Validation and Disease Risk Assessment of Previously Reported Genome-Wide Genetic Variants Associated with Brugada Syndrome: SADS-TW BrS Registry

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Abstract:

Background - Brugada syndrome (BrS) is an oligogenic arrhythmic disease with increased risk of sudden cardiac arrest (SCA). Several BrS or ECG traits-related single nucleotide polymorphisms (SNPs) were identified through previous genome-wide association studies in Caucasian patients. We aimed to validate these SNPs in BrS patients in the Taiwanese population, assessing the cumulative effect of risk alleles and the BrS polygenic risk score (BrS-PRS) in predicting cardiac events.

Methods - We genotyped 190 unrelated BrS patients using the TWB Array, and Taiwan Biobank was used as controls. SNPs not included in the array were imputed by IMPUTE2. Cox's proportional hazards model was utilized to evaluate the associations between each particular SNP, the collective BrS-PRS, and clinical outcomes.

Results - Of the 88 previously reported SNPs, 22 were validated in Taiwanese BrS patients ($P<0.05$). Of the 22 SNPs, 2 (rs10428132, rs9388451) were linked with susceptibility to BrS, 10 were SNPs previously reaching genome-wide significance, and 10 were SNPs associated with electrocardiogram traits. For the 3 most commonly reported SNPs, disease risk increased consistently with the number of risk alleles (OR 3.54, Ptrend $= 1.38 * 10^{-9}$ for 5 risk alleles versus 1). Similar patterns were observed in both *SCN5A* mutation+ (OR 3.66, Ptrend $= 0.049$) and *SCN5A* mutation- (OR 3.75, Ptrend $= 8.54 * 10^{-9)}$ subgroups. Furthermore, BrS patients without *SCN5A* mutations had more risk alleles than BrS patients with *SCN5A* mutations regardless of the range of PRS. Three SNPs (rs4687718, rs7784776, rs2968863) showed significant associations with the composite outcome (SCA plus syncope, HR 2.13, 1.48, 0.41; P=0.02, 0.006, 0.008, respectively).

Conclusions - Our findings suggested that some SNPs associated with BrS or electrocardiogram traits exist across multiple populations. The cumulative risk of the BrS-related SNPs is similar to that in Caucasian BrS patients, but it appears to correlate with the absence of *SCN5A* mutations.

Key words: bioinformatics; Brugada syndrome; genetic polymorphism; genetics; genotype; ethnic differences; allele frequency; disease risk

Nonstandard abbreviations and Acronyms:

BrS: Brugada Syndrome

SCA: Sudden Cardiac Arrest

BrS-PRS: Brugada Syndrome-polygenic risk score

Introduction

Brugada syndrome (BrS), an oligogenic arrhythmic disease responsible for sudden cardiac arrest (SCA) in patients with structurally normal hearts, was first reported by Brugada et al. in 1992.[1] BrS accounts for 4% of all sudden deaths and for up to 20% of sudden deaths in patients without structural cardiac disease.[2] The prevalence of BrS is estimated to be 1–5 per 10,000 people in Caucasians[3], but is higher in Southeast Asians (12 per 10,000).[4,5]

In 1998, the *SCN5A*-encoded alpha-subunit of the voltage-gated Nav1.5 cardiac sodium channel was first associated with BrS.[6] Although *SCN5A* is the most common BrS-susceptibility gene, it is responsible for only 20% of BrS cases in Caucasian populations[7, 8] and even less, 7.5–8% of BrS cases, in the Han Chinese population.[9] BrS is generally considered a Mendelian disorder with autosomal dominant transmission and incomplete penetrance. Priori et al. estimated that the overall disease penetrance across 4 small BrS families harboring mutations in the *SCN5A* gene was 16% (range 12.5% to 50%) based on their electrocardiograms (ECGs).[10, 11] In the past 2 decades, several BrS-associated genes and modifier genes have been reported, and most of these primarily encode sodium, potassium, and calcium channels or the proteins associated with these channels. However, disease-causing genes remain unknown in approximately 80-85% of BrS patients. Additionally, the disease is sporadic in many patients.[12, 13] These observations suggest a more complex inheritance model.

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To identify new genetic variants, Dr. Bezzina et al. conducted a large-scale genome-wide association study (GWAS) with 312 BrS cases in the European populations and replicated the results in 594 cases from Europe and 208 cases from Japan.[14] They reported 3 common SNPs associated with BrS: rs11708996 in *SCN5A*, rs10428132 in *SCN10A*, and rs9388451 near *HEY2*. Furthermore, they analyzed the cumulative effect of these 3 SNPs on susceptibility to BrS with a BrS-polygenic risk score (BrS-PRS), and found that likelihood of BrS increased consistently with the number of risk alleles. However, it is unclear whether the previously identified SNPs are relevant in other racial groups such as the Taiwanese or whether population-specific SNPs influence BrS. In this study, we first aimed to validate the previously identified BrS-related SNPs in patients with BrS in Taiwan and assessed the cumulative risk of these SNPs on susceptibility to BrS. We then analyzed additional SNPs, including those reaching genome-wide significance in the GWAS from Dr. Bezzina et al. and those associated with ECG traits (ex. PR interval, QRS duration and QT interval). Since clinical outcomes of identified BrS-related common variants in the previous GWAS from Dr. Bezzina et al. were not investigated, we further investigated the association between the BrS-PRS and clinical outcomes, and then compared them between BrS patients with and without pathogenic *SCN5A* variants.

