Brugada syndrome : clinical and pathophysiological aspects

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Diagnostic Value of Flecainide Testing in Unmasking *SCN5A*-related Brugada Syndrome

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Abstract

Introduction: Provocation tests with sodium channel blockers are often required to unmask ECG abnormalities in Brugada syndrome. However, their diagnostic value is only partially established, while life-threatening ventricular arrhythmias during these tests were reported. We aimed to establish sensitivity, specificity and safety of flecainide testing, and to predict a positive test outcome from the baseline ECG.

Methods and Results: We performed 160 tests with flecainide in subjects determined to be at risk for Brugada syndrome. P wave width, PQ duration, QRS width, S wave amplitude and duration in leads II-III, in addition to ST morphology and J point elevation in V1-V3 were measured before and after flecainide administration. Moreover, leads were positioned over the third intercostal space ($V1_{IC3}$-$V2_{IC3}$). Flecainide tests were considered positive if criteria from the First Consensus Report on Brugada syndrome were fulfilled. In 64 cases the test was positive, while 95 were negative (1 test was prematurely interrupted). The sensitivity and specificity, calculated in *SCN5A*-positive probands and their family members, were 77% and 80%, respectively. Baseline ECGs exhibited significant group differences in P, PQ and QRS duration, J point elevation (leads V1-V2 and $V1_{IC3}$-$V2_{IC3}$), and S duration in II, but an attempt to predict the outcome of flecainide testing from these baseline ECG parameters failed. No malignant arrhythmias were observed.

Conclusion: Flecainide testing is a valid and safe tool to identify *SCN5A*-related Brugada syndrome patients. Baseline ECGs do not predict test outcomes, but point to conduction slowing as a core mechanism in Brugada Syndrome.

Introduction

Brugada syndrome is characterized by an augmented risk of sudden death from malignant ventricular tachyarrhythmias. Its pathognomonic electrocardiographic (ECG) signature is ST segment elevation in right precordial leads [1]. Because structural cardiac abnormalities are not detected by routine cardiac examinations, Brugada syndrome is considered a primary electrical disease [2], although some studies provided evidence that it may involve ultra-structural derangements [for review see [3]] and may share features with arrhythmogenic right ventricular cardiomyopathy [4, 5]. Importantly, the differential diagnosis of Brugada syndrome includes various other diseases [1, 6]. Therefore, genetic confirmation (i.e., carriership of a causative mutation) is useful to provide final confirmation of this disease beyond the clinical phenotype alone. So far, the only gene with a proven causal involvement is *SCN5A*, encoding the α-subunit of the cardiac sodium (Na^+) channel (I_{Na})[7]. More than 50 *SCN5A* mutations have been linked to Brugada syndrome until now [8, 9]. Their common effect is I_{Na} reduction, resulting from changes in functional properties (gating) of the mutant Na^+ channels or failure of expression in the sarcolemma (trafficking) [10, 11]. The reduction in Na^+ current through the mutant Na^+ channels [12] points to reduction in cardiac excitability as the underlying electrophysiological mechanism. Accordingly, evidence of conduction slowing in the right ventricle is present [13]. Of note, ECG abnormalities may vary in time and may be modulated by factors such as body temperature or the use of sodium channel blockers [14, 15]. Consequently, provocation tests using Na^+ channel blockers are often required to unmask the Brugada syndrome [16]. While these tests are generally considered helpful for diagnosis and risk stratification in this syndrome [8, 16, 17] and ajmaline was shown to possess a high diagnostic yield [18, 19], several issues still need to be resolved and remain the object of international debate [20, 21]. First, large studies on the effectiveness of drug testing in uncovering Brugada syndrome are lacking, as reported studies were conducted either in the absence of molecular-genetic confirmation of Brugada syndrome [19, 22], or only among few families in whom *SCN5A* mutations had already been identified[18]. Secondly, their safety has been questioned, as case studies have reported the induction of life-

threatening ventricular tachyarrhythmias [23, 24]. With the present study, we aimed to investigate the capacity of flecainide testing to identify *SCN5A*-related Brugada syndrome subjects and to assess its safety in a large series of patients. Furthermore, we conducted analysis of the baseline ECGs in an effort to identify baseline ECG parameters, in addition to J point elevation, that could predict a positive outcome of flecainide testing.

