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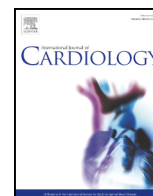
Short communication

# SCN5A mutation type and topology are associated with the risk of ventricular arrhythmia by sodium channel blockers

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## ARTICLE INFO

## ABSTRACT

Background: Ventricular fibrillation in patients with Brugada syndrome (BrS) is often initiated by premature ventricular contractions (PVCs). Presence of SCN5A mutation increases the risk of PVCs upon exposure to sodium channel blockers (SCB) in patients with baseline type-1 ECG. In patients without baseline type-1 ECG, however, the effect of SCN5A mutation on the risk of SCB-induced arrhythmia is unknown. We aimed to establish whether presence/absence, type, and topology of SCN5A mutation correlates with PVC occurrence during ajmaline infusion.

Methods and results: We investigated 416 patients without baseline type-1 ECG who underwent ajmaline testing and SCN5A mutation analysis. A SCN5A mutation was identified in 88 patients ($S^+$). Ajmaline-induced PVCs occurred more often in patients with non-missense mutations ($S^{non\text{-}missense}$) or missense mutations in transmembrane or pore regions of SCN5A-encoded channel protein ($S^{missense\text{-}TP}$) than patients with missense mutations in intra-/extracellular channel regions ($S^{missense\text{-}IE}$) and patients without SCN5A mutation ($S^-$) (29%, 24%, 9%, and 3%, respectively; $P < 0.001$). The proportion of patients with ajmaline-induced BrS was similar in different mutation groups but lower in $S^-$ (71% $S^{non\text{-}missense}$, 63% $S^{missense\text{-}TP}$, 70% $S^{missense\text{-}IE}$, and 34% $S^-$; $P < 0.001$). Logistic regression indicated $S^{non\text{-}missense}$ and $S^{missense\text{-}TP}$ as predictors of ajmaline-induced PVCs.

Conclusions: SCN5A mutation is associated with an increased risk of drug-induced ventricular arrhythmia in patients without baseline type-1 ECG. In particular, $S^{non\text{-}missense}$ and $S^{missense\text{-}TP}$ are at high risk.

## 1. Introduction

Ventricular fibrillation in patients with Brugada syndrome (BrS) is often initiated by premature ventricular contractions (PVCs) [1]. Mutations in SCN5A, the gene encoding the cardiac sodium channel protein Na$_v$1.5, are an important cause of BrS, and BrS patients with baseline type-1 ECG who carry such mutations have increased risk of PVCs after exposure to sodium channel blockers (SCB) [2]. However, the impact of SCN5A mutations on the risk of drug-induced ventricular arrhythmia in BrS patients without baseline type-1 ECG (BrS in these patients is diagnosed through SCB testing) is unknown. As a result, no guidelines or consensus recommendations exist regarding the use of SCB in SCN5A mutation carriers without baseline type-1 ECG.

Because conduction slowing is a key pathomechanism in BrS and Na$_v$1.5 is critical for impulse propagation [3], clinical severity should be greatest in patients who carry SCN5A mutations that disrupt Na$_v$1.5 function the most. Accordingly, we previously showed that non-missense mutations leading to premature truncation of Na$_v$1.5 increased the sensitivity of the cardiac conduction system to SCB more than missense mutations, as reflected by more PR and QRS prolongation during SCB testings [4]. Similarly, missense mutations that cause severe loss of Na$_v$1.5 current ($I_{Na}$) caused more conduction slowing than mutations that reduced $I_{Na}$ less. In our study, we derived the magnitude of $I_{Na}$ from published biophysical studies [4]. However, such studies are labor-intensive and not available for many mutations. While magnitude of $I_{Na}$ reduction and clinical severity may be easy to predict for non-missense mutations (severe), we hypothesized that this can also be estimated for missense mutations based on their topology. In support of this hypothesis, recent evidence suggests that SCN5A missense mutations affecting the transmembrane or pore regions of Na$_v$1.5 (severe $I_{Na}$ reduction) are more likely to be pathogenic than mutations in intracellular or

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extracellular regions (i.e., N-terminus, C-terminus, interdomain or intersegment linkers) (limited $I_{Na}$ reduction) [5].

