# Brugada syndrome : clinical and pathophysiological aspects

Meregalli, P.G.

**Publication date**
2009

# 4

# Type of *SCN5A* Mutation Determines Clinical Severity and Degree of Conduction Slowing in Loss-of-Function Sodium Channelopathies

Paola G. Meregalli*, Hanno L. Tan*, Vincent Probst*,
Tamara T. Koopmann, Michael W. Tanck, Zahurul A. Bhuiyan,
Frederic Sacher, Florence Kyndt, Jean-Jacques Schott,
Juliette Albuisson, Philippe Mabo, Connie R. Bezzina,
Hervé Le Marec and Arthur A.M. Wilde

*Heart Rhythm 2009; 6:341-8*

## Abstract

**Background:** Patients carrying loss-of-function *SCN5A* mutations linked to Brugada syndrome or progressive cardiac conduction disease (PCCD) are at risk of sudden cardiac death at young age. The penetrance and expressivity of the disease are variable and new tools for risk stratification are needed.

**Objectives:** We aimed to establish whether the type of *SCN5A* mutation correlates with the clinical and electrocardiographic phenotype.

**Methods:** We studied probands affected by Brugada syndrome or PCCD and their relatives, who carried a *SCN5A* mutation. Mutations were divided into 2 main groups: missense mutations (M) or mutations leading to premature truncation of the protein (T). The M group was subdivided according to available biophysical properties: M mutations with ≤ 90% ($M_{active}$) or > 90% ($M_{inactive}$) peak $I_{Na}$ reduction were analysed separately.

**Results:** The study group was composed of 147 individuals with 32 different mutations. No differences in age and sex distribution were found between the groups. Subjects carrying a T mutation had significantly more syncopes than those with an $M_{active}$ mutation (19 of 75 vs. 2 of 35, p=0.03). Also, mutations associated with drastic peak $I_{Na}$ reduction (T and $M_{inactive}$ mutants) had a significantly longer PR interval, compared with $M_{active}$ mutations. All other ECG parameters were comparable. After drug provocation testing, both PR and QRS intervals were significantly longer in the T and $M_{inactive}$ group than in the $M_{active}$ group.

**Conclusions:** In loss-of-function *SCN5A* channelopathies, patients carrying T and $M_{inactive}$ mutations develop a more severe phenotype than those with $M_{active}$ mutations. This is associated with more severe conduction disorders. This is the first time that genetic data are proposed for risk stratification in Brugada syndrome.

## Introduction

Cardiac sodium channels, whose pore-forming subunit is encoded by *SCN5A*, control cardiac excitability by triggering the action potential. Loss-of-function sodium channelopathies are autosomal-dominant inherited conditions in which *SCN5A* mutations produce sodium channels that conduct less sodium current ($I_{Na}$). Such channelopathies lead to aberrant rhythm phenotypes [1], mainly Brugada syndrome [2] and progressive cardiac conduction disease (PCCD) [3]. PCCD and Brugada syndrome display significant overlap and can coexist in the same family and even in the same individual [4-6]. Sudden cardiac death (SCD) and syncope, a harbinger of SCD in these diseases [7], are associated with reduced cardiac excitability as witnessed by ECG signs of conduction slowing [8]. Moreover, Brugada syndrome is characterized by signature ST segment elevation [9] which seems also to be related to conduction slowing [10, 11], although this is still a matter of debate [12]. In support of the role of $I_{Na}$ reduction, these ECG abnormalities are provoked and/or exacerbated by sodium channel blockers. Accordingly, such drug challenges have proven to be a good and safe tool to unmask silent disease carriers [13, 14].

A clinical dilemma arises from the fact that disease penetrance and expressivity are highly variable, notably in Brugada syndrome [15]. This is particularly troublesome as the only generally accepted treatment to prevent SCD in Brugada syndrome is an implantable cardioverter-defibrillator. Clearly, new tools for risk stratification are highly needed. Given the role of $I_{Na}$ reduction in the pathophysiology of Brugada syndrome and PCCD, we hypothesized that those *SCN5A* mutations which reduce $I_{Na}$ the most cause the most severe phenotype. *SCN5A* mutations may reduce $I_{Na}$ by changing the functional properties (gating) of the sodium channel protein [16] or by resulting in its failure to express in the sarcolemma (trafficking) [17]. Missense (M) mutations, in which a single amino acid is replaced by an aberrant amino acid, most often disrupt gating of the channel. In contrast, truncation (T) mutations, in which the sodium channel protein is truncated due to the presence of a premature stop codon, are usually not inserted into the sarcolemma, and cause haploinsufficiency. Because of this and the fact that mutant sodium channels with gating abnormalities confer no dominant-negative effects on normal sodium

channels, T mutations potentially reduce $I_{Na}$ more than M mutations. In order to test our hypothesis, we retrospectively studied clinical and ECG data of a cohort of patients affected by Brugada syndrome and PCCD carrying *SCN5A* mutations that have known or predictable effects on $I_{Na}$ magnitude. Although this regards only a minority of Brugada syndrome patients (up to now, only in around 30% of Brugada syndrome patients a *SCN5A* mutation is found [18]), we believe that this innovative use of the genetic substrate for risk stratification is highly promising. Also, it is likely that in the remaining Brugada syndrome patients, in which no mutations in *SCN5A* are found, an involvement of other gene(s) with modulating effects on cardiac $I_{Na}$ may be present [19].