Methods

Study Population

We retrospectively analyzed 160 consecutive flecainide tests, performed between 1999 and 2004 at our institution (n=137) or referred for evaluation from elsewhere (n=23), and collected clinical and family history of all subjects. Patients who had exhibited a diagnostic ECG (type I ECG, see below) at any time, in the absence of flecainide, were excluded from this study.

All subjects except one were suspected to have Brugada syndrome and underwent flecainide testing for the following reasons: 1) unexplained aborted sudden death from a documented ventricular tachyarrhythmia (n=17), 2) syncope of unknown origin (n=29), 3) family screening (diagnosis of Brugada syndrome in a relative; n=82), 4) ECG, recorded for other medical reasons, that was suspicious [see [1]] but not diagnostic (type I ECG, see below) for Brugada syndrome (n=28), 5) episodes of ventricular tachyarrhythmias during treatment with flecainide (n=3). In one subject, provocation testing with flecainide was performed to investigate the characteristics of an accessory pathway and the test resulted in a diagnostic outcome for Brugada syndrome. Structural cardiac abnormalities were excluded in all subjects by physical examination and echocardiography. Laboratory tests were done to exclude electrolyte or metabolic disturbances. Flecainide infusion (2 mg/kg) was conducted in repeated boluses of 10 mg/min until a maximum dose of 150 mg [1]. The tests were performed in a quiet room, equipped for cardiopulmonary resuscitation. The infusion was terminated when a positive ECG response was elicited or major adverse events occurred, defined as ventricular arrhythmias or important conduction slowing (prolongation of the QRS ≥ 30% of the basal value [1]).

A positive response was defined as the occurrence of a type I ECG: coved-type ST segment elevation (≥ 2 mm at its peak, followed by a negative T wave, without isoelectric separation) in conventional leads V1-V3 or in leads $V1_{IC3}$-$V2_{IC3}$, positioned over the third intercostal space [1].
The subjects were monitored until return of ST segments to the baseline, in case of a positive response, or for 30 min in case of a negative response.

Calculation of the Diagnostic Yield of Flecainide Testing

We expressed the diagnostic value of the flecainide test by its sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV). To calculate these parameters, we defined that Brugada syndrome was present when, in addition to clinical criteria, a *SCN5A* mutation was identified [6]. Although *SCN5A* mutations are present in only 20-30% of Brugada syndrome patients [17, 25, 26], we required carriership of a *SCN5A* mutation, as no other test provides equally strong confirmation of Brugada syndrome in the presence of clinical criteria for this disease. Given this assumption, we selected, between all tested subjects in whom genetic results were also known (n=110), the ones coming from families with a documented *SCN5A* mutation (n=35).

ECG Analysis

ECG tracings were enlarged to 141% to facilitate manual analysis. The following ECG variables were analyzed at baseline and at the maximal flecainide dose: heart rate (HR), P wave width (P), PQ duration (PQ), QRS duration in leads V1 (QRSV1) and V6 (QRSV6), ST segment morphology and amount of J point elevation in leads V1-V3 (JV1-JV3). J point elevation was also investigated in 144/159 patients (in all negative and in 49/64 positive responders) in leads positioned over the third intercostal space, cranial to leads V1 and V2 ($JV1_{IC3}$-$JV2_{IC3}$), since their usefulness in diagnosing Brugada syndrome has been demonstrated [27-29]. Baseline ST segment morphology was examined in all patients according to the classification reported in the first Consensus paper [1]. Furthermore, we examined R wave amplitude in leads V1-V2 (RV1-RV2) as measurement of the leading depolarizing forces in the right ventricle outflow tract (RVOT) and the amplitude and duration of S

waves in leads II and III (aSII; dSII; aSIII; and dSIII), as they might be reciprocal representations of the delayed electrical activity in the RVOT. Because flecainide testing in Brugada syndrome may elicit changes in QT duration [30], we analyzed QT duration (QT) and rate-corrected (Bazett's formula) QT duration (QTc), taking their largest value among leads V2-V4.

