of studies on both sides of the mean was symmetrical. The Egger's test was non-significant for small-study bias in overall analysis (p=0.518), symptomatic BrS (p=0.787), Caucasians (p=0.756), and Asians (p=0.095). Egger's test could not be performed in asymptomatic BrS, and spontaneous Type-1 pattern subgroups since there were only 2 studies. Meta-regression confirmed that the studies published before 2010 were significant sources of heterogeneity (p=0.035) but available verification status of *SCN5A* disease-causing variants was not a significant source of heterogeneity (p=0.968)

Discussion

We have analyzed 1,049 subjects with BrS from seven studies and showed an association between the presence of *SCN5A* mutations and a risk for developing MAE in Asian populations, patients with symptomatic BrS, and individuals with spontaneous Type-1 Brugada pattern. Increased but not statistically significant risk was found in Caucasians, all BrS individuals, and asymptomatic BrS subjects. The non-significant association in overall BrS may be due to inter-study and intra-study demographic and genetic variations.

After performing subgroup analyses, the association between *SCN5A* status and MAE in some groups appeared more significant with decreased heterogeneities. Overall BrS individual's analysis showed moderate heterogeneity of 38.0% whereas subgroups analysis showed low heterogeneity (0% in Asian, 23% in symptomatic, 0% in spontaneous Type-1, 15% in Caucasian, and 0% in asymptomatic). These results reflected the effect of individual basic characteristics to the outcome of MAE, which is more homogenized when analyzed by each subgroup.

Additionally, to explore the possible sources of heterogeneity in our meta-analysis we used sensitivity analysis by omitting one study at a time. When omitting the studies published by Eckardt et al. and Priori et al. from the overall analysis, we found a significantly increased risk of MAE in BrS patients with *SCN5A* mutations compared to those without the mutations, with a pooled RR of 1.65 (95% CI: 1.09-2.51, p= 0.019) and 1.89 (95% CI: 1.31-2.74, p= 0.001, I^2=0.0%), consecutively (Figure 5). Heterogeneity decreased from moderate (38.0%)

to low (24.3%) when we omitted only Eckardt et al. and from moderate (38.0%) to none (0.0%) when we omitted only Priori et al. These sensitivity analysis results can be explained by several possible reasons. First, since the early publications in 2002 and 2005, more then 300 novel *SCN5A* mutations have been discovered (Juang & Horie, 2016). Hence, Eckardt et al. and Priori et al. may not have studied several mutations included in our study and meta-regression confirmed that the studies published before 2010 (Eckardt et al., 2005; Priori et al., 2002) are significant sources of heterogeneity (p=0.035). Second, their study populations were mostly asymptomatic Caucasian individuals (58% in Eckardt et al. and 72% in Priori et al.). The subgroup analyses from our study indicated significant associations of *SCN5A* mutations and MAE in symptomatic and Asian groups, but not in the Caucasian group. These aforementioned factors are thus suggestive of existing heterogeneity interfering with the results from our analyses. The cause of heterogeneity is also noted in the study done by Makarawate et al. which correlated *SCN5A* mutation status with cardiac conduction disturbances and resultant appropriate ICD shocks (Makarawate et al., 2017). Their study included a geographically and genetically isolated population: most patients were of northeastern Thai origin; only symptomatic patients were included; and only two polymorphisms were identified (R1193Q and H558R). A degree of pathogenicity of these two variants were questionable and may be more, or less, malignant than those reported in other studies.

It is well recognized that a history of aborted cardiac arrest is one of the strongest predictors for future MAE in BrS patients (Eckardt et al., 2005; Priori et al., 2002; Probst et al., 2010; Yamagata et al., 2017); in fact, the current guidelines recommends that those who survive episodes of cardiac arrest should undergo ICD implantation (Priori et al., 2013) since the risk of subsequent cardiac events was highest among this patient subgroup and was estimated as 7.7% per year in one study (Probst et al., 2010). On the other hand, the risk of MAE in asymptomatic BrS individuals is low, approximately 0.5% per year (Probst et al., 2010). Other reported potential risks include male sex, presence of spontaneous ST-segment elevation in the precordial leads, positive electrophysiological study, presence of atrial fibrillation, and certain electrocardiographic conduction abnormalities such as prolonged P-wave, prolonged QRS duration, and fragmented QRS (Chen et al., 1998; Gehi et al., 2006; Priori et al., 2002; Rattanawong, Riangwiwat, et al., 2017; Yamagata et al., 2017). Priori et al. demonstrated that the presence of both syncope and spontaneous ST-segment elevation have a sensitivity of 36% and a high specificity of 94% in predicting the occurrence of