In this study, we aimed to establish whether *SCN5A* mutation presence/absence, type and topology determine the risk of PVC occurrence during SCB (ajmaline) testing in patients without baseline type-1 ECG. Such knowledge may drive clinical management strategies.

## 2. Methods

### 2.1. Patient inclusion

In this study, we included 416 consecutive subjects (>15-years-old) who had undergone ajmaline testing and *SCN5A* mutation analysis. No subject displayed type-1 ECG at baseline. Indications for the test were aborted cardiac arrest (ACA), ventricular arrhythmia, syncope, palpitations, family history of BrS and/or sudden cardiac death (FH-SCD), or an ECG suspicious but not diagnostic for BrS.

### 2.2. Mutation analysis, ajmaline testing and ECG analysis

Informed consent was obtained. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Genomic DNA extraction from peripheral blood lymphocytes and *SCN5A* mutation analysis was performed as described previously [4]. Ajmaline testing was performed using the protocol of the BrS consensus conference [6]. Ajmaline infusion was stopped when type-1 ECG appeared or immediately after occurrence of PVCs. Twelve-lead ECGs were analyzed at baseline and peak ajmaline dose (ajmaline$^{peak}$; i.e., at maximum dose of ~1 mg/kg or at the first dose when type-1 ECG or PVCs occurred). Ajmaline testing was considered positive if type-1 ST elevation ≥2-mm appeared in ≥1 right-precordial lead [6].

### 2.3. Statistical analysis

Differences between groups were compared using Fisher exact test or χ [2] test (categorical variables), or Student $t$-tests or analysis of variance (continuous variables). Homogeneous subsets of groups were determined with the Standardized Residual Methods (categorical variables) and Student-Newman-Keuls post hoc multiple comparison of groups (continuous variables). For ECG parameters, since multiple tests were performed, the significance level was set at 0.001. In the Tables, homogeneous subsets (no statistical difference) are indicated by an equals (=) sign. A logistic regression analysis was performed to identify predictors for PVCs during ajmaline infusion, and variables with $P < 0.05$ were selected for multivariable analysis. A correction for the relatedness among individuals was applied and the linearity assumption for the numerical predictors was checked. Results of the logistic regression are expressed as odds ratio (OR) with confidence interval (CI). Data are expressed as number (percentage) or mean ± standard deviation (SD), where appropriate.

## 3. Results

The study population included 210 men (age 43 ± 15 years) and 206 women (age 44 ± 14 years). Twenty-eight (6.7%) and 52 (12.5%) patients had experienced ACA or syncope, respectively, and 89 patients (21.4%) had a FH-SCD. Ajmaline induced type-1 ECG in 171 patients (41.1%). A *SCN5A* mutation was identified in 88 patients (21.2%) (see Supplementary Table 1 for a list of mutations). No patient developed sustained arrhythmia or high-degree AV block during ajmaline testing. Twenty-six patients (6.3%) developed PVCs during ajmaline infusion.

### 3.1. Comparisons between patients according to the occurrence of ajmaline-induced type-1 ECG

First we studied whether ajmaline-induced BrS was associated with the occurrence of PVCs. We therefore compared patients with ajmaline-induced type-1 ECG (Ajmaline$^{positive}$; n = 171) with those without ajmaline-induced type-1 ECG (Ajmaline$^{negative}$; n = 245) (Supplementary Table 2). Compared to Ajmaline$^{negative}$, Ajmaline$^{positive}$ were more often probands (41 [16.7%] vs. 52 [30.4%], $P = 0.002$), and had experienced more often syncope (23 [9.4%] vs. 29 [17.0%], $P = 0.032$). The proportion of patients with a *SCN5A* mutation ($S^+$) was higher in Ajmaline$^{positive}$ than Ajmaline$^{negative}$ (59 [34.5%] vs. 29 [11.8%], $P \leq 0.001$). Both at baseline and at ajmaline$^{peak}$, PR and QRS were longer in Ajmaline$^{positive}$ than Ajmaline$^{negative}$. The proportion of patients with ajmaline-induced PVCs did not differ between