## Methods

### Clinical and ECG analysis

We retrospectively included patients whose data were collected between 1999 and 2007 in six tertiary hospitals (Academic Medical Center of the University of Amsterdam, Nantes University Hospital, Bordeaux University Hospital, Rennes University Hospital, Tours University Hospital, and Brest University Hospital) in the Netherlands and in France. We included all Brugada syndrome and/or PCCD probands that carried a loss-of-function *SCN5A* mutation whose effect on $I_{Na}$ was known or could be predicted. Family members of the probands, who carried the familial *SCN5A* mutation, were also included. Subjects with a Long QT syndrome phenotype, alone or in combination with Brugada syndrome, were excluded, as were compound heterozygous mutation carriers. Since children affected by Brugada syndrome or PCCD differ from adults with regards to ECG variables (different normal values) and clinical presentation (more predominant role of fever as precipitating factor of SCD [20] and a lower incidence of conduction disorders [21]), only subjects > 16 years of age were included. We studied the following demographic and clinical data: 1) sex; 2) age at diagnosis; 3) history of aborted SCD or unexplained syncope; 4) familial history of SCD at a young age (< 45 years); 5) outcome of drug provocation test, where performed [9]. Twelve-lead ECGs were analyzed by three expert cardiologists (AW, HT, VP) for heart rate, PR

interval duration (lead II), QRS width (maximum QRS duration in leads V1 or V6), QTc duration (Bazett's formula), amount of ST segment elevation (maximum among leads V1-V3), and type of Brugada syndrome-associated ST segment abnormality (type I, II or III) [9].

**Genetic Analysis**

The *SCN5A* gene was screened as follows: genomic DNA was first extracted from peripheral blood lymphocytes using standard protocols. Then, all *SCN5A* exons were amplified using primers located in flanking intronic sequences [2], and analyzed for mutations using denaturing high performance liquid chromatography (dHPLC)-DNA sequencing or direct sequencing [22]. It was verified that these DNA variants were disease-causing mutations, rather than polymorphisms, by generally accepted criteria: 1) location in highly conserved regions of *SCN5A*; 2) absence in at least 100 control individuals (200 alleles); 3) co-segregation with the disease phenotype. Amino acid numbering was made according to transcription variant 1 of *SCN5A* (http://www.ncbi.nlm.nih.gov/; NM_198056) and the predicted structure reported by Wang et al.[2], according to which the protein consists of 4 transmembrane domains (D), each composed of 6 segments (S).

**Determination of Degree of $I_{Na}$ Reduction**



In order to test our hypothesis that a possible correlation was present between the severity of the clinical outcome and the amount of reduction in $I_{Na}$ caused by the mutant sodium channels, *SCN5A* mutations were divided into two main groups: T mutations and M mutations. T mutations were those that generated a premature stop codon due to nonsense mutations or frameshifts (insertions, deletions, splice site mutations). Splice site mutations with an equivocal effect were excluded from further analysis. For the single amino acid changing mutations (M), we studied the biophysical properties of the mutant sodium channels, as published previously (Table 1, these *in vitro* data were found by performing a literature search in PubMed and selecting only papers written in English) or studied in the laboratory of the Academic Medical Centre (unpublished data, Supplementary Figure). According to their biophysical data, M mutations were subdivided into two groups. M

mutations that resulted in ≥ 90% peak $I_{Na}$ reduction [23] in patch-clamp studies were classified as "functionally inactive" M mutations ($M_{inactive}$), while M mutations with < 90% peak $I_{Na}$ reduction constitute the "partially active" M group ($M_{active}$). While arbitrary, the cut-off value of 90% was chosen because of previous studies into the relation between $I_{Na}$ magnitude and impulse propagation in the heart [23]. We analyzed T, $M_{active}$, and $M_{inactive}$ mutations in separate groups. We then focused on comparisons between T and $M_{active}$ mutations and did not describe the results of the $M_{inactive}$ group in detail, because the reduction in net $I_{Na}$ magnitude of $M_{inactive}$ mutations is not known. Although *in vitro* data indicate that $I_{Na}$ reduction is clearly larger in $M_{inactive}$ than $M_{active}$ mutations, it is also probably significantly smaller than in T mutations, because $M_{inactive}$ mutations are expressed in the sarcolemma and may be at least partially functional there, as opposed to T mutations.

**Statistical Analysis**

Continuous variables were checked for normality using the Shapiro Wilk test ($W > 0.90$). Normally distributed variables are presented as mean ± SD. Not-normally distributed variables (QRS width and ST elevation) are presented as median with the Interquartile range (IQR) between brackets. In the regression models, rank-transformed values were used for these variables. The effect of sodium channel blockade on ECG parameteres was expressed as absolute change (the difference between the post-test and pre-test values). Categorical variables are presented as number and percentages. Differences between groups were analysed using linear and logistic regression for continuous and categorical variables, respectively, with age as covariate, followed by post-hoc analysis in case of a significant overall p-value. Generalised Estimation Equations were used in the regression models to correct for correlations between individuals due to family relations. Two sided p-values < 0.05 were regarded significant. All analyses were carried out using SAS version 9.

## Results

### Study Group

In total, 147 individuals (45 families) met the inclusion criteria. These subjects constitute the study group. The population study comes from a total population of 232 carriers of a loss-of-function *SCN5A* mutation (147 French and 85 Dutch). There were 32 different *SCN5A* mutations, including two insertions (in-frame insertion 1570insI and frameshift insertion c.3142-3143insTG), four deletions (c.2582-2583delTT, c.3816delG, c.4299delG, c.5131delG), seven nonsense mutations (p.W156X, p.E346X, p.R535X, p.E1208X, p.L1393X, p.R1638X, p.Q1695X), and 4 frameshift/splice site mutations that caused premature termination of the sodium channel protein: c.2320delT (p.Tyr774fsX28), c.1983-1993dupAla665GfsX16, c.3840 +1 G>A and c.4437+5G>A. In the M group, there were 10 $M_{active}$ mutations (p.E161K, p.R225W, p.G514C, p.R1232W, p.D1275N, p.G1319V, p.R1512W, p.D1714G, p.G1740R, p.A1924T) and 5 $M_{inactive}$ mutations (p.R367C, p.R367H, p.G752R, p.G1408R, p.G1743E) (Table 1). Figure 1 shows the location of all mutations within the topology of the sodium channel protein. The demographic and ECG data of all patients are shown in Table 2.