Methods

This study was approved by the local ethical committee of National Taiwan University Hospital, and all participants gave informed consent before participating. The imputation workflow is illustrated in **Figure 1** and the details of the methods are shown in the supplemental data. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Results

Patient population

The basic demographic and clinical presentation of the 190 Taiwanese patients with BrS is

shown in **Table 1**. The mean age of the patients with BrS was 45.6 ± 15.7 years and 87.4% of

the patients were male. Regarding their symptoms, 62.6 % of the study patients were

symptomatic (either SCA or unexplained syncope), and 71.2% presented with spontaneous type

1 Brugada ECG.

Validation of the imputation method by *in silico* approaches and direct sequencing

To ensure high quality of the imputed genotyping calls of the variants in this study, we verified

the accuracy of the imputation method by using it on sequences for which microarray data were

available. We found that the concordance of genotypes obtained by the imputation algorithm and

the actual microarray was more than 90%. In addition to the *in silico* analyses, we performed

direct sequencing to validate the genotyping calls obtained by imputation. We randomly selected

3 SNP loci in 20 samples for this validation, and all of the genotypes were the same as those

obtained by using the imputation approach. These results indicate that imputation is a feasible

and efficient approach to obtain genotyping calls for the SNP loci that were originally not

detectable in the microarrays.

Comparisons of the variants with significance in BrS patients between Caucasian and

Taiwanese populations

Among the 3 SNPs previously shown to cause susceptibility to Brugada syndrome (set 1), only

rs9388451 was available on the Affymetrix TWB chip. As shown in **Table 2**, the allele

frequency of rs9388451 was significantly higher in the Taiwanese BrS patients than the healthy

controls (*P*=0.003). We obtained the genotyping calls of the other 2 SNPs (rs11708996,

rs10428132) by using the imputation approach which was validated by Sanger sequencing. Only

rs10428132 showed significant differences between BrS patients and controls (P=5.92*10^{-8}).

Among the 12 SNPs reaching genome-wide significance in the previous GWAS from Dr.

Bezzina et al. (set 2), most of them are located in *SCN10A*.[14] As shown in **Table 2**, 10 out of the

12 SNPs had significantly higher allele frequencies in Taiwanese BrS patients than in the healthy

controls. In general, the results were similar to those reported in the Caucasian BrS patients.

Among the 75 SNPs associated with ECG traits (set 3), 10 showed significant differences in

Taiwanese BrS patients versus healthy controls (**Table 2**). Among the 10 SNPs, only 4—

rs11129795, rs6795970, rs6798015, and rs314370—reached significance in the GWAS

significance threshold ($< 5*$ 10^{-8}). In conclusion, the results suggested that the general patterns of

the important SNPs in BrS patients were similar between Caucasian and Taiwanese populations,

but the low number of replicated SNPs associated with ECG traits implied they were not

important targets for BrS in Taiwanese patients.

Comparisons of the PRS between BrS patients and the healthy controls from Taiwan

In addition to the single marker tests, we developed PRS models using the 3 sets of SNPs. The

weighting for the PRS models was obtained by using all BrS patients in Taiwan versus the

healthy controls. The healthy controls were classified into 5 subgroups with equal PRS ranges.

The group with the lowest PRS (0%-20%) was utilized as the reference to calculate the ORs of

disease risk. We calculated the ORs by dividing the number of BrS patients by the number of

healthy controls in each subgroup. The results are summarized in **Table 3**. For the 3 SNPs in set

1 (BrS-PRS[14]), the BrS patients with the relatively higher PRS (61%-80%) had a significantly

higher risk of disease (OR 2.03) than the healthy controls, but this OR was not the highest. For

the 12 SNPs reaching genome-wide significance (set 2), only rs10428132 and rs12638572

showed a high correlation ($R^2 > 0.7$) in the LD calculation. We selected rs10428132 for further

analyses because rs10428132 has lower p-value in **Table 2**. After excluded rs12638572, other

SNPs in set 2 were left for further analyses. To further address the issue of genomic inflation, we

performed a condition analysis for each SNP set 2 by conditioning on rs10428132, which was

the leading SNP reported by the previous GWAS. The results of the conditional analyses are

summarized in **Table S1.** Consequently, we used the 7 significant SNPs in **Table S1** along with

rs10428132 to develop the PRS model. As shown in **Table 3**, the BrS patients with the highest

PRS (81%-100%) showed significantly higher disease risk (likelihood of BrS diagnosis) than

controls (OR: 3.61 (2.14-6.10)). For the 75 SNPs associated with ECG traits (set 3), the BrS

patients with relatively higher scores (41%-100%) reported significantly higher disease risk

(ORs 4.83-11.67). Similar to the second set, the BrS patients with the highest PRS (81%-100%)

displayed the highest OR (11.67). In summary, the results of the three different PRS models

demonstrated that the cumulative effects of the reported SNPs, as reflected in the PRSs, were

effective markers in distinguishing Taiwanese BrS patients and healthy controls.