Ajmaline$^{positive}$ and Ajmaline$^{negative}$ (15 [8.8%] vs. 11 [4.4%], respectively; $P = 0.117$).

Next, we studied the role of the *SCN5A* mutation in relation to the occurrence of type-1 ECG and PVCs during ajmaline testing by comparing Ajmaline$^{positive}$ without a *SCN5A* mutation (Ajmaline$^{positive}$/$S^-$; n = 112) with Ajmaline$^{positive}$ with a *SCN5A* mutation (Ajmaline$^{positive}$/$S^+$; n = 59) and Ajmaline$^{negative}$ with a *SCN5A* mutation (Ajmaline$^{negative}$/ $S^+$; n = 29) (Table 1). Ajmaline$^{positive}$/$S^-$ and Ajmaline$^{positive}$/$S^+$ (i.e., BrS patients) were younger, and more often probands and symptomatic compared to Ajmaline$^{negative}$/$S^+$. At baseline, PR was longer in Ajmaline$^{positive}$/$S^+$ and Ajmaline$^{negative}$/$S^+$ (i.e., mutation carriers) than Ajmaline$^{positive}$/$S^-$. At ajmaline$^{peak}$, PR and QRS were longer in Ajmaline$^{positive}$/$S^+$ and Ajmaline$^{negative}$/$S^+$ (mutation carriers) than Ajmaline$^{positive}$/$S^-$. The proportion of patients with ajmaline-induced PVCs was higher in Ajmaline$^{positive}$/$S^+$ and Ajmaline$^{negative}$/$S^+$ (mutation carriers) than Ajmaline$^{positive}$/$S^-$ (10 [16.9%] and 7 [24.1%] vs. 5 [4.4%], respectively; $P = 0.002$).

### 3.2. Comparisons between SCN5A mutation carriers and non-carriers

We next compared *SCN5A* mutation carriers ($S^+$; n = 88) vs. non-carriers ($S^-$; n = 328); regardless of the occurrence of type-1 ECG during ajmaline testing. $S^+$ were more often probands than $S^-$ (29 [33.0%] vs. 64 [19.5%], $P = 0.011$). Other clinical characteristics (age, ACA, syncope, and FH-SCD) did not differ between $S^+$ and $S^-$ (Supplementary Table 3).

At baseline, $S^+$ had longer PR than $S^-$, while baseline heart rate (HR), QRS and QTc did not differ. Ajmaline$^{peak}$ was lower in $S^+$ than $S^-$. At ajmaline$^{peak}$, $S^+$ had slower HR and longer PR and QRS than $S^-$. Ajmaline induced type-1 ECG more often in $S^+$ than $S^-$ (59 [67.0%] vs. 112 [34.1%], $P < 0.001$).

Ajmaline also induced PVCs more often in $S^+$ than $S^-$ (17 [19.3%] vs. 9 [2.7%], $P < 0.001$). Except for baseline PR (212 ± 28 ms. in $S^+$ vs. 147 ± 29 ms. in $S^-$, $P < 0.001$), other ECG parameters at baseline or ajmaline$^{peak}$ and clinical characteristics (age, sex, history of ACA or syncope, and FH-SCD) did not differ between $S^+$ with PVC and $S^-$ with PVC. PVCs occurred immediately after the appearance of type-1 ECG in 10/17 $S^+$ (58.8%) and 5/9 $S^-$ (55.5%). The remaining patients with PVCs did not develop type-1 ECG.