Predictive Value of the Fecainide Test Based on Pre-test ECG Parameters

To determine whether the result of the flecainide test could be predicted from a combination of observed baseline ECG values, a logistic regression analysis was carried out. To this end, the ECG parameters that, at baseline, showed a significant difference between the positive and negative test group were entered into the logistic regression model in a forward conditional way (SPSS 11.5.1). The addition of parameters to the model was stopped when no new parameter could significantly ($p<0.05$) contribute to the prediction. The group membership resulting from the final model was saved and used to calculate the sensitivity and specificity, as well as the positive and negative predictive value, of the prediction of the flecainide test result from these baseline ECG parameters.

Genetic Analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. All *SCN5A* exons were amplified using primers located in flanking intronic sequences [7] and analysed for mutations using denaturing high performance liquid chromatography (dHPLC)-DNA sequencing, as described previously [31]. We verified that these DNA variants were disease causing mutations, rather than polymorphisms, by generally accepted criteria, including the following: their presence in highly conserved regions of *SCN5A*, their absence in 100 control individuals and, where possible, co-segregation with the disease phenotype.

Statistical Analysis

ECG parameter values are expressed as mean ± SEM. For comparison between the positive and the negative responders groups, the non-parametric Mann-Whitney-U Test was used. The effect of flecainide on each ECG parameter was

calculated as percentage (the difference between the pre-test and post-test values divided by the pre-test value, x 100). Statistical analysis was performed using SPSS (version 11.5.0). All tests were two-sided and statistical significance was defined as $p< 0.05$.

Results

Demographic and Clinical Data

Demographic and clinical characteristics are summarized in Table 1. A total of 160 flecainide tests were analysed. One test in a male was prematurely interrupted (see below) and excluded from further analysis. Of the remaining 159 patients, 64 (40%) had a positive response, while 95 (60%) were negative. There was a statistically significant difference in the average age between both groups (positive: 48±2 years, negative: 42±1 years, p=0.005), albeit with a large overlap (quartile range positive: 35-61, negative: 32-52 years). While there was a clear predominance of males, their proportion was similar in both groups (positive: 40/64 [62%], negative: 59/95 [62%], p=1.0). Baseline ST-segment morphology before flecainide administration (Table 1), as well as the proportion of patients with arrhythmic events at the time of testing was not significantly different between the groups (positive responders: 21/64 [33%], negative responders: 21/95 [22%], p= 0.13). Given that infusion was stopped when the test became positive, the patients in the positive group received significantly less flecainide (positive: 111±5 mg, negative: 140±2 mg, p<0.0001).

Diagnostic Yield of Flecainide Testing

Results of DNA analysis were available in 110/159 patients (59 in the positive and 51 in the negative group) while DNA analysis was not conducted (negative responders with low pre-test likelihood of Brugada syndrome) or declined in the remaining subjects. The patient whose test was prematurely interrupted had no *SCN5A* mutation. In all, *SCN5A* mutations (Table 2) were found in 23/59 positive subjects (39%) and in 7/51 negative ones (14%). Among these 110 patients, the analysis of the diagnostic yield of flecainide testing was restricted to subjects coming from families in which a *SCN5A* mutation, responsible for the Brugada

syndrome phenotype was found. Accordingly, 35 subjects, belonging to twelve *SCN5A*-positive families, were included. Among these 35 subjects 24 had a positive and 11 a negative outcome upon flecainide. Twenty-three out the 24 positive-responders had a *SCN5A* mutation, while a *SCN5A* mutation was found in 7 out the 11 negative responders. The sensitivity and specificity of flecainide testing, calculated in carriers of *SCN5A* mutation, were 77% and 80%, and its positive and negative predictive values 96% and 36%, respectively.

Table 1: Demographic and clinical characteristics of the study population.

	Positive test	Negative test	p-value
Number	64 (40%)	95 (60%)	-
Age, years (quartile range)	48 ± 2 (35-61)	42 ± 1 (32-52)	0.005
Gender, male/female[$]	40/24	59/36	1
Spontaneous events°	21	21	0.13
Baseline ST segment pattern[#]			
Type II	26	30	0.24
Type III	28	36	0.46
None between Types I-II-III	10	29	0.03
Flecainide dose (mg)	111 ± 5	140 ± 2	<0.0001

[$] Both in the positive test and negative test groups: male 62,5% and female 37,5%.

° Events before flecainide testing.

[#] Types of repolarization patterns have been classified as described in the first Consensus Report on Brugada syndrome [1].