Cumulative effects of the 3 major risk alleles (set 1, BrS-PRS[14]) on susceptibility to BrS in

Taiwanese BrS patients

To compare the cumulative effect of the 3 previously reported major risk alleles in Taiwanese

BrS patients, the allele frequencies of these 3 SNPs were evaluated between BrS patients and

healthy controls. The result is illustrated in **Figure 2**. First, we compared the disease risk

between all BrS patients and healthy controls and found that the risk increased consistently with

the number of risk alleles present (*P*trend=$1.38 * 10^{-9}$), with the estimated OR reaching 3.54 in

the presence of more than 4 risk allele copies versus only 1 risk allele (**Figure 2A**). Second, we

divided the BrS patients into two groups based on the presence or absence of *SCN5A* mutations.

Similar patterns were observed in both *SCN5A* mutation+ (OR=3.66, *P*trend=0.049) and *SCN5A* mutation- (OR=3.75, *P*trend=8.54 * 10^{-9}) subgroups (**Figure 2B and Figure 2C**). However, some discrepancies of the ORs can be observed between the *SCN5A* mutation+ and *SCN5A* mutation- subgroups, suggesting some genetic differences may exist in the 2 subgroups.

Comparisons of the previously reported SNPs in BrS patients with and without *SCN5A* mutations

Since *SCN5A* has been reported as the most dominant gene in BrS, albeit still accounting for only 20% of BrS in Caucasians, we divided the BrS patients into two subgroups accordingly (*SCN5A* mutation+ versus *SCN5A* mutation-). The results are shown in **Table 4**. In set 1 SNPs, rs10428132 was significant in BrS patients in both subgroups (*P*<0.05). However, rs9388451 was only significant in the *SCN5A* mutation- BrS patients. In set 2 SNPs, the 10 SNPs which were significant in all combined BrS patients were still significant in the *SCN5A* mutation- BrS patients. Intriguingly, 3 SNPs (rs6599240, rs1268070, rs9388451) were only significant in the *SCN5A* mutation- BrS patients but not in the *SCN5A* mutation+ BrS patients. Similar patterns were observed in the 75 SNPs associated with ECG traits (set 3 SNPs). Among the 10 significant SNPs of set 3 in **Table 2**, all of them were significant in the *SCN5A* mutation- BrS patients, but only 3 were significant in the *SCN5A* mutation+ BrS patients. Notably, two SNPs (rs37062 and rs7784776) were significant in the *SCN5A* mutation+ BrS patients alone, which were not in the 10 significant SNPs, and intriguingly they were both protective SNPs in the *SCN5A* mutation+ BrS patients. In addition, rs2074518 was not significant in all combined BrS patients, but it was significant in both BrS mutation+ and mutation- patients. However, rs2074518 was a risk allele in BrS *SCN5A* mutation- patients but a protective allele in BrS *SCN5A* mutation+ patients. Therefore, these differences between the *SCN5A* mutation+ and *SCN5A* mutation-BrS patients

hinted that future investigations should classify the patients into subgroups instead of combining them into one unit.

In addition to the single marker tests, we examined the cumulative proportions of the 3 BrS-associated SNPs (set 1, BrS-PRS[14]) in the Taiwanese BrS patients after being stratified by the presence/absence of *SCN5A* pathogenic variants (**Figure 3**). Notably, the *SCN5A* mutation-BrS patients had more risk alleles (at least 4 risk alleles). Furthermore, we developed the PRS models using weighting schemes generated from the *SCN5A* mutation-BrS patients versus healthy controls. The results are summarized in **Figure 4** and **Table S2**. Using the PRS model generated from the 3 SNPs in set 1 (BrS-PRS[14]), the ORs of the disease risk showed differences in BrS patients with and without *SCN5A* variants (**Figure 4A**) in the 21%-80% PRS groups. However, the results have negative ORs in the 21%-40% PRS group whereas positive ORs in the 41%-80% PRS groups. These fluctuating ORs may result from the low number of SNPs analyzed in the PRS model. Alternatively, obvious differences were observed in the ORs obtained from the PRS models generated from set 2 SNPs or set 3 SNPs (**Figure 4B and Figure 4C**). In general, most ORs were higher in the *SCN5A* mutation- BrS patients than in the *SCN5A* mutation+ BrS patients, regardless of the high or low range of PRS. These results further indicated that the genetic markers differ between BrS1 patients with a pathogenic *SCN5A* variant and those patients diagnosed with BrS who are *SCN5A* negative.