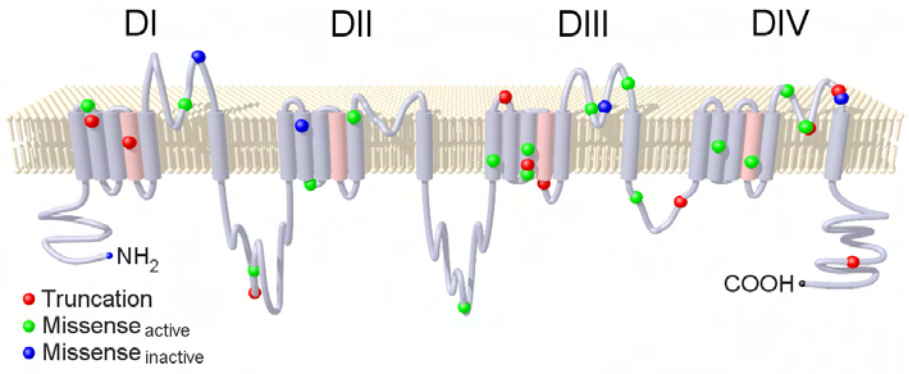



***Figure 1:*** *Schematic representation of the α-subunit of SCN5A protein in which the position of the 32 included mutations is shown. Courtesy of Dr. A. Linnenbank.*

**Table 1:** *SCN5A* mutations included in the study. Location is given. Functional data are reported when available. Last column indicates reported reduction in peak sodium current ($I_{Na}$). In cases where peak $I_{Na}$ reduction is 0% or not available (n.a.), biophysical studies that are referenced in column 1 indicate that net $I_{Na}$ is reduced because of gating changes.

| *SCN5A* mutation | Type | Location | Pore(P)/ Nonpore (NP) | Patients, n (%) | N. of fam | N. of SCD | Reduction in peak $I_{Na}$ |
|---|---|---|---|---|---|---|---|
| **Missense mutations with ≤90% peak $I_{Na}$ reduction ($M_{active}$)** | | | | | | | |
| p.Glu161Lys [4] | Missense | Exon 4; IS2 | NP | 12 (8.1) | 2 | 0 | 60%[#] |
| p.Arg225Trp [24] | Missense | Exon 6; IS4 | NP | 7 (4.7) | 2 | 0 | 90% |
| p.Gly514Cys[8] | Missense | Exon 12; IS6-IIS1 | NP | 6 (4.0) | 1 | 1 | 0% |
| p.Arg1232Trp [25] | Missense | Exon 21; IIIS1-IIIS2 | NP | 1 (0.7) | 1 | 0 | 0% |
| p.Asp1275Asn [26] | Missense | Exon 21; IIIS3 | NP | 2 (1.3) | 2 | 0 | 0% |
| p.Arg1319Val[27] | Missense | Exon 22; DIII S4-S5 | NP | 4 (2.7) | 2 | 1 | 0%[#] |
| p.Arg1512Trp[28] | Missense | Exon 26; IIIS6-IVS1 | NP | 2 (1.3) | 2 | 1 | n.a. |
| p.Asp1714Gly[29] | Missense | Exon 28; IVS5-IVS6 | P | 1 (0.7) | 1 | 0 | 80% |
| p.Gly1740Arg[30] | Missense | Exon 28; IVS5-IVS6 | P | 1 (0.7) | 1 | 0 | 75% |
| p.Ala1924Thr [31] | Missense | Exon 28; C term. | NP | 1 (0.7) | 1 | 0 | n.a. |
| **Truncation mutations (T)** | | | | | | | |
| p.Trp156X[24] | Nonsense | Exon 4 ; IS1-IS2 | NP | 1 (0.7) | 1 | 0 | 100% |
| p.Glu346X | Nonsense | Exon 9, IS5-IS6 | P | 1 (0.7) | 1 | 0 | 100% |
| p.Arg535X [32] | Nonsense | Exon 12, IS6-IIS1 | P | 8 (5.3) | 1 | 0 | 100% |
| p.Glu1208X | Nonsense | Exon 20; IIIS1 | NP | 1 (0.7) | 1 | 0 | 100% |
| p.Leu1393X | Nonsense | Exon 23; IIIS5-IIIS6 | P | 10 (6.7) | 2 | 0 | 100% |
| p.Arg1638X | Nonsense | Exon 28; IV4 | NP | 6 (4.0) | 2 | 0 | 100% |
| p.Gln1695X | Nonsense | Exon 28; IVS5-IVS6 | P | 1 (0.7) | 1 | 0 | 100% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1570insI ^ | In frame insertion | Exon 27; IVS2 | NP | 6 (4.0) | 1 | 0 | 100%^ |
| c.1983_1993 dupp. Ala665GlyfsX16 | Frameshift, Stop | Exon13; IS6-IIS1/Exon14; IS6-IIS1 | NP | 8 (5.4) | 1 | 0 | * |
| c.2320delT (p.Tyr774fsX28) | Frameshift, Stop | Exon 15; IIS2-S3 | NP | 2 (1.3) | 1 | 0 | * |
| c.2582_2583 del TT | Deletion | Exon 16; IIS5/ Exon 17; IIS6-IIIS1 | NP | 14 (9.4) | 4 | 2 | * |
| c.3142_3143 insTG | Insertion frameshift | Exon17 ;IIS6-IIIS1 | NP | 4 (2.7) | 1 | 1 | * |
| c.3816 del G | Deletion | Exon 21; IIIS3 | NP | 4 (2.7) | 1 | 1 | * |
| c.3840+1G>A | Frameshift, stop | Exon 21; IIIS1 | NP | 1 (0.7) | 1 | 1 | * |
| c.4299 del G | Deletion | Exon 25; IIIS5-S6 | P | 1 (0.7) | 1 | 1 | * |
| c.4437 +5G>A | Frameshift, Stop | Exon 25; IIIS6-IVS1 | NP | 2 (1.3) | 1 | 0 | * |
| c.5131 del G | Deletion | Exon 28, IV S5-S6 | P | 5 (3.4) | 1 | 0 | * |
| **Missense mutations with > 90% peak $I_{Na}$ reduction ($M_{inactive}$)** | | | | | | | |
| p.Arg367Cys ^ | Missense | Exon 9; IS5-IS6 | P | 7 (4.7) | 2 | 1 | 100%^ |
| p.Arg367His [33] | Missense | Exon 9; IS5-IS6 | P | 2 (1.3) | 2 | 0 | 100%§ |
| p.Gly752Arg [34] | Missense | Exon 14; IIS2 | NP | 6 (4.0) | 1 | 0 | 95%# |
| p.Gly1408Arg [6] | Missense | Exon 23; IIIS5-S6 | P | 14 (9.4) | 1 | 0 | 100%# |
| p.Gly1743Glu [35] | Missense | Exon 28; IVS5-S6 | P | 6 (4.0) | 2 | 0 | 98% |