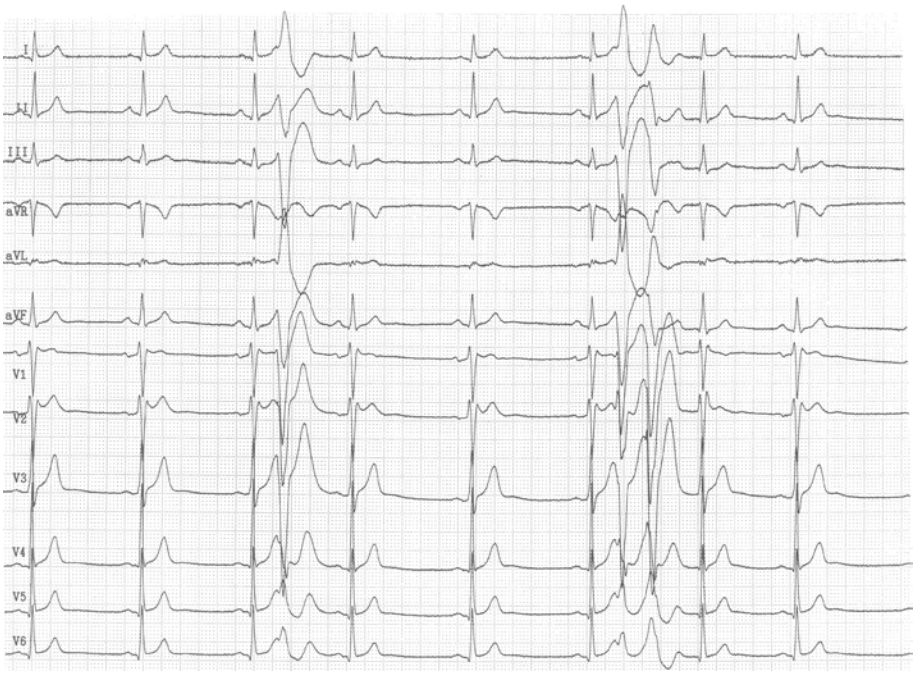


Figure 1: *ECG recorded after intravenous infusion of 80 mg flecainide in a 45 year old male subject showing a saddle-back ST segment elevation in leads V1 and V2 (type II ECG) and the appearance of premature ventricular beats, isolated and in couples, from the right ventricle. The ectopic beats show a short coupling interval. In this subject, an* SCN5A *mutation was not identified. Investigation of the leads* $V1_{IC3}$ *and* $V2_{IC3}$ *was not performed.*

Table 2: Identified *SCN5A* mutations in the study population

W156X (n=1)	C1363Y (n=1)
E161K (n=10)	R1638X (n=2)
L860fsx89 (n=1)	1570insI (n=5)
N927S (n=1)	G1743E (n=5)
R965H (n=1)	1795 insD (n=3)

Mutations E161K and G1743E were identified in two non-related families

Safety of the Flecainide Testing

No malignant arrhythmias or side effects were observed during flecainide tests. Ten of 160 patients (6%) showed premature ventricular complexes (PVCs), usually solitary and, rarely, in couplets. In one male patient, who had no *SCN5A* mutation, the increasing incidence of PVCs prompted premature termination of the test (Figure 1). Of the 9 remaining subjects, 4 had a positive and 5 a negative test. Among the 4 positive responders, 3 had a *SCN5A* mutation (N927S, R965H, W156X), and showed PVCs at 30, 120, and 150 mg, respectively; the fourth had no *SCN5A* mutation and PVCs at baseline, which disappeared during flecainide administration. Among the 5 negative responders upon flecainide, 2 had PVCs at high doses, 2 only at baseline, while the fifth exhibited frequent supraventicular ectopic beats, but no ventricular arrhythmias. In these 5 patients, DNA analysis was not conducted.

Moreover, 24 subjects (15%) showed, during flecainide infusion, signs of conduction slowing, defined by an increase in QRS duration by more than 30% of the baseline value. Eleven of these patients had a *SCN5A* mutation, including 6 with a positive test and 5 with a negative test. In seven subjects a *SCN5A* mutation was not identified (4 in the positive, 3 in the negative group), while no DNA analysis was conducted in the 6 other patients (5 negative, 1 positive).