Associations of the previously reported SNPs and PRS with clinical outcomes

We evaluated whether these SNPs were able to predict clinical outcomes (SCA or unexplained syncope or both) in BrS patients. First, a single marker test was utilized for all previously reported SNPs (set 1-3) in **Table S3**. Of these SNPs, 3 SNPs (rs4687718 in *TKT*, rs7784776 in *IGFBP3*, rs2968863 in *KCNH2*) showed significant associations with the composite clinical

outcome (SCA plus syncope) (*P*<0.05, **Table S4**). Notably, rs7784776 was only significant in the *SCN5A* mutation- BrS patients, whereas rs4687718 was significant only in the *SCN5A* mutation+ BrS patients. Only the SNP, rs2968863, showed protective effects in all BrS patients no matter whether they had a *SCN5A* variant or not. We also examined whether the PRS models generated from the 3 sets of previously reported SNPs were able to predict the clinical outcomes or not. Unfortunately, none of the results of the 3 PRS models were significant, and thus they cannot serve as a predictor for the clinical outcomes in Taiwanese BrS patients.

Discussion

In this study, we successfully validated 22 of the 88 previously identified BrS- or ECG traits-related SNPs in patients with BrS in Taiwan. We assessed the cumulative effects of the SNPs on the risk of BrS and their association with clinical outcomes.

In 2013, Bezzina and colleagues reported 3 important common SNPs associated with BrS, including rs11708996 in *SCN5A*, rs10428132 in *SCN10A*, and rs9388451 near *HEY2*,[14] and found that disease risk (likelihood of BrS diagnosis) increased consistently with the number of risk alleles. In the current study, rs10428132 and rs9388451 were successfully validated in Taiwanese BrS patients, demonstrating that the same variants can affect BrS across different populations. Intriguingly, the MAF difference of rs9388451 between the cases and controls was only 0.08 in the Taiwanese population, which was lower than that in both the Japanese (0.11) and Caucasian populations (0.15),[14] suggesting that racial differences should be taken into consideration because of the differing allele frequencies in the BrS patients with different ethnic backgrounds.

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In addition, the cumulative effect of the 3 major risk alleles on susceptibility to BrS increased with the number of risk alleles present in the Taiwanese BrS patients, consistent with the results in Caucasian BrS patients.[14] However, the cumulative effect was smaller in Taiwanese BrS patients than in Caucasian BrS patients; this might be caused by the smaller sample size of our study cohort or by ethnic differences.

Validation of the 12 SNPs reaching genome-wide significance in Caucasian BrS patients

Among the 12 SNPs which reached genome-wide significance in Caucasian BrS patients (set 2), 10 were successfully validated in Taiwanese BrS patients, providing further evidence that *SCN10A* and *HEY2* are highly associated with BrS. The *P*-values were relatively higher in the Taiwanese population, which may have resulted from the limited sample size of BrS patients or from genetic differences between the two populations.

Comparisons of the reported SNPs (set 1, 2 and 3) and PRS between the BrS patients with *SCN5A* mutation and the BrS patients without *SCN5A* mutations

SCN5A is the major BrS-causing gene, but is responsible for only 20% of BrS cases in Caucasian populations[7, 8] and even less in the BrS patients in Japanese and Taiwanese population. (7.5–8%)[9,15], which might imply that the genetic background of BrS is partly different in different populations. In this study, we found that BrS patients without *SCN5A* mutations had more risk alleles (4 or 5 risk alleles) in the BrS-PRS[14] than BrS patients with *SCN5A* mutations. In addition, the cumulative effect of the 3 common SNPs on susceptibility to BrS was larger in the BrS patients without *SCN5A* mutations than in BrS patients with *SCN5A* mutations. This may suggest that *SCN5A* mutations are dominant drivers of BrS whereas other minor variants/genes need higher quantities to display their effects. However, when the PRS generated by the 3 common SNPs was applied, there was no significant difference in disease risk between the two groups.

This discrepancy might be explained by the limited number of the SNPs in this 3-SNP model or the assumption that the genetic effect of each SNP is the same. When the PRS generated by more SNPs (10 SNPs (set 2) or 75 SNPs (set 3), all ORs of disease risk were consistently higher in the BrS patients without *SCN5A* mutations than in the BrS patients with *SCN5A* mutations, regardless of the range of PRS. Furthermore, we found that more SNPs reaching genome-wide significance in the GWAS threshold in this study (set 2) and more SNPs associated with ECG traits (set 3) were validated in the BrS patients without *SCN5A* mutations than in the BrS patients with *SCN5A* mutations, although some of the SNPs were overlapped. These results indicated that it is necessary to consider the effects of the SNPs in BrS patients in the context of *SCN5A* variants, because the genetic architecture between BrS patients with SCN5A mutations and without SCN5A mutations may be different. In addition, the cumulative effects of SNP set 2 from Dr. Bezzina et al., which were mostly located on chromosome 3, showed different ORs between *SCN5A* (+) and *SCN5A* (-) BrS patients (**Figure 4B**). This indicated that a *SCN5A* (-) BrS patient tends to have more risk alleles in SNP set 2. However, due to the limitations of the experimental techniques utilized in this study, we could only consider the effects of multiple SNPs by an additive model instead of a haplotype. Therefore, further investigations are warranted to explore whether chromosome 3 haplotype can provide synergetic effects in BrS patients.

