Genetic basis of cardiac ion channel diseases

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Chapter 4

A common sodium channel promoter haplotype in Asian subjects underlies variability in cardiac conduction

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Abstract

Background Reduced cardiac sodium current slows conduction and renders the heart susceptible to ventricular fibrillation (VF). Loss of function mutations in *SCN5A*, encoding the cardiac sodium channel, are one cause of the Brugada syndrome, associated with slow conduction and a high incidence of VF, especially in Asians. In this study, we tested the hypothesis that an *SCN5A* promoter polymorphism common in Asians modulates variability in cardiac conduction.
Methods and Results Resequencing 2.8 kb of *SCN5A* promoter identified a haplotype variant, consisting of 6 polymorphisms in near-complete linkage disequilibrium, occurring at an allele frequency of 22% in Asian subjects, and absent in Caucasians and African-Americans. Reporter activity of this variant haplotype, designated HapB, in cardiomyocytes was reduced 62% compared to wild-type (P=0.006). The relationship between *SCN5A* promoter haplotype and PR and QRS durations, indices of conduction velocity, was then analyzed in a cohort of 71 Japanese Brugada syndrome subjects without *SCN5A* mutations and in 102 Japanese controls. In both groups, PR and QRS durations were significantly longer in HapB individuals ($P \leq 0.002$) with a gene-dose effect. In addition, up to 28% and 48% of variability in PR and QRS durations respectively was attributable to this haplotype. The extent of QRS widening during challenge with sodium channel blockers, known to be arrhythmogenic in Brugada syndrome and other settings, was also genotype-dependent (P=0.002).
Conclusion These data demonstrate that genetically-determined variable sodium channel transcription occurs in the human heart, and is associated with variable conduction velocity, an important contributor to arrhythmia susceptibility.

Keywords

Sodium, conduction, arrhythmia, sudden death, genetics

4.1 Introduction

Sudden cardiac death (SCD) accounts for 20% of all mortality in Western countries.[1] One key determinant of normal excitation and conduction of the cardiac impulse is the cardiac sodium channel, responsible for rapid depolarization in most cardiomyocytes. Reduced sodium current predisposes to SCD. For example, while sodium channel blockers have been used for antiarrhythmic therapy, the Cardiac Arrhythmia Suppression Trial showed that these agents increase the incidence of SCD.[2] Loss of function mutations in *SCN5A*, the cardiac sodium channel gene, cause ~20% of cases of the Brugada syndrome, associated with a high risk of SCD.[3] Furthermore, there is evidence that such sodium channel mutations may also lead to enhanced fibrosis in myocardial tissue.[4,5] The overall hypothesis underlying the work presented here is that variability in regulation of sodium channel expression contributes to inter-individual variability in cardiac conduction, and consequently can be considered a candidate modulator of arrhythmia susceptibility, especially in the presence of other stressors such as drugs or acute myocardial ischemia.[6] As a first step in testing this hypothesis, we cloned and characterized the proximal promoter region of *SCN5A* and identified multiple cis-acting elements regulating gene expression.[7] We report here identification of an ethnic-specific, common *SCN5A* promoter variant that modulates PR and QRS durations, indices of cardiac conduction.

4.2 Patients and Methods

Identification of polymorphisms

Resequencing 2.8 kb of the *SCN5A* promoter region in a single individual of Asian origin identified him as a homozygote for 6 DNA polymorphisms in the region: T-1418C, T-1062C, T-847G, -835insGC, G-354C, and C287T (Figure 1). The resequenced region encompassed positions -2190 to +613, relative to major transcription initiation site[7] of the *SCN5A* promoter including 2.2 kb upstream of exon 1, exon 1 (which is 173 bp and non-coding), and the proximal 439 bp of intron 1. The fragment was amplified by LA PCR (TaKaRa kit) with primers F1 and R1 (Table 1). Further studies described below established that these polymorphisms were common and in near-total linkage disequilibrium, thereby identifying 2 common haplotype blocks, designated HapA and HapB. We also detected a third combination of polymorphisms, designated HapC, in <1% of subjects. In addition to the study populations, 150 Caucasian and 100 African-American individuals were tested for these haplotypes.

Generation of constructs

The 2.8 kb fragment described above was amplified from genomic DNA of HapA and HapB homozygous individuals. These fragments were cloned into the pGEM-T Easy vector (Promega) and inserts were subsequently subcloned into the pGL3-Basic vector (Promega), which contains the firefly luciferase coding sequence, to generate *SCN5A* promoter-luciferase fusion constructs for reporter assays. These constructs were designated pGL3-HapA and pGL3-HapB.