Analysis of Baseline ECG Parameters

Detailed analysis of ECG parameters at baseline and after flecainide is summarized in Table 3. No statistically significant difference between the two groups was found in mean heart rate (HR) before and after flecainide infusion.

At baseline, patients with a positive test had, on average, wider P waves, longer PQ intervals, wider QRS complexes in leads V1 and V6, and S waves in lead II. Also, positive patients had more prominent J point elevation in leads V1, V2, $V1_{IC3'}$ and $V2_{IC3'}$, but not in lead V3.

After flecainide, all these differences were maintained, while S wave duration in III also became significantly longer in the positive group.

R wave amplitude in V1 and V2 was not significantly different between the groups, neither at baseline, nor after flecainide.

Table 3: ECG parameters measured at baseline and at maximal flecainide dose, as well as the effect of flecainide (calculated as percentage of the baseline value) on ECG parameters in both groups. Numbers are given as mean ± SEM. P values between positive and negative responders are reported.

	Baseline			Maximal flecainide dose			Flecainide Effect (%)		
ECG-parameter	Positive responders	Negative responders	p-value	Positive responders	Negative responders	p-value	Positive responders	Negative responders	p-value
Heart rate (HR) (bpm)	70 ± 2	65 ± 1	0.085	71 ± 1.5	70 ± 1	0.356			
P wave duration (P) (msec)	95 ± 2	89 ± 2	0.023*	109 ± 3	105 ± 2	0.027*	15 ± 3	18 ± 2	0.696
PQ time (PQ) (msec)	177 ± 3	166 ± 2	0.007*	205 ± 4	197 ± 3	0.039*	16 ± 2	19 ± 1	0.201
QRS duration in V1 (QRSV1) (msec)	108 ± 2	98 ± 2	0.0001*	125 ± 3	116 ± 2	0.0008*	15 ± 2	18 ± 1	0.209
QRS duration in V6 (QRSV6) (msec)	100 ± 2	92 ± 1	0.001*	121 ± 3	109 ± 2	0.0002*	21 ± 2	18 ± 2	0.146
QT interval (QT) (msec)	397 ± 5	407 ± 4	0.121	428 ± 6	423 ± 3	0.379	8 ± 1	4 ± 1	0.009*
QTc interval (QTc) (msec)	424 ± 4	423 ± 4	0.649	462 ± 6	454 ± 3	0.242	9 ± 1	8 ± 1	0.302
R wave in V1 (RV1) (mm)	1.6 ± 0.1	1.9 ± 0.1	0.218	1.4 ± 0.1	1.6 ± 0.1	0.440	-16 ± 5	-17 ± 4	0.149
R wave in V2 (RV2) (mm)	5.2 ± 0.4	4.8 ± 0.3	0.539	5.5 ± 0.4	4.7 ± 0.3	0.073	5 ± 6	-2 ± 3	0.165
S wave amplitude II (aSII) (mm)	1.9 ± 0.2	1.6 ± 0.2	0.068	3.6 ± 0.3	2.9 ± 0.2	0.051	86 ± 12	83 ± 7	0.705
S wave duration II (dSII) (msec)	45 ± 4	29 ± 2	0.0007*	70 ± 4	45 ± 2	0.0001*	57 ± 8	55 ± 6	0.013*
S wave amplitude III (aSIII) (mm)	2.3 ± 0.4	2.0 ± 0.3	0.528	3.1 ± 0.4	2.6 ± 0.4	0.363	35 ± 12	46 ± 14	0.343
S wave duration III (dSIII) (msec)	35 ± 4	27 ± 3	0.109	59 ± 6	37 ± 3	0.001*	68 ± 12	39 ± 7	0.039*
J point in V1 (JV1) (mm)	1.0 ± 0.1	0.3 ± 0.01	< 0.0001*	1.6 ± 0.2	0.3 ± 0.004	< 0.0001*	65 ± 14	11 ± 12	< 0.0001*
J point in V2 (JV2) (mm)	1.8 ± 0.2	0.9 ± 0.1	< 0.0001*	3.3 ± 0.2	1.4 ± 0.1	< 0.0001*	83 ± 11	54 ± 7	< 0.0001*
J point in V3 (JV3) (mm)	0.9 ± 0.1	0.6 ± 0.1	0.112	1.2 ± 0.2	0.9 ± 0.1	0.279	39 ± 15	50 ± 11	0.889
J point V1IS3 ($JV1_{IC3}$) (mm)	1.3 ± 0.2	0.4 ± 0.1	< 0.0001*	2.2 ± 0.2	0.6 ± 0.1	< 0.0001*	61 ± 11	32 ± 13	< 0.0001*
J point V2IS3 ($JV2_{IC3}$) (mm)	1.9 ± 0.3	0.8 ± 0.1	0.0002*	3.5 ± 0.2	1.1 ± 0.1	< 0.0001*	84 ± 11	39 ± 12	< 0.0001*