Limitations

Some limitations exist in this study. First, although the sample size for all BrS patients was almost 200, it may still have lacked sufficient power to do the patient stratification by *SCN5A* mutations. It is well known that only around 20% of the BrS patients have pathogenic *SCN5A* variants, which resulted in less than 50 patients with *SCN5A* variants in this study. However, the

current data indicated huge differences in MAFs between BrS patients with and without *SCN5A* mutations, suggest that it is worth doing stratification in future investigations. Second, we used the 1000 Genomes East Asian population as the reference in the imputation approach. This reference is perhaps not the best one, because the genetics of the Taiwanese population may be closer to the Han Chinese population. However, the sample size would be relatively limited if we used the Han Chinese population only, and thus we decided to use all East Asian populations to include more diverse haplotypes in the reference. Based on the high accuracy values validated by the imputation algorithm and the PCR experiment, we believe this is not a critical issue in this study. In addition to the validations of DNA variants associated with BrS from a previous GWAS, we performed the analysis of all SNPs examined in the TWB chip in the current dataset containing 190 BrS cases and around 16,000 healthy Taiwanese controls in Taiwan. The preliminary results of our primary GWAS were summarized in **Table S5**. However, notably some of the p-values were very low (**Table S5**). We were afraid that these astounding findings may be too good to be true. These extremely low p-values may be the result of the probe design, technical issues in the GWAS chip or the hugely imbalanced sample sizes in our GWAS (190 cases vs. 16,000 controls). Therefore, validations of these DNA loci in independent cohorts are prerequisite to further apply them into advanced researchers and/or applications.

Conclusions

Of the 88 previously reported SNPs, 22 SNPs were validated in Taiwanese BrS patients, and 3 SNPs (rs4687718, rs7784776, rs2968863) were associated with composite clinical outcomes (SCA plus syncope). The cumulative effect of the 3 major risk alleles on susceptibility to BrS was larger in BrS patients than in healthy controls. Furthermore, this effect was also larger in *SCN5A* genotype negative BrS patients than those with *SCN5A*-mediated BrS (BrS1). PRSs

showed that all ORs of disease risk were consistently higher in BrS patients without *SCN5A*

mutations than in those with *SCN5A* mutations, regardless of the range of PRS.

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Table 1. Demographic data and clinical manifestations of the BrS patients

	BrS patients **(n = 190)**
Male, *n* (%)	166 (87.4)
Mean age, *years* ± SD	45.6 ± 15.7
Asymptomatic, *n* (%)	30 (15.8)
Palpitation or chest pain, *n* (%)	41 (21.6)
Unexplained syncope, *n* (%)	70 (36.8)
SCD, *n* (%)	49 (25.8)
Family history of SCD, *n* (%)	32 (16.8)
Spontaneous type 1 Brugada ECG, *n* (%)	136 (71.2)

BrS = Brugada syndrome; ECG = electrocardiogram; SCD = sudden cardiac death; SD = standard deviation.

Table 2. Validation of the previously reported SNPs in the Taiwanese BrS patients