Ten of 17 $S^+$ and 8/9 $S^-$ had PVCs with left bundle branch block (LBBB) morphology: 8/17 $S^+$ (47.1%) and 7/9 $S^-$ (77.8%) with LBBB and inferior axis, and 2/17 $S^+$ (11.8%) and 1/9 $S^-$ (11.1%) with LBBB and superior axis. Seven $S^+$ (41.2%) and 1 $S^-$ (11.1%) had PVCs with right bundle branch block (RBBB) morphology.

### 3.3. Comparisons between patients with different SCN5A mutations and non-carriers

To further study the role of the *SCN5A* mutation on the occurrence of ajmaline-induced PVCs, we compared patients with non-missense mutations ($S^{non-missense}$; n = 14), patients with missense mutations in transmembrane/pore regions ($S^{missense-TP}$; n = 41), patients with missense mutations in intra−/extracellular regions ($S^{missense-IE}$; n = 33), and $S^-$ (Table 2). Except for FH-SCD, other clinical characteristics did not differ between groups.

At baseline, PR was longer in $S^{non-missense}$ and $S^{missense-TP}$ than $S^{missense-IE}$ and $S^-$. Other baseline ECG parameters did not differ between the groups. Ajmaline$^{peak}$ was lower in $S^{non-missense}$ and $S^{missense-TP}$ than $S^{missense-IE}$ and $S^-$. At ajmaline$^{peak}$, $S^{non-missense}$ had slower HR and longer QRS than other $S^+$ and $S^-$. The proportion of patients with ajmaline-induced type-1 ECG did not differ between different mutation groups.

Ajmaline induced PVCs more often in $S^{non-missense}$ and $S^{missense-TP}$ than $S^{missense-IE}$ and $S^-$. Expect for baseline PR (212 ± 33 ms. in $S^{non-missense}$, 223 ± 21 in $S^{missense-TP}$, 175 ± 16 ms. in $S^{missense-IE}$, and 147 ± 26 ms. in $S^-$, $P < 0.001$), other ECG parameters at baseline or ajmaline$^{peak}$ and clinical characteristics did not differ between

**Table 1**
Comparisons between subjects according to the occurrence of ajmaline-induced type-1 ECG and *SCN5A* mutation status.

| Parameter | Positive ajmaline test & $S^-$ (n = 112) | Positive ajmaline test & $S^+$ (n = 59) | Negative ajmaline test & $S^+$ (n = 29) | P value |
|---|---|---|---|---|
| *Clinical parameters* | | | | |
| Male gender, n (%) | 55 (49) | 32 (54) | 15 (52) | 0.813 |
| Proband, n (%) | 26 (23) | 26 (44) | 3 (10) | 0.001 (1 = 2 > 3) |
| Age at ajmaline test, years | 44 ± 13 | 44 ± 12 | 51 ± 14 | 0.033 (1 = 2 < 3) |
| Aborted cardiac arrest, n (%) | 10 (9) | 3 (3) | 0 | 0.192 |
| Syncope, n (%) | 16 (14) | 13 (22) | 0 | 0.022 (1 = 2 > 3) |
| Family history of SCD, n (%) | 20 (18) | 18 (31) | 8 (28) | 0.143 |
| Body weight, kg | 74 ± 14 | 76 ± 17 | 81 ± 6 | 0.053 |
| *Baseline* | | | | |
| Heart rate, beats/min | 68 ± 13 | 65 ± 13 | 63 ± 11 | 0.045 |
| PR, ms | 164 ± 26 | 191 ± 36 | 197 ± 39 | <0.001 (1 < 2 = 3) |
| QRS, ms | 100 ± 12 | 102 ± 16 | 97 ± 25 | 0.230 |
| QTc, ms | 406 ± 22 | 404 ± 24 | 416 ± 31 | 0.077 |
| *Peak ajmaline dose* | | | | |
| Ajmaline dose/weight, mg/kg | 0.99 ± 0.19 | 0.82 ± 0.27 | 1.00 ± 0.20 | <0.001 (2 < 1 = 3) |
| Heart rate, beats/min | 79 ± 11 | 73 ± 12 | 70 ± 12 | <0.001 (1 > 2 = 3) |
| PR, ms | 220 ± 31 | 243 ± 53 | 254 ± 51 | <0.001 (1 < 2 = 3) |
| QRS, ms | 133 ± 17 | 146 ± 25 | 150 ± 28 | <0.001 (1 < 2 = 3) |
| QTc, ms | 470 ± 29 | 471 ± 34 | 470 ± 27 | 0.981 |
| PVC, n (%) | 5 (4) | 10 (17) | 7 (24) | 0.002 (1 < 2 = 3) |
| *Δ Peak ajmaline - baseline* | | | | |
| Δ HR, ms | 11 ± 12 | 7 ± 9 | 7 ± 8 | (1 > 2 = 3) |
| Δ PR, ms | 56 ± 20 | 51 ± 36 | 57 ± 25 | 0.108 |
| Δ QRS, ms | 33 ± 16 | 45 ± 28 | 53 ± 24 | <0.001 (1 < 2 = 3) |
| Δ QTc, ms | 64 ± 19 | 67 ± 31 | 55 ± 27 | 0.062 |