**: significant at 0.05 level. $V1_{IC3}$ and $V2_{IC3}$ third intercostal space, cranial from V1 and V2.*

Prediction of the Outcome of Flecainide Testing

Because of the significant differences, at baseline, in several ECG parameters between positive and negative responders upon flecainide administration (Table 3), an attempt was made to predict the test result from baseline ECGs. The logistic regression approach identified three ECG parameters (QRSV1, JV2, and dSII) that significantly contributed to the prediction of the flecainide test outcome (Table 4). When these three parameters were included in the prediction model, the addition of other pre-test ECG parameters did not bring any further significant contribution to the prediction. The resulting logistic regression model correctly predicted 78 out of 95 cases in the negative group and 40 out of 64 cases in the positive group, which corresponds to a sensitivity of 63% and a specificity of 82%. The positive and negative predictive values of the model (Table 4) are 70% and 76%, respectively.

Table 4: Results of the Logistic Regression approach to identify pre-test ECG parameters that could be used to predict the flecainide test outcome.

Parameters in Equation	B	S.E.	Wald	df	P value	Exp(B)
QRSV1	0.031	0.014	4.908	1	0.027	1.032
JV2	0.751	0.203	13.73	1	0.000	2.119
dSII	0.020	0.009	4.895	1	0.027	1.020
Constant	-5.426	1.451	13.98	1	0.000	0.004

The parameters that showed significant pre-test differences between the positive and negative responders where entered into a forward conditional logistic regression approach. After 3 steps QRSV1, dSII and JV2 were found to significantly contribute to the prediction. After inclusion of these three parameters in the model, none of the other ECG parameters added significantly to the prediction.

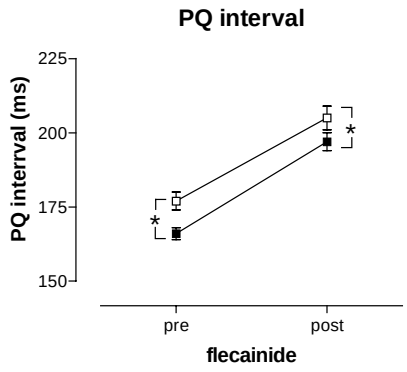
Effect of Flecainide on ECG

Possible differences in the effect of flecainide on ECG parameters between the two groups were analyzed. These differences, expressed as percentage effect of flecainide on all ECG parameters, are reported in Table 3.

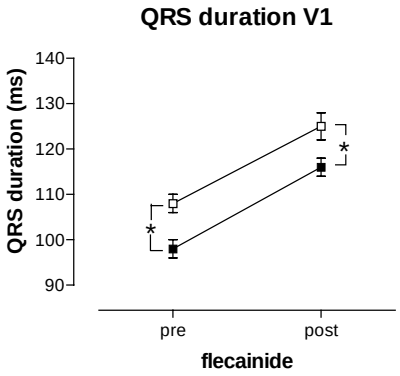
Flecainide elicited, by definition, more J point elevation in the positive than in the negative group in V1, V2, $V1_{IC3}$ and $V2_{IC3}$, but not in V3.
Flecainide elicited similar prolongations of P wave duration, PQ interval, QRSV1 and QRSV6 in both groups (Figures 2A, 2B, 2C). However, flecainide increased dSII and dSIII more in the positive group than in the negative group (Figures 2D and 2E), but not aSII or aSIII.