	Chr. Position (Build 37)	Imputed or original	Gene or nearest gene	Risk allele	Protective allele	RAF (190 cases /15,981 controls) in Taiwanese	MAF (case/control) in Caucasians	All BrS patients in Taiwan vs. Healthy controls		OR in the previous GWAS[1]
SNPs causing susceptibility to Brugada syndrome (set 1)								**P-value***	**OR**	**OR**
rs11708996	Chr3:38633923	original	*SCN5A*	C	G	0.011/0.007	0.23/0.15	4.27E-01	1.5 (0.46-3.57)	1.64 (1.30–2.07)
rs10428132	Chr3:38777554	imputed	*SCN10A*	T	G	0.305/0.193	0.69/0.41	5.92E-08[†]	1.84 (1.47-2.29)	3.00 (2.45–3.69)
rs9388451	Chr6:126090377	original	*HEY2, NCOA7*	C	T	0.818/0.742	0.65/0.50	7.94E-04[†]	1.56 (1.21-2.05)	1.83 (1.51–2.22)
SNPs reaching genome-wide significance in the GWAS threshold in this study (set 2)										
rs6599240	Chr3:38738717	original	*SCN10A*	A	G	0.195/0.138	NA/0.435	1.65E-03[†]	1.51 (1.16-1.94)	2.07 [1.71-2.51]
rs11129801	Chr3:38750375	imputed	*SCN10A*	G	A	0.645/0.535	NA/0.72	8.91E-06[†]	1.56 (1.28-1.89)	2.00 [1.56-2.55]
rs9874633	Chr3:38771994	imputed	*SCN10A*	A	G	0.789/0.784	NA/NA	9.94E-01	1.00 (0.79-1.28)	2.70 [2.07-3.52]
rs10428132	Chr3:38777554	imputed	*SCN10A*	T	G	0.305/0.193	NA/NA	5.92E-08[†]	1.84 (1.47-2.29)	3.00 [2.45-3.69]
rs7428167	Chr3:38778191	original	*SCN10A*	T	C	0.521/0.396	NA/NA	1.08E-06[†]	1.66 (1.35-2.03)	2.86 [2.32-3.53]
rs10428168	Chr3:38780059	original	*SCN10A*	T	C	0.663/0.669	NA/NA	8.08E-01	0.97 (0.79-1.21)	2.62 [2.06-3.32]
rs12638572	Chr3:38787797	original	*SCN10A*	A	G	0.318/0.205	NA/NA	9.04E-08[†]	1.81 (1.45-2.25)	2.12 [1.68-2.67]
rs7641844	Chr3:38802251	original	*SCN10A*	A	G	0.447/0.318	NA/NA	1.05E-07[†]	1.74 (1.42-2.13)	2.02 [1.57-2.59]
rs7430439	Chr3:38803639	original	*SCN10A*	G	A	0.418/0.294	NA/NA	1.91E-07[†]	1.73 (1.4-2.12)	1.75 [1.45-2.13]
rs6599257	Chr3:38804588	original	*SCN10A*	C	T	0.226/0.13	NA/NA	4.78E-08[†]	1.97 (1.54-2.5)	2.17 [1.79-2.65]
rs1268070	Chr6:126041164	imputed	*HEY2*	C	T	0.853/0.804	NA/NA	3.79E-02[†]	1.33 (1.02-1.75)	1.80 [1.48-2.20]
rs9388451	Chr6:126090377	original	*HEY2, NCOA7*	C	T	0.818/0.742	NA/0.49	7.94E-04[†]	1.56 (1.21-2.05)	1.83 [1.51-2.22]
SNPs previously associated with ECG traits (set 3)†										
rs17020136	Chr2:37248015	imputed	*HEATR5B, STRN*	T	C	0.443/0.509	0.213/0.182	7.36E-03[†]	0.76 (0.62-0.93)	NA

SNP	Position	Type	Gene	Risk Allele	Other Allele	RAF (BrS/Control)	MAF	P-value*	OR (95% CI)	OR[1]
rs10865355	Chr2:66764997	imputed	*MEIS1*	A	G	0.221/0.174	0.402/0.377	1.62E-02[†]	1.35 (1.05-1.72)	NA
rs11897119	Chr2:66772000	original	*MEIS1*	T	C	0.779/0.825	0.401/0.377	1.14E-02[†]	0.73 (0.58-0.94)	NA
rs11129795	Chr3:38589163	original	*SCN5A, SCN10A*	G	A	0.932/0.91	0.284/0.233	1.77E-17[†]	3.89 (2.91-5.46)	NA
rs6795970	Chr3:38766675	original	*SCN5A, SCN10A*	A	G	0.263/0.152	0.668/0.410	5.10E-09[†]	1.98 (1.57-2.49)	NA
rs6798015	Chr3:38798836	imputed	*SCN5A, SCN10A*	C	T	0.315/0.194	0.607/0.364	3.96E-09[†]	1.92 (1.54-2.39)	NA
rs314370	Chr7:100453208	original	*SLC12A9, UFSP1*	T	C	0.928/0.955	0.176/0.189	2.65E-09[†]	2.33 (1.8-3.15)	NA
rs7342028	Chr10:114479262	original	*VTI1A*	G	T	0.405/0.461	0.260/0.277	3.56E-02[†]	0.80 (0.65-0.98)	NA
rs1896312	Chr12:115346424	original	*TBX3,TBX5*	C	T	0.611/0.553	0.303/0.286	2.35E-02[†]	1.27 (1.03-1.56)	NA
rs10850409	Chr12:115381740	original	*TBX3,TBX5*	G	A	0.382/0.441	0.278/0.269	2.69E-02[†]	0.79 (0.64-0.97)	NA

OR: odds ratio; NA: not available; 1. Bezzina et. al. Nat Genet. 2013 Sep;45(9):1044-9

BrS: Brugada Syndrome, MAF: minor allele frequency, OR: Odds Ratio, RAF: risk allele frequency, SNP: single nucleotide polymorphism

NA: only one genotype exists

*P-value was obtained by using logistic regression. Red color indicates statistical significance.

[†] indicates statistical significance (p < 0.05).

[‡] Only significant SNPs are listed (for all SNPs, please refer to **Table S6**).