$S^+$, *SCN5A* mutation carriers; $S^-$, patients without *SCN5A* mutation. HR, heart rate; PVC, premature ventricular contractions; QTc, heart rate-corrected QT interval; SCD, sudden cardiac death. Data are expressed as number (percentage) or mean ± SD. N indicates number of patients. Δ indicates differences between ECG values at peak ajmaline dose – values at baseline. P values indicate results of statistical comparisons between the three groups (columns). In case of an overall statistical significant difference, homogeneous groups (with no statistical difference) are indicated by an equals (=) sign.

patients with PVCs in different groups (i.e., $S^{non-missense}$ and $S^{missense-TP}$, $S^{missense-IE}$, and $S^-$).

### 3.4. Predictors of PVCs during ajmaline infusion

Multivariable analysis included HR, PQ, QRS, and QTc at baseline and ajmaline$^{peak}$, weight-adjusted ajmaline$^{peak}$, $S^{non-missense}$, $S^{missense-TP}$, and $S^{missense-IE}$. Ajmaline-induced type-1 ECG was not included because it was not identified as a predictor for PVCs by logistic regression analysis. $S^{non-missense}$ (OR 10.15 [CI 2.14–48.02]) and $S^{missense-TP}$ (OR 7.25 [CI 1.68–31.38]) were identified as independent predictors for ajmaline-induced PVCs. Moreover, baseline HR (OR 1.05 [CI 1.02–1.09]) and QRS at ajmaline$^{peak}$ (OR 1.04 [1.01–1.06]) were found as independent predictors. Baseline HR was higher in patients with than without PVCs (74 ± 14 vs. 67 ± 13 beats/min, P = 0.007). QRS at ajmaline$^{peak}$ was longer in patients with than without PVCs (155 ± 33 vs. 131 ± 18 ms, P < 0.001).

## 4. Discussion

In the last 15 years, several studies have attempted to identify predictors for drug-induced ventricular arrhythmia in patients undergoing SCB testing. However, these studies did not investigate *SCN5A* mutation status and/or included patients with baseline type-1 ECG (who are well-recognized to be at high risk for adverse events in the presence of SCB) [2,7]. As a result, it is still unknown whether carriers of a *SCN5A* mutation without baseline type-1 ECG are at higher risk of drug-induced ventricular arrhythmia.