cardiac arrest in BrS patients (Priori et al., 2002). On the contrary, many studies have assessed family history of sudden cardiac death as a predictor for poorer outcomes, and the results were reproducibly unrevealing (Gehi et al., 2006; Makarawate et al., 2017; Nishii et al., 2010; Priori et al., 2002). Clinicians have long been intrigued by the concept of using *SCN5A* mutation status to predict MAE in BrS patients.

Adler et al. have recently reviewed the risk stratification strategy in patients with BrS and stated that, according to the large registries, the use of genetic data to risk stratify BrS patients are not well-defined and challenging (Adler et al., 2016). Even though a risk score based on the mutations and other polymorphisms has been developed (Sommariva et al., 2013), the authors suggested that the tool needs to be validated before being adopted. In 2006, Gehi et al. analyzed 383 patients from two publications (Eckardt et al., 2005; Priori et al., 2002) and found no link between *SCN5A* mutations and increased risk of sudden cardiac death, syncope, or ICD shock (relative risk 0.60, CI: 0.29-1.26) (Gehi et al., 2006). We speculate that the non-significant result in their study was due to a lower number of recruited patients, lower number of included studies, and limited power to identify a minimal increase in risk. To our knowledge, our study is the first meta-analysis to demonstrate the potential utilization of *SCN5A* mutation in the risk stratification scheme, particularly in certain subgroups, of BrS.

SCN5A mutations were reported in approximately 20-25% of BrS patients and known as the most common BrS-associated gene. Almost 300 *SCN5A* mutations have been identified in BrS, including missense mutations, nonsense mutations, nucleotide insertion/deletions, and splice site mutations, and the number of *SCN5A* mutations is still increasing (Juang & Horie, 2016). An *SCN5A* mutation does not necessarily indicate BrS (Probst et al., 2009). The functional loss of NaV1.5 cardiac sodium channel with subsequent reduced sodium current is typically described in BrS patients with *SCN5A* mutations (Juang & Horie, 2016). This is supported by the fact that BrS-associated *SCN5A* mutations usually result in frameshift errors, splice-site defects, or premature stop codons that lead to nonfunctional channels (Chen et al., 1998). BrS-causing missense mutations were observed to be nonfunctional due to either disrupted protein trafficking to the cell membrane or impaired sodium conductance (George, 2005). Although some missense mutations are functional, they may cause defective gated properties of the channels involving activation and/or inactivation kinetics (Andorin et al., 2016; Rook et al., 1999). Meregalli et al. corroborated this speculation by studying 147

mutation-positive BrS individuals and divided them into three groups: 1) those with prematurely truncated proteins (group 1); 2) those with missense mutations resulting in significantly (>90%) reduced peak sodium current (group 2); and 3) those with missense mutations resulting in mildly (≤90%) reduced peak sodium current (group 3) (Meregalli et al., 2009). They found that patients in group 1 and group 2, in which drastic peak sodium current were noted, developed a more severe conduction disorders. The underlying electrophysiological mechanisms of how altered sodium current causes BrS is still under investigation, and two models have been proposed (Meregalli, Wilde, & Tan, 2005). In the "repolarization disorder" model, the defective sodium channel reduces the myocardial sodium current and causes a disproportionate shortening of the right ventricular epicardial action potentials, leading to an exaggerated transmural (i.e. epicardium-to-myocardium) voltage gradient and the characteristics finding on electrocardiogram (George, 2005; Juang & Horie, 2016; Smits et al., 2002). The "depolarization disorder" model theorizes that conduction delay in the right ventricular outflow tract, with respect to the right ventricle, causes the electrocardiogram changes in BrS. The arrhythmogenicity of BrS is likely multifactorial and other pathophysiology may play a role: for instance, a recent study proposed epicardial surface fibrosis and reduced gap junction expression in the right ventricular outflow tract as arrhythmogenic mechanisms in BrS (Nademanee et al., 2015).