**denotes that sodium current reduction is predicted to be 100% (based on type of mutation, i.e., truncation and/or frameshift). ^ These two mutations have been studied in the laboratoty of the AMC. Both mutant channels did not reach the cell membrane (unpublished data, see Supplementary figure).*
*# Results obtained from mammalian transfection system. § Results obtained from* Xenopus *oocytes.*

**Missense *versus* Truncation Mutations**

The T group included 75 individuals, the $M_{active}$ group 37 individuals, and the $M_{inactive}$ group 35 individuals (Table 2). We first conducted group comparisons between all groups with One-Way ANOVA. Demographic variables did not differ between groups. However, there was a group difference in the proportion of patients who had experienced syncope. These syncope episodes were considered to be due to arrhythmias, because they were otherwise unexplained. Moreover, PR duration at baseline and after drug testing, and QRS width after drug testing were different between groups. Post-hoc analysis revealed that these 4 variables (syncope, PR at baseline, PR after drug testing, QRS after drug testing) were different between the T and $M_{active}$ groups. Because the extent of net $I_{Na}$ reduction of $M_{inactive}$ mutations is not predictable, we limited our following analyses to the T and $M_{active}$ groups. The proportion of patients who had experienced syncope was significantly larger among those with a T mutation than those with a $M_{active}$ mutation. This difference remained after correction for age and/or gender (p=0.03). Moreover, although the proportion of patients with aborted SCD was similar in both groups, the proportion of families in whom SCD had occurred in a first-degree family member (at young age) was almost twice as high in families with a T mutation as in those with a $M_{active}$ mutation. Yet, with the number of families studied, this difference did not reach statistical significance (p=0.06).



Next, we studied whether these group differences in clinical severity were attended by disparities in ECG variables before (baseline) and/or after (post-test) a provocation test with a sodium channel blocker. The typical type-1 Brugada syndrome-ECG at baseline was equally prevalent in both $M_{active}$ and T groups. In the remaining individuals who were suspected of having Brugada syndrome, a provocation test was conducted and yielded a positive outcome for Brugada syndrome in 45 (84%). The proportion of positive tests (type-1 Brugada syndrome-ECG [9]) was similar among T and $M_{active}$ patients. Among these patients, the penetrance of the Brugada syndrome phenotype (type I ECG) increased with the use of sodium channel blockers from 28% to 77% in the T group, and from 36% to 77% in the $M_{active}$ group. Importantly, individuals with a T mutation had significantly longer PR intervals at baseline and post-test (Figure 2, panel A), and

longer QRS width post-test (Figure 2, panel B) than those with a $M_{active}$ mutation. The effect of sodium channel blockers on QRS duration, expressed as difference between the post-test and pre-test values, was significantly higher in the T group than in the $M_{active}$ group (p=0.03, Figure 2, panel B). All other ECG variables were similar in both groups, including QTc duration and the degree of ST segment elevation at baseline and post-test (Figure 2, panels C and D).

**Table 2**: Clinical and electrocardiographic characteristics of the study population.
ECG parameters are measured at baseline and at maximal sodium channel blockage. Data are given as number (%), mean ± SD, or median (IQR), when appropriate. Reported p values between the three groups are corrected for age.

| | Truncation (T) mutations | Missense ($M_{active}$) mutations | Missense ($M_{inactive}$) mutations* | p-values (ANOVA) |
|---|---|---|---|---|
| Total patients, n | 75 | 37 | 35 | - |
| Male gender, n (%) | 41 (54%) | 21 (57%) | 17 (49%) | 0.7 |
| Families, n | 22 | 15 | 8 | - |
| *SCN5A* mutations, n | 23 | 10 | 5 | - |
| Age at diagnosis, years | 44±16 | 46±12 | 47±14 | 0.7 |
| Sudden cardiac deaths, n (%) | 6 (8%) | 3 (8%) | 1 (3%) | 0.3 |
| Syncope,n (%) | 19 (25%)[a] | 2 (5%)[b] | 4 (11%)[ab] | **0.03** |
| Family history of SCD, number of families (%) | 14/22 (63) | 4/15 (26) | 3/8 (37) | 0.06 |
| Spontaneous type-I ECG, n (%) | 10 (13%) | 8 (22%) | 13 (37%) | 0.3 |
| HR at baseline, bmp | 65 ± 10 | 67 ± 11 | 66 ± 8 | 0.4 |
| PR interval at baseline, msec | 213 ± 35[a] | 190 ± 35[b] | 213 ± 27[a] | **0.03** |
| QRS interval at baseline, msec | 115 (14) | 116 (24) | 120 (14) | 0.6 |
| QTc interval at baseline, msec | 410 ± 35 | 414 ± 30 | 402 ±39 | 0.5 |
| ST max interval at baseline, mm | 1.2 (1.8) | 1.2 (2) | 1.2 (2) | 0.9 |
| ECG after drug test, n | 35 | 22 | 17 | - |
| HR after drug test, bpm | 69 ± 8 | 70 ± 11 | 75 ± 12 | 0.3 |
| PR after drug test, msec | 238 ± 38[a] | 211 ± 36[b] | 221 ± 32[ab] | **0.03** |
| QRS after drug test, msec | 149 (25)[a] | 132 (16)[b] | 149 (30)[a] | **0.04** |
| QTc after drug test, msec | 458 ± 61 | 460 ± 40 | 424 ± 45 | 0.3 |
| ST max after drug test, mm | 3.4 (2.1) | 3.4 (2.5) | 3 (1.9) | 0.6 |
| Positive drug test, n (%) | 27/35 (77) | 18/22 (82) | 13/17 (72) | 0.8 |