In this study, we first systematically studied the impact of *SCN5A* mutation on the occurrence of PVCs during ajmaline testing in patients without baseline type-1 ECG. We found that presence of *SCN5A* mutation increases the likelihood that ajmaline exposes BrS and evokes

PVCs, and that the risk of PVCs in $S^+$ is regardless of the occurrence of type-1 BrS ECG during ajmaline testing (i.e., the occurrence of PVCs was similar between Ajmaline$^{positive}$-$S^+$ and Ajmaline$^{negative}$-$S^+$ and higher than Ajmaline$^{positive}$-$S^-$ [Table 1]). In line with earlier data [8], $S^-$ often had PVCs with LBBB morphology and inferior axis, suggesting a right-ventricular outflow tract origin, while $S^+$ had PVCs originating from both left and right ventricle.

In addition, we found that the risk for drug-induced PVCs depends on the type and topology of the *SCN5A* mutation. Multivariable analysis identified $S^{non-missense}$ and $S^{missense-TP}$ mutations as strong predictors for ajmaline-induced PVCs. Moreover, although the effect was weaker, baseline heart rate and QRS after ajmaline were also identified as predictors for PVCs. Ajmaline-induced type-1 ECG was not associated with PVC occurrence and did not predict PVCs, and the proportions of patients who developed type-1 ECG did not differ between mutation groups. Based on these findings, one may speculate that the mechanism underlying ajmaline-induced PVCs is large reduction of $I_{Na}$ and the depolarization reserve in the heart [3,4,9]. $I_{Na}$ reduction by $S^{non-missense}$ or $S^{missense-TP}$ mutations may be significant, but depolarization reserve is large enough to ensure that QRS in $S^{non-missense}$ and $S^{missense-TP}$ at baseline is not different from $S^{missense-IE}$ or $S^-$. However, the added presence of ajmaline results in further $I_{Na}$ reduction and decline in the depolarization reserve to a level that results in QRS prolongation [4] and ultimately PVCs [9]. Since ajmaline requires repetitive opening and closing of $Na_v1.5$ to act as a blocker ('use-dependent block'), $Na_v1.5$ is blocked more potently at higher heart rates. Together these processes may explain why *SCN5A* mutation type and topology, higher baseline heart rates and longer QRS intervals after ajmaline are associated with the risk for ajmaline-induced PVC occurrence.

The concept that the magnitude of $I_{Na}$ reduction due to a *SCN5A* mutation plays a crucial role in the occurrence of ajmaline-induced PVCs is in line with our finding that the peak ajmaline dose was