When compared to *SCN5A*-negative BrS patients, those with *SCN5A* mutations tend to exhibit significantly longer conduction intervals on electrocardiogram, such as PQ or His-to-ventricle intervals, and more fragmented QRS, both at baseline and throughout the follow-up (Makarawate et al., 2017; Nishii et al., 2010; Rattanawong, Riangwiwat, et al., 2017; Smits et al., 2002; Yokokawa et al., 2007). These parameters were also predictive of the presence of the mutation: for example, PQ duration of ≥ 210 milliseconds had a sensitivity of 48% and a specificity of 98% for detecting *SCN5A* mutation in BrS patients (Smits et al., 2002). The prognostic value of *SCN5A* status has become more apparent in recent well-designed studies. In a study of 415 BrS patients reported by Yamagata et al, *SCN5A* mutation carriers tended to experience their cardiac events more frequently and at younger ages (Yamagata et al., 2017). Apart from history of aborted cardiac arrest, harboring the mutation was the only independent predictor of MAE, with a hazard ratio of 2.0 (95%CI: 1.0-3.8). They also found that mutations in the pore region of the NaV1.5 cardiac sodium channel were more associated with MAE (Yamagata et al., 2017). Hence, these studies have confirmed the genotype-

phenotype correlations in *SCN5A* mutation positive BrS individuals, both at electrocardiographic level and clinical level.

Recently, Nadeemanee et al. reported right ventricular outflow tract epicardial ablation in recurrent symptomatic Brugada syndrome (Nademanee et al., 2011). The indication for right ventricular outflow tract epicardial ablation in symptomatic Brugada syndrome is still unclear. Right ventricular outflow tract fibrosis and conduction delay was identified in carriers of *SCN5A* mutations (Meregalli et al., 2009). Moreover, age-related fibrosis has also been seen in mouse models of *SCN5A* mutation (Jeevaratnam et al., 2012; Royer et al., 2005). Therefore, *SCN5A* mutations may contribute to substrate changes which may be treatable with epicardial ablation. In our study, we found that *SCN5A* mutations in symptomatic Brugada syndrome is two-fold associated with major arrhythmic events compared to symptomatic Brugada syndrome without *SCN5A* mutations. *SCN5A* mutation status may therefore enhance risk stratification of symptomatic Brugada syndrome.

Limitations

Although most recruited studies were of high quality, we recognize there are some limitations to our analysis. First, the studies are heterogeneous as discussed earlier. The potential sources of heterogeneity include age and gender of participants, definitions of MAE in each study, follow-up duration, inclusion of mutation-positive screened family members or of asymptomatic carriers, geographic difference, and recruiting protocol (e.g. multicenter registry vs. single center). Second, genetic heterogeneity also existed among studies. For instance, Yamagata et al. identified 55 different mutations in 60 affected individuals in their multicenter cohort, whereas Makarawate et al. found only two different mutated alleles in 13 *SCN5A* mutation carriers (Makarawate et al., 2017; Yamagata et al., 2017). However, since BrS is uncommon and large-scale genetic studies have been rarely performed, the possibility of small-study bias due to the small number of included studies and small sample size is not negligible. A larger study with a more homogeneous population is needed to confirm our results. Unfortunately, there was not enough information reported in 2 articles that we could use to calculate multivariate adjusted RR. Risk ratios were calculated based on number of the patients without multivariate adjustment. Third, large genomic imbalances, such as copy-number variants (CNVs), in *SCN5A* were recently shown to underlie a portion of genotype-negative patients and should be screened (Mademont-Soler et al., 2016; Sonoda et al., 2018). However, all of the recruited studies have used traditional methods of sequencing which could not detect CNVs; hence, the control group may include those CNV-harboring

sequencing-negative patients and did not truly represent unaffected individuals. Fourth, three (Makarawate et al., 2017; Priori et al., 2013; Yamagata et al., 2017) of seven studies reported information on how the authors verified if the variants were disease-causing (Table 1); however, meta-regression confirmed that verification status of *SCN5A* disease-causing variants was not a significant source of heterogeneity in overall results. Lastly, this is a meta-analysis of observational studies with the inherent limitation of being able to confirm an association, but not a causal relationship.