*Letters in superscript denote significant ($p < 0.05$) differences between the groups if they are discordant; concordant letters indicate that group differences are not significantly different.* * in-vitro *experiments have shown that these mutants result in > 90% peak* $I_{Na}$ *reduction.*

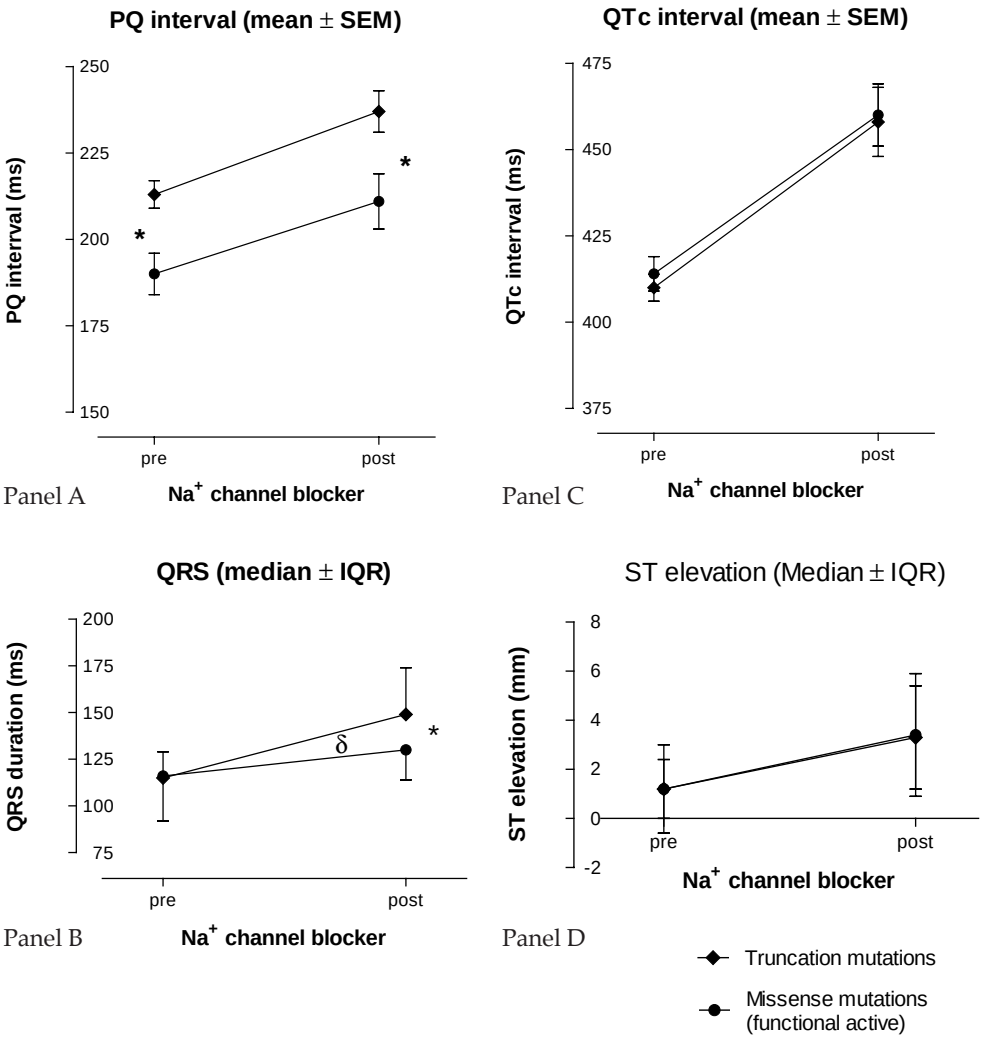
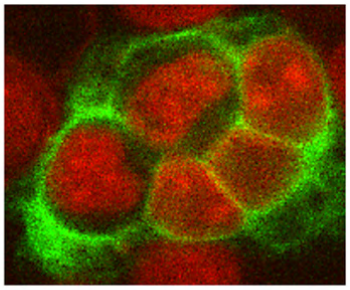




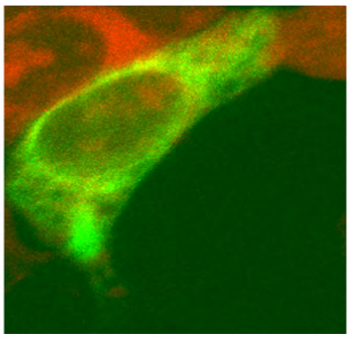

***Figure 2:*** *Effect of sodium channel blockers on ECG parameters: PR interval (panel A), QRS duration (panel B), QTc duration (panel C) and ST segment elevation (panel D).*

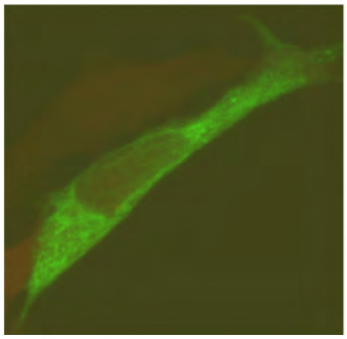
** indicates a statistically significant difference in pre-test and/or post-test values between the groups;* δ *indicates a statistically significant difference in the effect of sodium channel blockers between both groups.*