**Table 2**
Comparisons between subjects according to *SCN5A* mutation status, type and location.

| Parameter | $S^+$ non-missense (n = 14) | $S^+$ missense-TP (n = 41) | $S^+$ missense-IE (n = 33) | $S^-$ (n = 328) | P value |
|---|---|---|---|---|---|
| *Clinical parameters* | | | | | |
| Male gender, n (%) | 7 (50) | 17 (41) | 23 (70) | 163 (50) | 0.098 |
| Proband, n (%) | 5 (36) | 13 (32) | 11 (33) | 64 (20) | 0.062 |
| Age at ajmaline test, years | 47 ± 10 | 44 ± 14 | 49 ± 13 | 43 ± 14 | 0.089 |
| Aborted cardiac arrest, n (%) | 0 | 1 (2) | 2 (6) | 25 (8) | 0.448 |
| Syncope, n (%) | 3 (21) | 7 (17) | 3 (9) | 39 (12) | 0.519 |
| Family history of SCD, n (%) | 9 (64) | 9 (22) | 8 (27) | 63 (19) | <0.001 (1 > 2 = 3 = 4) |
| Body weight, kg | 74 ± 20 | 76 ± 17 | 82 ± 14 | 76 ± 16 | 0.074 |
| *Baseline* | | | | | |
| Heart rate, beats/min | 61 ± 9 | 66 ± 14 | 64 ± 11 | 68 ± 13 | 0.037 |
| PR, ms | 212 ± 23 | 198 ± 41 | 179 ± 33 | 160 ± 24 | <0.001 (1 = 2 > 3 = 4) |
| QRS, ms | 104 ± 20 | 99 ± 21 | 99 ± 17 | 98 ± 12 | 0.292 |
| QTc, ms | 406 ± 29 | 411 ± 28 | 404 ± 24 | 408 ± 25 | 0.543 |
| *Peak ajmaline dose* | | | | | |
| Ajmaline dose/weight, mg/kg | 0.79 ± 0.21 | 0.84 ± 0.26 | 0.96 ± 0.27 | 1.03 ± 0.14 | <0.001 (1 = 2 < 3 = 4) |
| HR, beats/min | 66 ± 12 | 73 ± 13 | 73 ± 12 | 78 ± 11 | <0.001 (1 < 2 = 3 = 4) |
| PR, ms | 260 ± 44 | 253 ± 62 | 234 ± 38 | 212 ± 30 | <0.001 (1 = 2 > 3 = 4) |
| QRS, ms | 162 ± 21 | 150 ± 29 | 138 ± 19 | 129 ± 17 | <0.001 (1 > 2 = 3 = 4) |
| QTc, ms | 481 ± 39 | 475 ± 31 | 460 ± 29 | 466 ± 32 | 0.090 |
| Brugada ECG, n (%) | 10 (71) | 26 (63) | 23 (70) | 112 (34) | <0.001 (1 = 2 = 3 > 4) |
| PVC, n (%) | 4 (29) | 10 (24) | 3 (9) | 9 (3) | <0.001 (1 = 2 > 4 = 3) |
| *Δ Peak ajmaline - baseline* | | | | | |
| Δ HR, ms | 5 ± 5 | 7 ± 10 | 8 ± 6 | 10 ± 9 | 0.004 |
| Δ PR, ms | 48 ± 33 | 53 ± 37 | 55 ± 26 | 52 ± 21 | 0.843 |
| Δ QRS, ms | 58 ± 27 | 51 ± 27 | 40 ± 25 | 31 ± 15 | <0.001 (1 > 2 > 3 = 4) |
| Δ QTc, ms | 75 ± 29 | 64 ± 32 | 57 ± 28 | 58 ± 26 | 0.005 |

$S^+$, *SCN5A* mutation carriers; $S^-$, patients without *SCN5A* mutation; non-missense, non-missense mutations; missense-TP, missense mutations in transmembrane segments or pore region of $Na_v1.5$; missense-IE, missense mutation in intra- or extracellular regions of $Na_v1.5$. HR, heart rate; PVC, premature ventricular contractions; QTc, heart rate-corrected QT interval; SCD, sudden cardiac death. Data are expressed as number (percentage) or mean ± SD. N indicates number of patients. Δ indicates differences between ECG values at peak ajmaline dose – values at baseline. P values indicate results of statistical comparisons between the four groups (columns). In case of an overall statistical significant difference, homogeneous groups (with no statistical difference) are indicated by an equals (=) sign.

lower in $S^{\text{non-missense}}$ and $S^{\text{missense-TP}}$ than $S^{\text{missense-IE}}$ or $S^-$, while the proportion of patients with PVCs was much larger in $S^{\text{non-missense}}$ and $S^{\text{missense-TP}}$. This suggests that $S^{\text{non-missense}}$ and $S^{\text{missense-TP}}$ mutations cause more $I_{Na}$ reduction and therefore require less $Na_v1.5$ block by ajmaline for PVCs to occur. Interestingly, the same concept may also apply for the occurrence of ajmaline-induced type-1 ECG. While the proportion of ajmaline-induced type-1 ECG did not differ between $S^{\text{non-missense}}$, $S^{\text{missense-TP}}$, and $S^{\text{missense-IE}}$, the required peak ajmaline dose was higher in the latter, suggesting that the degree of $I_{Na}$ reduction may also play an important role in the pathophysiology of BrS, and that $S^{\text{non-missense}}$ and $S^{\text{missense-TP}}$ are at higher risk of developing BrS in the presence of SCB than $S^{\text{missense-IE}}$ (or $S^-$).