Conclusion

From our study, we found that mutation status may help predict MAE and guide treatment decisions in certain subgroups of BrS, especially in Asian population, symptomatic patients, and individuals with spontaneous Type-1 Brugada pattern. The presence of *SCN5A* mutations may be an important tool to prognosticate risk and guide treatment in patients with BrS in the future.

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Figure and table legends

Figure 1 Search methodology and selection process

Figure 2 Forest plot of the included studies assessing the association between *SCN5A* mutation and major arrhythmic events and fatal arrhythmia among Asian, Caucasian, and overall analysis.

Figure 3 Forest plot of the included studies assessing the association between *SCN5A* mutation and major arrhythmic events and fatal arrhythmia in subgroup analysis of 1) symptomatic BrS, 2) asymptomatic BrS, and 3)spontaneous Type-1 BrS.

Figure 4 Funnel plot of *SCN5A* mutation and major arrhythmic events in 1) overall analysis, Asian, and Caucasian, 2) symptomatic, 3) asymptomatic, and 4) spontaneous Type-1. Circles represent observed published studies.

Figure 5 Sensitivity analysis graph to explore source of heterogeneity by omitting one study at a time.

Table 1: The characteristics and summary of included studies.

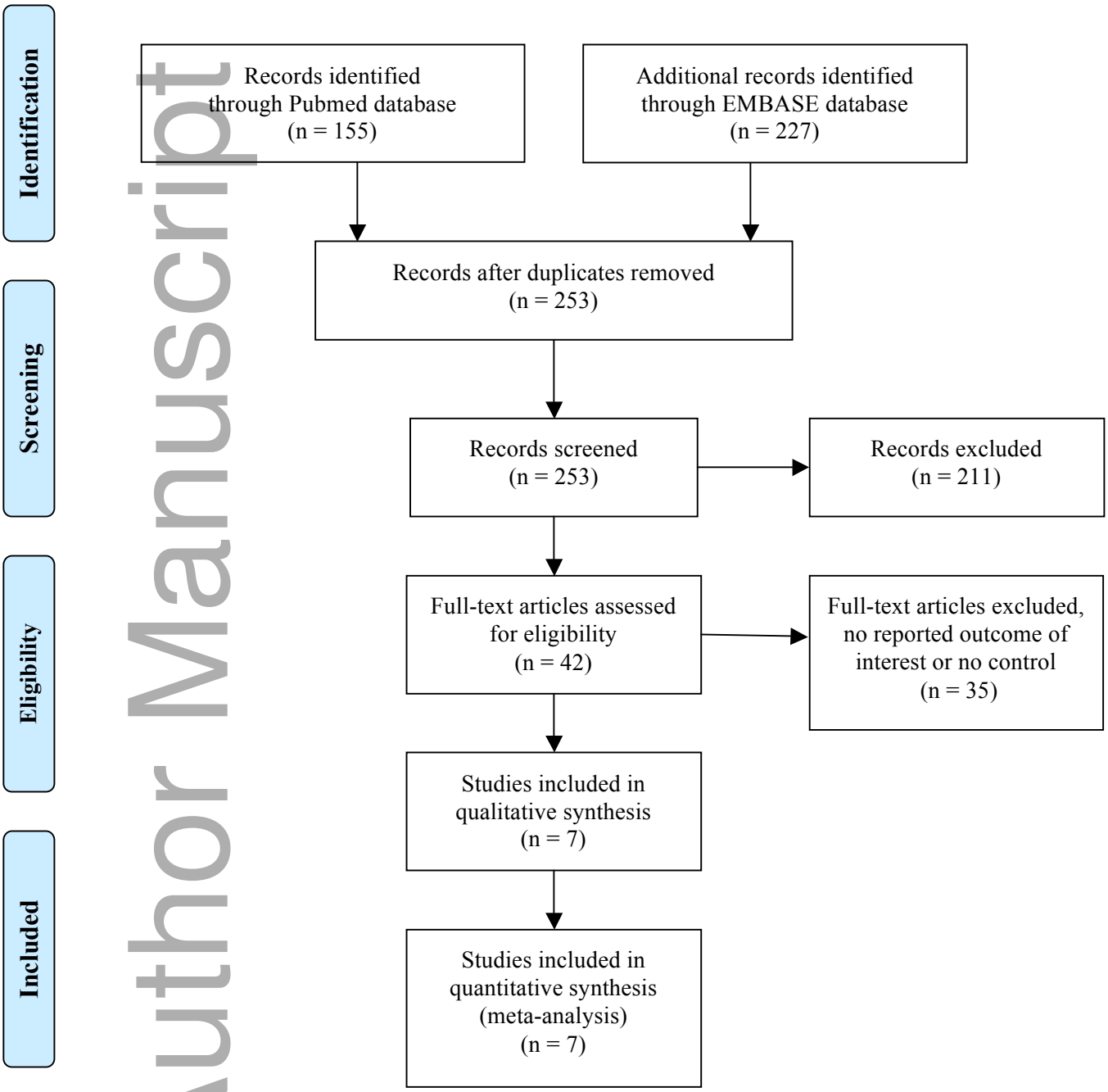
Table 1: The clinical characteristics and summary of included studies