Third Intercostal Space

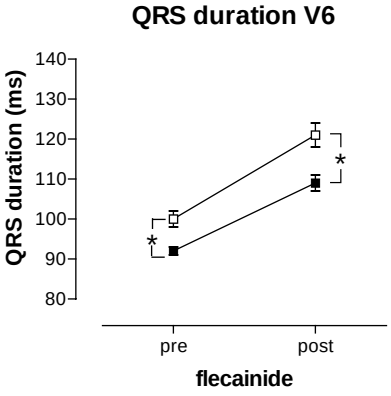
In our study population, 47/64 positive tests and all negative tests were conducted using leads positioned over the third intercostal space ($V1_{IC3}$-$V2_{IC3}$).
In 21/47 (45%) a type I ECG was only obtained when these leads were investigated, while in 26/47 (55%) a type I ECG was recorded also in the conventional leads V1-V3. Of note, the proportion of mutation carriers was similar in both subgroups: 9/21 (43%) in the group where a positive outcome for Brugada syndrome was seen only in $V1_{IC3}$ and $V2_{IC3}$, and 10/26 (38%) in the group where a positive ECG was unmasked in V1-V3 as well (p=0.8).



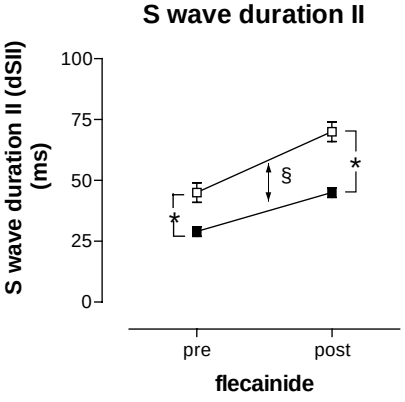
Panel A



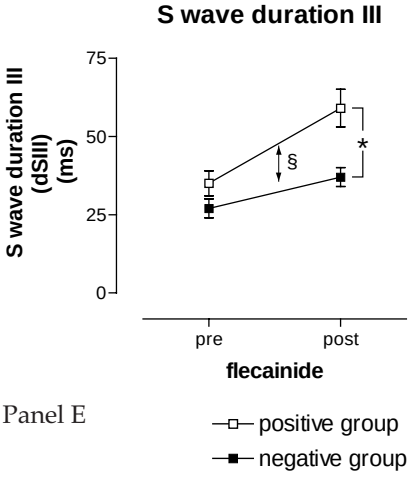
Panel B



Panel C



Panel D



Panel E

Figure 2: *Effect of flecainide on the ECG parameters: PQ interval (Panel A), QRS duration in leads V1 and V6 (Panel B and C), S wave duration in leads II and III (Panel D and E) in both groups.*

** indicates a statistical significant difference ($p<0.05$) in pre-test or post-test values between the groups; § indicates a statistical significant difference ($p<0.05$) in the effect of flecainide (post-pre test value) between both groups.*

Discussion

In the present study, we observed that flecainide challenge possesses a reasonably high sensitivity and specificity to identify a *SCN5A*-related Brugada syndrome patient. These findings with flecainide are similar to the reported ajmaline's sensitivity and specificity, calculated among members of four Brugada syndrome families with documented *SCN5A* mutations [18] and, as such, represent a confirmation that provocative tests with Na^+ channel blockers are useful and equipped with good quality. Based on these encouraging results, and considering the safety of drug provocation tests, we believe that their use in the identification of affected Brugada syndrome subjects should be highly promoted. Yet, we observed that a negative outcome of flecainide test does not exclude the absence of a *SCN5A* mutation (7 *SCN5A*-positive subjects in the negative-responder group). Several explanations can be proposed: (1) the lack of power of flecainide, in comparison, for instance, with the more powerful ajmaline [19]; (2) the overlap between Brugada syndrome and other syndromes, e.g., Long QT Syndrome type III and inherited cardiac conduction disorders. These disorders are also associated with *SCN5A* mutations [10, 12, 32], but Na^+ channel blockers do not necessarily provoke ST elevation here; (3) the presence of specific *SCN5A* mutations that might provoke variable clinical phenotypes resulting in combinations of different diseases, as demonstrated for the E161K mutation [33]; (4) the presence of an effect of age and/or gender on disease penetrance, including the ability of a Na^+ channel blocker to unmask ST segment elevation in *SCN5A* mutation carriers. For instance, women with Brugada syndrome may be less likely to sustain sudden death than men and the aging process may contribute to conduction delays at the level of the RVOT, which are likely to be implicated in the pathogenesis of this disease [3].