Wild type

1570insl





R367C

***Supplementary figure***

*Confocal laser microscopic analysis of the R367C (p.Arg367Cys) and 1570insI mutants. We transfected HEK-293 cells with GFP-tagged wild-type* SCN5A *or the mutants R367C and 1570insI. Cells transfected with GFP-hH1 (wild-type) showed sharp fluorescence on the plasma membrane (upper panel). A similar type of fluorescence was obtained when* $\beta_1$*-subunit expressing HEK-293 cells were transfected with the same construct. In contrast, cells transfected with GFP-R367C or GFP-1570insI showed fluorescence in a uniform distribution all over the cytoplasm with a lower intensity on the plasma membrane (lower panels). No change in fluorescence distribution was found for the same mutants transfected into HEK-293 cells expressing the* $\beta_1$*- subunit.*

## Discussion

The cardiac sodium channel is the main determinant of impulse formation and propagation in the heart. Accordingly, $I_{Na}$ reduction by loss-of-function *SCN5A* mutations slows cardiac conduction velocity at various levels in patients with Brugada syndrome [36] and PCCD [3].

Given the fact that Brugada syndrome and PCCD are both associated with conduction slowing and that a significant overlap exists between them, even in the same individual, it is likely that Brugada syndrome and PCCD represent two aspects of the same disease rather than separate clinical entities [37]. Still, especially in Brugada syndrome, many notions on the pathophysiology and the risk factors for development of ventricular arrhythmias remain unclear.

We investigated whether a genotype-phenotype relationship exists in loss-of-function sodium channelopathies according to the type and biophysical properties of the *SCN5A* mutation. Our main finding is that the disease expressivity was more severe in patients with more severe $I_{Na}$ reduction than in those with smaller $I_{Na}$ reduction (T and $M_{inactive}$ vs. $M_{active}$), as witnessed by larger proportions of patients with syncope (presumed arrhythmic). Moreover, the proportion of families with SCD were clearly larger in the T group than in the $M_{active}$ group, although this difference did not reach statistical significance, due to the relatively small overall numbers. Yet, the number of patients who had sustained aborted SCD was not different between groups.



Our retrospective study is composed of 147 genotyped individuals. The analysis was first conducted between three groups and focused further on the differences between the T and the $M_{active}$ group. Our strategy to subdivide the M mutations into two groups ($M_{active}$ and $M_{inactive}$) was made according to the reported biophysical properties of the M mutants, in particular, the degree of reduction in peak $I_{Na}$, as found in patch-clamp experiments. This reduction could vary from 0% [27] to 100% [33] in HEK-293 cells or Xenopus oocytes. We anticipated that M mutants with ≥ 90% peak $I_{Na}$ reduction behaved similarly to the T mutants. This was also in part confirmed by our study, which showed that individuals in the $M_{inactive}$ group had, on average, similar ECG conduction parameters as the T

group (Table 2). Still, the $M_{inactive}$ group is composed of point mutations that are probably expressed in the sarcolemma. However, the available data are limited and the true reduction in net $I_{Na}$ of these mutant channels is not fully resolved. For this reason, the focus of the present investigation is the differences between T mutants and $M_{active}$ mutants. The finding that T mutant carriers have more severe phenotypes than $M_{active}$ mutant carriers of the same age can be explained by the notion that they have haploinsufficiency, and that the remaining 50% of normally functioning sodium channels (normal allele), are incapable of fully compensating for the deficit in peak $I_{Na}$. Experimental studies have revealed that the heart has a large conduction reserve, i.e., conduction velocity can be mantained at almost normal values even when cardiac excitability (peak $I_{Na}$) is markedly reduced [38]. The depolarization reserve declines in various conditions, e.g., with advancing age [39] and the associated increases in fibrosis and changes in cell geometry [40], or by the administration of class 1A or 1C sodium channel blockers, as used in drug provocation tests [13]. Conduction slowing results from the added effects of intrinsic (loss-of-function *SCN5A* mutations) and extrinsic (sodium channel blockers) reduction in $I_{Na}$ magnitude on conduction reserve. Thus, the degree of intrinsic $I_{Na}$ reduction determines whether or not the added presence of extrinsic $I_{Na}$ reduction is sufficient for significant conduction slowing, which has been well-established as a pro-arrhythmic factor [23, 41]. For example, $I_{Na}$ reduction caused by T mutations alone may be significant, but conduction reserve is sufficiently large to ensure that QRS width at baseline associated with T mutations is not different from M mutations. Yet, the added presence of sodium channel blockers results in significantly longer QRS width in T than in $M_{active}$ mutants (Figure 2, panel B).



The ECG hallmark of Brugada syndrome (type I Brugada syndrome-ECG) is, on the other hand, equally expressed in T and $M_{active}$ mutation carriers. In our study population, the maximal degree of ST segment elevation does not seem to be influenced by the type of mutation, since subjects carrying a $M_{active}$ mutation (with a less severe reduction in $I_{Na}$ compared with T carriers) reached an average of 3.4 mm ST segment elevation after administration of sodium channel blockers, similar to the T group. That means that the presence of a spontaneous type I Brugada syndrome-ECG or the amount of ST segment elevation, alone, is not a strong