	Priori SG et al.	**Eckardt et al.**	**Nishii et al.**	**Conte al.**	**Andorin et al.**	**Makarawate et al.**	**Yamagata et al.**
Country	Italy	Netherlands, Germany, and France	Japan	Belgium	European countries	Thailand	Japan
Study design	Prospective cohort	Prospective cohort	Prospective cohort	Retrospective cohort	Prospective cohort	Prospective cohort	Prospective cohort
Year of Publication	2002	2005	2010	2015	2016	2017	2017
Study subjects	BrS patient (130 probands, 70 Affected family members)	BrS with Type-1 BrP patients from 4 university hospitals	BrS with Type-1 BrP patients who were admitted to 5 hospitals in Japan during January 1997 to December 2009	BrS with spontaneous or drug-induced Brugada Type-1 BrP who underwent ICD implantation and follow-up at a single study	BrS with Type-1 BrP patients who are younger than 19 years from 16 European tertiary centers	Symptomatic BrS patients with Type-1 BrP with ICD implantations in Khon Kaen, Thailand, between 2008 and 2011	BrS with Type-1 BrP patients who underwent genetic testing for SCN5A mutation and were followed up between 1988 and 2013.
Exclusion criteria	Right ventricular cardiomyopathy by echocardiography	Structural heart diseases, acute ischemia and metabolic or electrolyte disturbances	Abnormalities found by echocardiography and chest X-ray	None	Structural cardiac abnormalities, electrolyte or metabolic disturbances at the time of ECG	Structural heart diseases	Structural heart disease

