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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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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 ($S^{\text{non-missense}}$) or missense mutations in transmembrane or pore regions of *SCN5A*-encoded channel protein ($S^{\text{missense-TP}}$) than patients with missense mutations in intra-/extracellular channel regions ($S^{\text{missense-IE}}$) 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% $S^{\text{non-missense}}$, 63% $S^{\text{missense-TP}}$, 70% $S^{\text{missense-IE}}$, and 34% S^{-}; $P < 0.001$). Logistic regression indicated $S^{\text{non-missense}}$ and $S^{\text{missense-TP}}$ 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, $S^{\text{non-missense}}$ and $S^{\text{missense-TP}}$ 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 $Na_v1.5$, 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 $Na_v1.5$ is critical for impulse propagation [3], clinical severity should be greatest in patients who carry *SCN5A* mutations that disrupt $Na_v1.5$ function the most. Accordingly, we previously showed that non-missense mutations leading to premature truncation of $Na_v1.5$ 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 $Na_v1.5$ 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 $Na_v1.5$ (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 ($S^{non\text{-}missense}$; n = 14), patients with missense mutations in transmembrane/pore regions ($S^{missense\text{-}TP}$; n = 41), patients with missense mutations in intra−/extracellular regions ($S^{missense\text{-}IE}$; n = 33), and S^- (Table 2). Except for FH-SCD, other clinical characteristics did not differ between groups.

At baseline, PR was longer in $S^{non\text{-}missense}$ and $S^{missense\text{-}TP}$ than $S^{missense\text{-}IE}$ and S^-. Other baseline ECG parameters did not differ between the groups. Ajmaline^{peak} was lower in $S^{non\text{-}missense}$ and $S^{missense\text{-}TP}$ than $S^{missense\text{-}IE}$ and S^-. At ajmaline^{peak}, $S^{non\text{-}missense}$ 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 $S^{non\text{-}missense}$ and $S^{missense\text{-}TP}$ than $S^{missense\text{-}IE}$ and S^-. Expect for baseline PR (212 ± 33 ms. in $S^{non\text{-}missense}$, 223 ± 21 in $S^{missense\text{-}TP}$, 175 ± 16 ms. in $S^{missense\text{-}IE}$, and 147 ± 26 ms. in S^-, $P < 0.001$), other ECG parameters at baseline or ajmaline^{peak} and clinical characteristics did not differ between

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