Due to the small number of *SCN5A* mutation carriers in the negative group, we could not analyze age and gender as variables able to influence the result of the provocation test. More subjects with a *SCN5A* mutation are also needed to analyze whether particular *SCN5A* mutations may cause specific clinical manifestations. For instance, we have previously shown that two *SCN5A* variants reported here, mutations 1795insD [34] and E161K [33] respectively, exhibit gating properties changes

that may explain particular ECG features. While we did not conduct biophysical studies of all other *SCN5A* variants reported in the present study, it is likely that at least the 3 variants that are predicted to produce prematurely truncated Na^+ channel proteins (W156X, L860fsx89 and R1638X) result in reduced Na^+ current, in accordance with the notion that Brugada syndrome is explained by reductions in depolarizing forces [3].

Safety of Flecainide Testing

In contrast to previous reports [23, 24], we did not observe significant arrhythmias in this relatively large series of patients, including those with *SCN5A* mutations. The reason for this apparent discrepancy could reside in the selection criteria of the patients to expose to drug challenge and in the criteria to stop the infusion. Using the recommended dosing scheme[1], we did not conduct/continue flecainide challenge in the presence of a type I ECG [1], a condition known for its vulnerability to ventricular arrhythmias [35], and we suspended the infusion when frequent PVCs occurred.

ECG Analysis

We found ECG signs of conduction slowing at baseline at all levels (P wave, PQ interval, QRS duration in right and left precordial leads) in the subjects with a positive flecainide test. Of note, these ECG signs included reciprocal changes in the inferior leads, mirroring the electrical activity in the RVOT. The width of the S waves in inferior leads may be more important than their amplitude. At baseline, P wave duration, PQ duration, QRS width, and duration of the S wave in II were significantly longer in the positive group than in the negative group. These differences were maintained after flecainide while an additional effect on the duration of the S wave in II and III was seen in the positive group. This finding suggests that conduction delay is strongly related to the pathogenesis of the Brugada syndrome [for review see [3]] and proposes S waves in inferior leads as new ECG indicators for the diagnosis of affected patients.

Finding these baseline ECG differences prompted us to attempt to develop a model able to predict the result of flecainide testing, suitable for patients

coming to the attention of a cardiologist and who are suspected to have Brugada syndrome. However, logistic regression analysis based on pre-test parameters led us to conclude that, although a combination of three parameters resulted in a statistically significant prediction model, the sensitivity and specificity of this prediction model is still too low to be of clinical use. As such, this analysis of the predictive value of baseline ECG parameters, represents a further confirmation of the necessity of flecainide testing.

We found the analysis of ECG leads positioned over the third intercostal space, cranial to V1 and V2 highly useful because in the majority of our positive tests, coved-type ST elevation appeared in these leads exclusively or in combination with conventional right precordial leads. Although we cannot exclude that coved-type ST elevations would also have appeared in these conventional leads, had we continued flecainde infusion, it is clear that $V1_{IC3}$ and $V2_{IC3}$ have a higher sensitivity in the diagnosis of Brugada syndrome and should be always explored when Brugada syndrome is suspected [28, 29]. This observation may also be relevant in the investigation of the possible mechanism of Brugada syndrome, because it places the RVOT at the core of the disease process which underlies this syndrome.

Conclusion

We present one of the largest series of drug provocation challenge in Brugada syndrome. We conclude that flecainide testing is a useful, valid and safe tool in diagnosing SCN5A-related Brugada syndrome for those patients who do not display its pathognomonic coved-type ECG pattern spontaneously.

According to our findings, drug testing when perfomed with flecainide, an inexpensive, generally available Na^+ channel blocker, represents, in combination with accurate investigation of clinical symptoms and family history, a very valuable means in the diagnostic strategy and in risk stratification of the *SCN5A*-related Brugada syndrome patients and their relatives [8, 36].

Finally, the baseline ECG offers intriguing clues to differentiate the subjects with a positive flecainide test from those with a negative one, but an analysis of the predictive value of pre-test ECG parameters failed to provide a prediction of the

test outcome, good enough to be of clinical use. Still, through a detailed ECG analysis, we propose the inclusion of the measurements of the width of the S wave in inferior leads as a new important ECG criterion in Brugada syndrome and we confirm the validity of the investigation of ST segment elevation in leads positioned over the third intercostal space whenever a Brugada syndrome case is suspected [27-29].

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