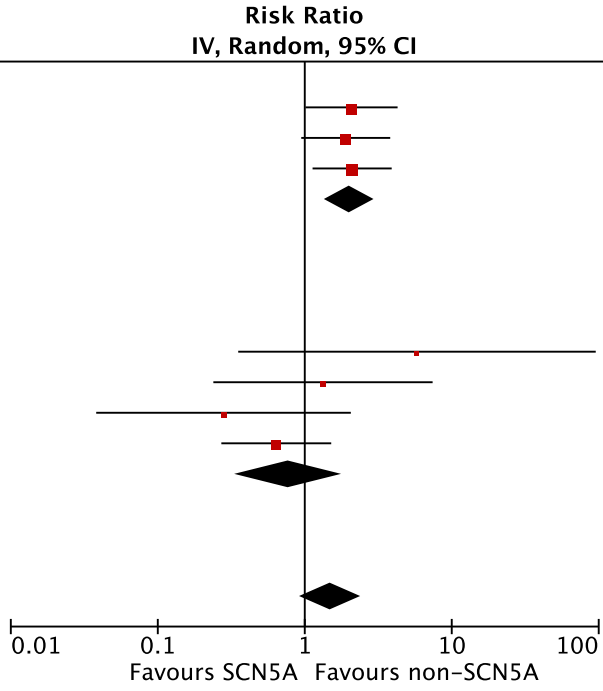
					recording		
Number of subjects (% male, mean age)	200 patients (76% male, mean age 41±18 years)	212 patients (71.7% male, mean age 45±6 years)	108 patients (97.2% male, mean age 46.8±11.6 years)	176 patients (67.0% male, mean age 43 years±16.8 years)	106 patients (54.7% male, mean age 11.1±5.7 years)	40 patients [97.5% male, median age 43 years (range 22–66)]	415 patients (97% male, mean age 46±14 years)
Methods of mutation detection	DHPLC and/or SSCA	DNA sequencing	DNA sequencing	DNA sequencing	DHPLC and/or DNA sequencing	DNA sequencing	DHPLC, SSCA, and DNA sequencing
Verification if the variants were disease-causing	A panel of 400 healthy white individuals (800 alleles) were used as control.	Not described	Not described	Not described	Not described	Only H558R and R1193Q variants were reported; both of which were known to relate to Brugada syndrome.	The frequencies and statuses of the mutations (using an in silico phenotype prediction algorithm) were evaluated.
Presence of SCN5A mutations	84 Positive (42%) 116 Negative	57 Positive (26.8%) 126 Negative 29 No genetic testing	17 Positive (15.7%) 91 Negative	23 Positive (21.9%) 82 Negative	58 Positive (54.7%) 17 Negative 31 No genetic testing	13 Positive (32.5%) 27 Negative	60 Positive (14.5%) 355 Negative

Number of symptomatic patients at diagnosis (%)	56 (28)	89 (42)	65 (60)	130 (73.8)	21 (20)	40 (100)	187 (45)
Endpoints	Documented VF or sudden death	SCD or documented VF	Appropriate ICD shock therapy	Appropriate ICD shock therapy	SD, documented VT or VF, appropriate ICD shock	Appropriate ICD shock therapy	Appropriate ICD shock, aborted cardiac arrest, or SCD
Average follow-up	Mean 34 ± 44 months	Mean 40 ± 50 months	Mean 71.9 ± 41.3 months	Mean 83.8 ± 57.3	Median 54 months (1st–3rd quartile 15–99)	Median 24 months (range 13–52 months)	Mean 72 months (range 1–249 months)
Conclusion by authors	SCN5A mutation was not associated with a higher risk of events and showed 32% sensitivity and 57% specificity to identify patients with cardiac arrest	Previous histories of aborted SCD and syncope were predictors for adverse outcome	SCN5A mutation is not associated with initial episodes of VF in BS, but is associated with early and frequent recurrence of VF in symptomatic patients	Risk stratification by means of electrophysiology study might identify asymptomatic patients at risk for arrhythmic events	SCN5A mutation may be necessary but is insufficient on its own for the development of lethal arrhythmia	R1193Q variant may be a genetic marker for ventricular arrhythmia in symptomatic BrS patients with ICD treatment	BrS patients with SCN5A mutations exhibit more conduction abnormalities on ECG and have higher risk for cardiac events
NOS	7	9	7	8	8	8	6

BrS: Brugada syndrome; ECG: Electrocardiogram; ICD: Implantable cardioverter-defibrillator; SCD: Sudden cardiac death; SD: Sudden death; VT: ventricular tachycardia; VF: Ventricular fibrillation; DHPLC: Denaturing high-performance liquid chromatography; SSCA: Single-strand conformational polymorphism analysis; NOS: Newcastle-Ottawa Scale