Table 3. Comparisons of disease risk using the PRS models generated by the 3 sets of SNPs

PRS range	Odds ratio
Set 1 SNPs	
0%-20% [*]	1
21%-40%	0.16 (0.06-0.42)
41%-60%	2.10 (1.36-3.22) [‡]
61%-80%	2.03 (1.32-3.13) [‡]
81%-100%	0.77 (0.45-1.32)
Set 2 SNPs [†]	
0%-20% [*]	1
21%-40%	1.67 (0.93-3.00)
41%-60%	1.67 (0.93-3.00)
61%-80%	1.56 (0.86-2.82)
81%-100%	3.61 (2.14-6.1) [‡]
Set 3 SNPs	
0%-20% [*]	1
21%-40%	2.17 (0.82-5.74)
41%-60%	4.83 (1.99-11.72) [‡]
61%-80%	5.84 (2.44-13.98) [‡]
81%-100%	11.67 (5.03-27.04) [‡]

PRS, polygenic risk score; SNP, single nucleotide polymorphism

[*]Reference group

[†]only included rs10428132, rs11129801, rs7428167, rs10428168, rs7641844 and rs7430439 on chromosome 3, and rs1268070 and rs9388451 on chromosome 6.

[‡]indicates statistical significance ($p < 0.05$).

Table 4. Summary of the significant previously reported SNPs in Taiwanese BrS patients with or without *SCN5A* mutations

	Without *SCN5A* mutations		With *SCN5A* mutations	
	P-value[*]	Odds ratio	*P*-value[*]	Odds ratio
SNPs causing susceptibility to Brugada syndrome (set 1)				
rs10428132	1.97E-06	1.79 (1.4-2.27)	6.68E-03[‡]	2.16 (1.21-3.72)
rs9388451	5.69E-04	1.66 (1.25-2.24)	6.57E-01	1.15 (0.64-2.22)
SNPs reaching genome-wide significance in the GWAS threshold in this study (set 2)				
rs6599240	3.79E-03	1.51 (1.13-1.97)	2.10E-01	1.53 (0.75-2.84)
rs11129801	7.81E-05	1.53 (1.24-1.89)	3.68E-02[‡]	1.73 (1.04-2.93)
rs10428132	1.97E-06	1.79 (1.4-2.27)	6.68E-03[‡]	2.16 (1.21-3.72)
rs7428167	2.69E-05	1.6 (1.28-1.99)	8.81E-03[‡]	2.03 (1.2-3.48)
rs12638572	1.68E-06	1.78 (1.4-2.25)	1.47E-02[‡]	2 (1.12-3.43)
rs7641844	2.68E-06	1.7 (1.36-2.12)	9.60E-03[‡]	2 (1.18-3.39)
rs7430439	3.64E-06	1.69 (1.35-2.11)	1.41E-02[‡]	1.94 (1.13-3.28)
rs6599257	5.81E-07	1.96 (1.49-2.53)	2.51E-02[‡]	2.04 (1.05-3.69)
rs1268070	1.00E-02	1.49 (1.11-2.04)	4.21E-01	0.79 (0.45-1.47)
rs9388451	5.69E-04	1.66 (1.25-2.24)	6.57E-01	1.15 (0.64-2.22)
SNPs previously associated with ECG traits (set 3) [†]				
rs17020136	2.72E-03	0.71 (0.57-0.89)	8.11E-01	1.07 (0.63-1.8)
rs10865355	6.72E-03	1.44 (1.1-1.86)	7.92E-01	0.91 (0.42-1.77)
rs11897119	3.99E-03	0.69 (0.54-0.89)	7.30E-01	1.13 (0.59-2.47)
rs11129795	3.95E-15	3.9 (2.85-5.66)	1.06E-03[‡]	3.77 (1.94-10.24)
rs11708996	3.85E-02	0.49 (0.27-1.08)	9.83E-01	NA (0-Inf) [ʃ]
rs6795970	1.79E-07	1.94 (1.51-2.48)	6.83E-03[‡]	2.22 (1.21-3.89)
rs6798015	1.54E-07	1.88 (1.48-2.38)	5.98E-03[‡]	2.17 (1.22-3.73)
rs7784776	8.72E-01	1.02 (0.79-1.31)	4.61E-02[‡]	0.45 (0.19-0.92)
rs314370	1.92E-08	2.48 (1.85-3.5)	5.68E-02	1.78 (1.05-3.57)
rs7342028	2.82E-02	0.78 (0.62-0.97)	8.42E-01	0.95 (0.56-1.6)
rs1896312	1.46E-02	1.32 (1.06-1.66)	9.81E-01	1.01 (0.6-1.71)
rs10850409	1.95E-02	0.76 (0.61-0.96)	8.80E-01	0.96 (0.56-1.62)
rs37062	2.95E-01	0.89 (0.71-1.11)	3.30E-02[‡]	0.57 (0.33-0.95)
rs2074518	2.52E-02	1.34 (1.04-1.74)	3.68E-02[‡]	0.57 (0.34-0.98)

SNP, single nucleotide polymorphism

[*]*P*-value was obtained by using a chi-square test. Red color indicates statistical significance.

[†]Only significant SNPs are listed. For all SNPs, please refer to **Table S7**.

[‡]indicates statistical significance ($p < 0.05$).

[ʃ]Unable to estimate due to extremely imbalanced sample distributions

Figure Legends:

Figure 1. The workflow for the imputation approach.