predictor of cardiac events. In both large series of Eckardt *et al.* [42] and Priori *et al.* [43], a type I Brugada syndrome-ECG was equally distributed between symptomatic and asymptomatic patients. Still, the smaller intrinsic conduction reserve (caused by the *SCN5A* mutation alone) in T mutation carriers, as compared with $M_{active}$ mutation carriers, may explain their higher disease expressivity, as a smaller extrinsic $I_{Na}$ reduction is sufficient to render them more symptomatic. Our notion that the biophysical properties (degree of $I_{Na}$ reduction) of the mutant ion channel in loss-of-function sodium channelopathies, as can be derived from analysis of the type of the mutation, could determine the extent of the pathophysiological derangement (conduction slowing) and the associated disease severity, gains support from two recent studies. In one study [44], the investigators found that disease severity in carriers of a loss-of-function mutation in the potassium channel encoding gene *KCNQ1* (Long QT syndrome type 1) was worse in those in whom the mutant ion channel had more reduction in current ($I_{Ks}$). Moreover, disease severity was also worse when the mutation was located in a transmembrane region of the ion channel than when located outside of it (C-terminus). In another study that included only missense mutations [45], severe myoclonic epilepsy of infancy, associated with loss-of-function mutations in *SCN1A*, which encodes the neuronal isoform of the sodium channel, was found to have an earlier onset if the mutations were located in the ion-conducting pore. Pore mutations have indeed shown, in previous experimental studies, to strongly reduce $I_{Na}$ because of impediment of ion permeation or abnormal trafficking [46, 47]. This is in agreement with our hypothesis and findings. However, we could not conduct an analysis of the potential effect of mutations in specific domains of the sodium channel gene, e.g., the pore region, the voltage sensor (S4) or the C/N termini, due to the small numbers of such mutations in our study cohort. Surely, the pioneer studies of Moss *et al.* [44] and Kanai *et al.* [45], mentioned above, together with our promising results, provide support for future studies to establish whether or not risk stratification in loss-of-function ion channelopathies should include the known and/or predicted biophysical properties of the mutant ion channel.

**Study limitations**

Although we excluded *SCN5A* mutations with unknown or unpredictable effects on peak $I_{Na}$ magnitude from our study, published biophysical data are not available for all included T mutations. We felt it reasonable to assume that T mutations reduce peak $I_{Na}$ magnitude by ~100%. Moreover, we did not screen for the contemporaneous presence of other *SCN5A* variants in the included patients, neither did we look at the possible co-presence of other known determinants of impulse conduction within the heart, e.g., variants in the connexin43 gene, which is involved in impulse transmission.

## Conclusions

In loss-of-function sodium channelopathies, individuals carrying a T mutation develop, at parity of age, a more severe phenotype compared with $M_{active}$ mutant carriers. Atrioventricular and intraventricular depolarization reserve is also significantly reduced in T mutant carriers, in comparison with $M_{active}$ mutant carriers, as unmasked by pharmacologic challenge. The $M_{inactive}$ group, with a drastic reduction in peak $I_{Na}$, shows the same behavior as T mutants with regards to the severity of conduction slowing. These innovative results show that genetic data might be proposed as a new tool for risk stratification in addition to the other existing criteria. The observation that those patients who were more symptomatic also had more ECG signs of conduction slowing provides further support for the notion that conduction slowing, mediated by loss-of-function *SCN5A* mutations, is a key pathophysiologic mechanism in these diseases, including Brugada syndrome.

## Reference List

(1) Tan HL. Sodium channel variants in heart disease: expanding horizons. *J Cardiovasc Electrophysiol* 2006;17 Suppl 1:S151-S157.

(2) Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. *Genomics* 1996;34(1):9-16.

(3) Schott JJ, Alshinawi C, Kyndt F et al. Cardiac conduction defects associate with mutations in SCN5A. *Nat Genet* 1999;23(1):20-1.

(4) Smits JP, Koopmann TT, Wilders R et al. A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in two families. *J Mol Cell Cardiol* 2005;38(6):969-81.

(5) Vorobiof G, Kroening D, Hall B, Brugada R, Huang D. Brugada syndrome with marked conduction disease: dual implications of a SCN5A mutation. *Pacing Clin Electrophysiol* 2008;31(5):630-4.



(6) Kyndt F, Probst V, Potet F et al. Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. *Circulation* 2001;104(25):3081-6.

(7) Gehi AK, Duong TD, Metz LD, Gomes JA, Metha D. Risk Stratification of Individuals with the Brugada Electrocardiogram: A Meta-Analysis. *Journal of cardiovascular electrophysiology* 2006;17:577-83.

(8) Tan HL, Bink-Boelkens MT, Bezzina CR et al. A sodium-channel mutation causes isolated cardiac conduction disease. *Nature* 2001;409(6823):1043-7.

(9) Wilde AA, Antzelevitch C, Borggrefe M et al. Proposed diagnostic criteria for the Brugada syndrome. *Eur Heart J* 2002;23(21):1648-54.

(10) Tukkie R, Sogaard P, Vleugels J, de Groot IK, Wilde AA, Tan HL. Delay in right ventricular activation contributes to Brugada syndrome. *Circulation* 2004;109(10): 1272-7.

(11) Zhang ZS, Tranquillo J, Neplioueva V, Bursac N, Grant AO. Sodium channel kinetic changes that produce Brugada syndrome or progressive cardiac conduction system disease. *Am J Physiol Heart Circ Physiol* 2007;292(1):H399-H407.

(12) Meregalli P.G., Wilde A.A.M., Tan H.L. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder or more? *Cardiovasc Res* 2005;67(3):367-78.

(13) Meregalli PG, Ruijter JM, Hofman N, Bezzina CR, Wilde AA, Tan HL. Diagnostic value of flecainide testing in unmasking SCN5A-related Brugada syndrome. *J Cardiovasc Electrophysiol* 2006;17(8):857-64.

(14) Hong K, Brugada J, Oliva A et al. Value of electrocardiographic parameters and ajmaline test in the diagnosis of Brugada syndrome caused by SCN5A mutations. *Circulation* 2004;110(19):3023-7.



(15) Antzelevitch C, Brugada P, Brugada J et al. Brugada syndrome: a decade of progress. *Circ Res* 2002;91(12):1114-8.

(16) Stuhmer W, Conti F, Suzuki H et al. Structural parts involved in activation and inactivation of the sodium channel. *Nature* 1989;339(6226):597-603.