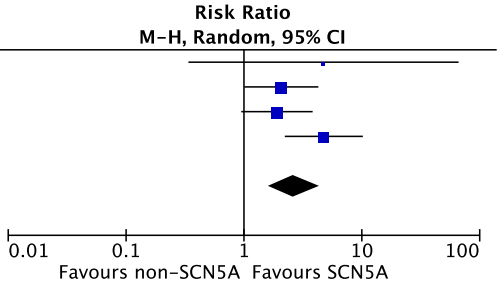
Study or Subgroup	log[Risk Ratio]	SE	SCN5A Events	SCN5A Total	non-SCN5A Events	non-SCN5A Total	Weight	Risk Ratio IV, Random, 95% CI
Asian								
Makarawate et al., 2017	0.73236789	0.36836803	8	13	8	27	21.4%	2.08 [1.01, 4.28]
Nishii et al., 2010	0.64185389	0.35565258	5	7	9	24	22.1%	1.90 [0.95, 3.81]
Yamagata et al., 2017	0.74193734	0.31624768	13	60	49	355	24.6%	2.10 [1.13, 3.90]
Subtotal (95% CI)				**80**		**406**	**68.1%**	**2.03 [1.37, 3.00]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.05$, df = 2 (P = 0.98); $I^2 = 0\%$								
Test for overall effect: Z = 3.56 (P = 0.0004)								
Caucasian								
Andorin et al., 2016	1.75785792	1.42895052	9	58	0	17	2.7%	5.80 [0.35, 95.44]
Conte et al., 2015	0.28517894	0.87635979	NA	23	NA	82	6.5%	1.33 [0.24, 7.41]
Eckardt et al., 2005	-1.27296568	1.01759797	1	57	8	126	5.0%	0.28 [0.04, 2.06]
Priori SG et al., 2002	-0.4462871	0.43914362	7	84	15	116	17.7%	0.64 [0.27, 1.51]
Subtotal (95% CI)				**222**		**341**	**31.9%**	**0.78 [0.34, 1.80]**
Heterogeneity: $Tau^2 = 0.13$; $Chi^2 = 3.54$, df = 3 (P = 0.32); $I^2 = 15\%$								
Test for overall effect: Z = 0.57 (P = 0.57)								
Total (95% CI)				**302**		**747**	**100.0%**	**1.50 [0.93, 2.41]**
Heterogeneity: $Tau^2 = 0.14$; $Chi^2 = 9.62$, df = 6 (P = 0.14); $I^2 = 38\%$								
Test for overall effect: Z = 1.66 (P = 0.10)								
Test for subgroup differences: $Chi^2 = 4.11$, df = 1 (P = 0.04), $I^2 = 75.7\%$								



1)

Study or Subgroup	SCN5A Events	SCN5A Total	non-SCN5A Events	non-SCN5A Total	Weight	Risk Ratio M-H, Random, 95% CI
Andorin et al., 2016	6	10	0	3	3.4%	4.73 [0.34, 66.18]
Makarawate et al., 2017	8	13	8	27	32.4%	2.08 [1.01, 4.28]
Nishii et al., 2010	5	7	9	24	34.1%	1.90 [0.95, 3.83]
Yamagata et al., 2017	10	30	11	157	30.1%	4.76 [2.22, 10.19]
Total (95% CI)		**60**		**211**	**100.0%**	**2.66 [1.62, 4.36]**
Total events	29		28			

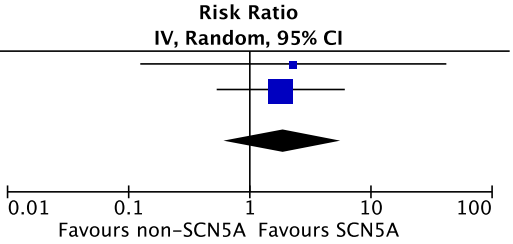
Heterogeneity: $Tau^2 = 0.06$; $Chi^2 = 3.92$, df = 3 (P = 0.27); $I^2 = 23\%$
Test for overall effect: Z = 3.88 (P = 0.0001)



2)

Study or Subgroup	SCN5A Events	SCN5A Total	non-SCN5A Events	non-SCN5A Total	Weight	Risk Ratio IV, Random, 95% CI
Andorin et al., 2016	3	48	0	15	14.9%	2.29 [0.12, 41.91]
Yamagata et al., 2017	3	30	11	198	85.1%	1.80 [0.53, 6.08]
Total (95% CI)		**78**		**213**	**100.0%**	**1.87 [0.61, 5.73]**
Total events	6		11			

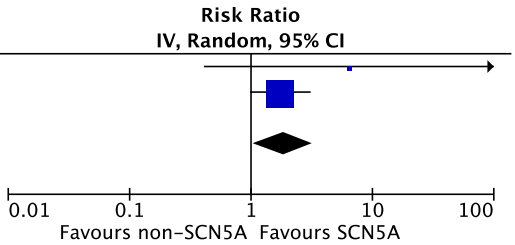
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.02$, df = 1 (P = 0.88); $I^2 = 0\%$
Test for overall effect: Z = 1.09 (P = 0.28)