Figure 2. Cumulative effect of the 3 reported major risk alleles (set 1, BrS-PRS[14]) on susceptibility to BrS in Taiwanese BrS patients. The x-axis shows the number of risk alleles in one individual, whereas the y-axis shows the odds ratios of the BrS patients on a log scale. (A) All BrS patients vs. controls (Ptrend=1.38×10^{-9}); (B) *SCN5A* mutation-positive BrS patients vs. controls (Ptrend=0.049); (C) *SCN5A* mutation-negative BrS patients vs. controls (Ptrend=8.54×10^{-9})

Figure 3. The distribution of the risk alleles in the Taiwanese BrS patients and healthy controls. The BrS patients were divided into two groups based on whether they possessed *SCN5A* mutations or not. The x-axis shows the number of risk alleles in one individual, whereas the y-axis shows the percentage of the people with the alleles.

Figure 4. Comparisons of the odds ratios for the PRS models between the Taiwanese BrS patients with and without *SCN5A* mutations. The x-axis shows the ranges of PRS scores in the BrS patients, whereas the y-axis shows the odds ratios on a log scale. (A) The PRS model developed using the 3 SNPs (set 1, BrS-PRS[14]); (B) The PRS model was developed using the 8 SNPs from set 2 (rs6599257, rs11129801, rs10428132, rs7428167, rs7641844 and rs7430439 in chromosome 3, and rs1268070 and rs9388451 in chromosome 6); (C) The PRS model developed
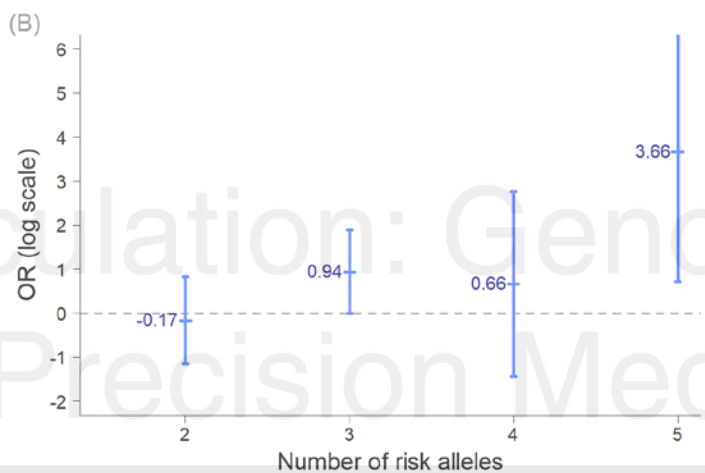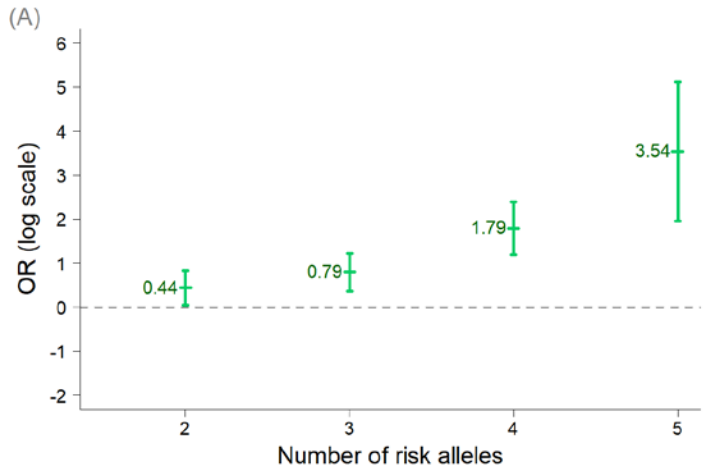
using the 75 SNPs (set 3). The BrS patients were divided into two groups based on whether they had pathogenic *SCN5A* mutations (blue color) or not (red color).
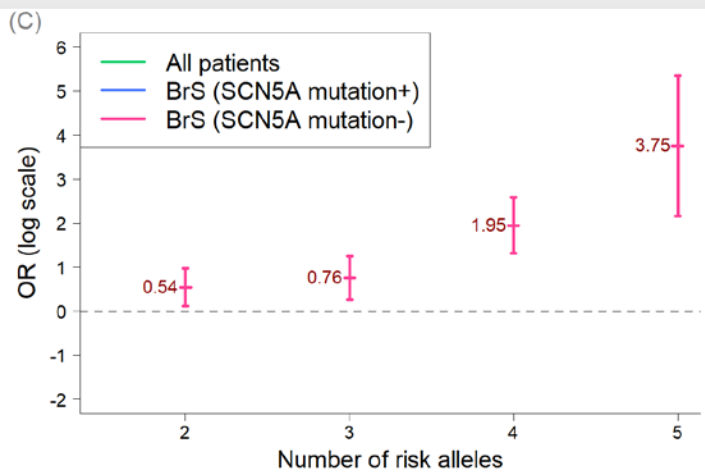
Pipeline

(A)

(B)

(C)

Legend:
- All patients
- BrS (SCN5A mutation+)
- BrS (SCN5A mutation-)