(17) Tan HL, Bezzina CR, Smits JP, Verkerk AO, Wilde AA. Genetic control of sodium channel function. *Cardiovasc Res* 2003;57(4):961-73.

(18) Priori SG, Napolitano C, Gasparini M et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. *Circulation* 2000;102(20):2509-15.

(19) London B, Sanyal S, Michalec M et al. A mutation in the glycerol-3-phosphate dehydrogenase 1-like gene (GPD1L) causes Brugada syndrome. *Heart Rhythm* 2006;3(1S):S32.

(20) Probst V, Denjoy I, Meregalli PG et al. Clinical aspects and prognosis of Brugada syndrome in children. *Circulation* 2007;115(15):2042-8.

(21) Chiu SN, Wang JK, Wu MH et al. Cardiac conduction disturbance detected in a pediatric population. *J Pediatr* 2008;152(1):85-9.

(22) Bezzina C, Veldkamp MW, van den Berg MP et al. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ Res* 1999;85(12):1206-13.

(23) Shaw RM, Rudy Y. Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. *Circ Res* 1997;81(5):727-41.

(24) Bezzina CR, Rook MB, Groenewegen WA et al. Compound heterozygosity for mutations (W156X and R225W) in SCN5A associated with severe cardiac conduction disturbances and degenerative changes in the conduction system. *Circ Res* 2003;92(2):159-68.

(25) Baroudi G, Acharfi S, Larouche C, Chahine M. Expression and intracellular localization of an SCN5A double mutant R1232W/T1620M implicated in Brugada syndrome. *Circ Res* 2002;90(1):E11-E16.

(26) Groenewegen WA, Firouzi M, Bezzina CR et al. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. *Circ Res* 2003;92(1):14-22.

(27) Casini S, Tan HL, Bhuiyan ZA et al. Characterization of a novel SCN5A mutation associated with Brugada syndrome reveals involvement of DIIIS4-S5 linker in slow inactivation. *Cardiovasc Res* 2007;76(3):418-29.

(28) Deschenes I, Baroudi G, Berthet M et al. Electrophysiological characterization of SCN5A mutations causing long QT (E1784K) and Brugada (R1512W and R1432G) syndromes. *Cardiovasc Res* 2000;46(1):55-65.

(29) Amin AS, Verkerk AO, Bhuiyan ZA, Wilde AA, Tan HL. Novel Brugada syndrome-causing mutation in ion-conducting pore of cardiac Na+ channel does not affect ion selectivity properties. *Acta Physiol Scand* 2005;185(4):291-301.

(30) Baroudi G, Napolitano C, Priori SG, Del BA, Chahine M. Loss of function associated with novel mutations of the SCN5A gene in patients with Brugada syndrome. *Can J Cardiol* 2004;20(4):425-30.

(31) Tan HL, Kupershmidt S, Zhang R et al. A calcium sensor in the sodium channel modulates cardiac excitability. *Nature* 2002;415(6870):442-7.

(32) Keller DI, Rougier JS, Kucera JP et al. Brugada syndrome and fever: genetic and molecular characterization of patients carrying SCN5A mutations. *Cardiovasc Res* 2005;67(3):510-9.

(33) Takehara N, Makita N, Kawabe J et al. A cardiac sodium channel mutation identified in Brugada syndrome associated with atrial standstill. *J Intern Med* 2004;255(1):137-42.

(34) Potet F, Mabo P, Le Coq G et al. Novel brugada SCN5A mutation leading to ST segment elevation in the inferior or the right precordial leads. *J Cardiovasc Electrophysiol* 2003;14(2):200-3.

(35) Vernooy K, Sicouri S, Dumaine R et al. Genetic and biophysical basis for bupivacaine-induced ST segment elevation and VT/VF. Anesthesia unmasked Brugada syndrome. *Heart Rhythm* 2006;3(9):1074-8.

(36) Smits JP, Eckardt L, Probst V et al. Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. *J Am Coll Cardiol* 2002;40(2):350-6.

(37) Herfst LJ, Potet F, Bezzina CR et al. Na+ channel mutation leading to loss of function and non-progressive cardiac conduction defects. *J Mol Cell Cardiol* 2003;35(5):549-57.

(38) van Veen TA, Stein M, Royer A et al. Impaired impulse propagation in Scn5a-knockout mice: combined contribution of excitability, connexin expression, and tissue architecture in relation to aging. *Circulation* 2005;112(13):1927-35.

(39) Probst V, Kyndt F, Potet F et al. Haploinsufficiency in combination with aging causes SCN5A-linked hereditary Lenegre disease. *J Am Coll Cardiol* 2003;41(4):643-52.

(40) Royer A, V Veen T, Le Bouter S et al. A Mouse model of SCN5A-linked hereditary Lenegre's Disease. *Circulation* 2005;111:1738-46.

(41) Shaw RM, Rudy Y. The vulnerable window for unidirectional block in cardiac tissue: characterization and dependence on membrane excitability and intercellular coupling. *J Cardiovasc Electrophysiol* 1995;6(2):115-31.

(42) Eckardt L, Probst V, Smits JP et al. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. *Circulation* 2005;111(3):257-63.



(43) Priori SG, Napolitano C, Gasparini M et al. Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation* 2002;105(11):1342-7.

(44) Moss AJ, Shimizu W, Wilde AA et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007;115(19):2481-9.

(45) Kanai K, Hirose S, Oguni H et al. Effect of localization of missense mutations in SCN1A on epilepsy phenotype severity. *Neurology* 2004;63(2):329-34.

(46) Itoh H, Shimizu M, Mabuchi H, Imoto K. Clinical and electrophysiological characteristics of Brugada syndrome caused by a missense mutation in the S5-pore site of SCN5A. *J Cardiovasc Electrophysiol* 2005;16(4):378-83.

(47) Pfahnl AE, Viswanathan PC, Weiss R et al. A sodium channel pore mutation causing Brugada syndrome. *Heart Rhythm* 2007;4(1):46-53.