The limitations of our study include its retrospective design and the small size of various *SCN5A* mutation groups. In addition, although type-1 ECG was absent in all patients on at least two time points, we cannot exclude the presence of transient type-1 ECG, e.g., during fever. Moreover, the study population was not screened for large genomic rearrangements or mutations in other genes that have been anecdotally linked to BrS. However, in this regard it is important to note that in an earlier genetic screening study in 38 BrS patients from our center, we have excluded large genomic rearrangements and mutations in other BrS-linked candidate genes [10].

## 5. Conclusions

The presence of a *SCN5A* mutation increases the likelihood that ajmaline exposes BrS and evokes ventricular arrhythmia in patients without baseline type-1 ECG. Moreover, the risk for drug-induced arrhythmia depends on type and topology of the *SCN5A* mutation, and patients with $S^{\text{non-missense}}$ or $S^{\text{missense-TP}}$ mutations may be at highest risk. We recommend *SCN5A* mutation analysis in individuals who experience ventricular arrhythmia while using SCB, particularly if arrhythmia is preceded by QRS prolongation, also in patients in whom type-1 ECG has not been achieved (yet). Moreover, we discourage prescription of SCB in patients with *SCN5A* mutation, including patients without BrS.

## Statement of authorship

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

## Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2017.09.010.

## References

[1] M. Kakisita, T. Kurita, K. Matsuo, et al., Mode of onset of ventricular fibrillation in patients with Brugada syndrome detected by implantable cardioverter defibrillator therapy, J. Am. Coll. Cardiol. 36 (2000) 1646–1653.

[2] M. Gasparini, S.G. Priori, M. Mantica, et al., Flecainide test in Brugada syndrome: a reproducible but risky tool, Pacing Clin. Electrophysiol. 26 (1 Pt II) (2003) 338–341.

[3] Z.S. Zhang, J. Tranquillo, V. Neplioueva, N. Bursac, A.O. Grant, Sodium channel kinetic changes that produce Brugada syndrome or progressive cardiac conduction system disease, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H399–407.

[4] P.G. Meregalli, H.L. Tan, V. Probst, et al., Type of *SCN5A* mutation determines clinical severity and degree of conduction slowing in loss-of-function sodium channelopathies, Heart Rhythm. 6 (2009) 341–348.

[5] S. Le Scouarnec, M. Karakachoff, J.B. Gourraud, et al., Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome, Hum. Mol. Genet. 24 (2015) 2757–2763.

[6] C. Antzelevitch, G.X. Yan, M.J. Ackerman, et al., J-wave syndromes expert consensus conference report: emerging concepts and gaps in knowledge, Heart Rhythm. 13 (2016) e295–324.

[7] B. Dobbels, D. De Cleen, J. Ector, Ventricular arrhythmia during ajmaline challenge for the Brugada syndrome, Europace 18 (2016) 1501–1506.

[8] H. Morita, S. Nagase, D. Miura, et al., Differential effects of cardiac sodium channel mutations on initiation of ventricular arrhythmias in patients with Brugada syndrome, Heart Rhythm. 6 (2009) 487–492.

[9] A. Bardai, A.S. Amin, M.T. Blom, et al., Sudden cardiac arrest associated with use of a non-cardiac drug that reduces cardiac excitability: evidence from bench, bedside, and community, Eur. Heart J. 34 (2013) 1506–1516.

[10] T.T. Koopmann, L. Beekman, M. Alders, et al., Exclusion of multiple candidate genes and large genomic rearrangements in *SCN5A* in a Dutch Brugada syndrome cohort, Heart Rhythm. 4 (2007) 752–755.