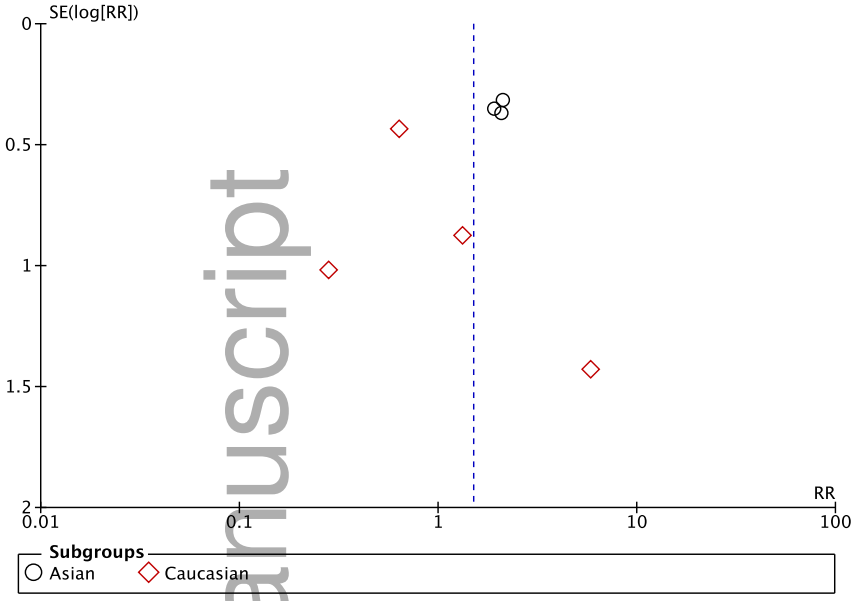
3)

Study or Subgroup	SCN5A Events	SCN5A Total	non-SCN5A Events	non-SCN5A Total	Weight	Risk Ratio IV, Random, 95% CI
Andorin et al., 2016	7	20	0	8	4.2%	6.43 [0.41, 100.98]
Yamagata et al., 2017	12	48	36	251	95.8%	1.74 [0.98, 3.10]
Total (95% CI)		**68**		**259**	**100.0%**	**1.84 [1.05, 3.23]**
Total events	19		36			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.83$, df = 1 (P = 0.36); $I^2 = 0\%$
Test for overall effect: Z = 2.12 (P = 0.03)



1)



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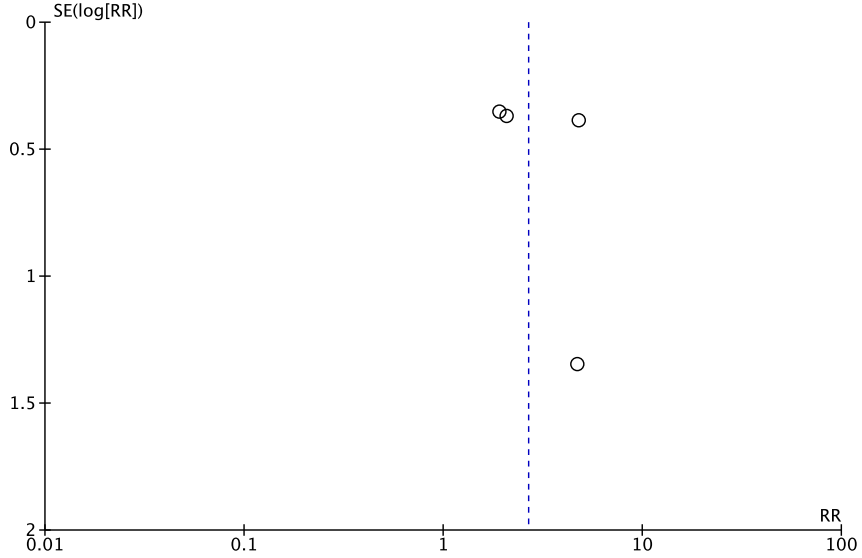

3)

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