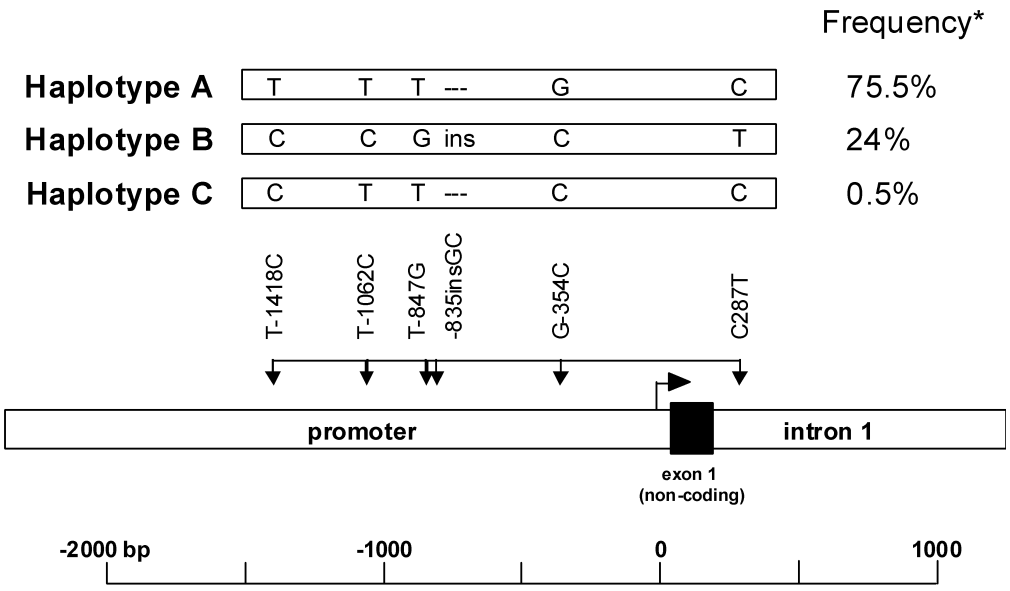

Figure 1: Haplotypes identified in the cardiac sodium channel gene (*SCN5A*) promoter. Nucleotide variations are indicated by their position relative to the major transcription initiation site (+1, reference[7]) with the most frequent nucleotide given below and the least frequent nucleotide given above from the position. *Frequency in the Japanese (control) population.

Reporter activity

Reporter activity was assayed in neonatal mouse cardiomyocytes and in Chinese Hamster Ovary (CHO) cells as described in detail previously.[7] In brief, 1 μg pGL3-HapA or pGL3-HapB was transfected into neonatal mouse cardiomyocytes or CHO cells. In each experiment, 0.05 μg pRL-TK plasmid (Promega) encoding Renilla luciferase was co-transfected to normalize for experimental variability caused by differences in cell viability or transfection efficiency. Luminescence was measured 48 hr post-transfection using the Dual-Luciferase Reporter Assay System (Promega). The pGL3-Basic (promoterless) plasmid was tested in each experiment and its activity level served as the baseline.

Study participants

Participants in the clinical study were ascertained at the National Cardiovascular Center, Osaka, Japan. All protocols (including molecular screening) were reviewed and approved by the Ethical Review Committee of the National Cardiovascular Center, and informed consent was obtained from all individuals.

The control population consisted of 102 subjects drawn from mutation-negative relatives in congenital Long QT syndrome families, in which the causative mutation had been identified. Only one person was drawn from each family. There were 67 males and 35 females ranging in age from 9 to 69, with a mean age of 40±14 years (mean±SD).

The Brugada syndrome population included 80 patients diagnosed with Brugada syndrome, defined as Type 1 "coved" ST-segment elevation in V1-V3 (spontaneous in 70 patients, sodium channel blocker-induced in 10 patients).[8] In all patients, physical examination, chest roentgenogram, laboratory values, echocardiography with wall motion analysis and Doppler screening excluded structural heart disease. Aborted cardiac arrest or ventricular fibrillation (VF) was documented in 30 patients, syncope in 20, and 30 were asymptomatic. All patients had been previously screened for *SCN5A* coding region mutations and a mutation had been identified in 9 patients. The patient group included 76 males and 4 females ranging in age from 1 to 76 years (mean$\pm$SD, 47$\pm$16).

ECG phenotypes

ECGs were assessed by an investigator (WS) blinded to age, gender, and genetic and clinical information. Phenotypes assessed included RR interval, PR interval measured in lead II (PR_{II}), QRS interval measured in leads V1 (QRS_{V1}) and V6 (QRS_{V6}), ST amplitude at J point (ST_J) and ST amplitude at 80 ms after the end of the QRS (ST_{80}).

The effects of intravenous administration of sodium channel blockers on these ECG parameters were examined in 49/80 Brugada syndrome patients. Pilsicainide (maximum 1 mg/kg at a rate of 0.1 mg/kg/min) was used in 37 patients, flecainide (maximum 2 mg/kg at a rate of 0.2 mg/kg/min) was used in 9 patients, and disopyramide (maximum 2 mg/kg at a rate of 0.2 mg/kg/min) was used in 3 patients.

Genotyping

Genomic DNA was prepared from blood leukocytes. Genotyping for the T-1418C and T-1062C single nucleotide polymorphisms (SNPs) was performed by restriction fragment length polymorphism analysis after PCR amplification, with *Ear* I and *Hae* III, respectively. PCR primers used to amplify the 161-bp fragment encompassing the T-1418C SNP were F2 and R2, and those used to amplify the 123-bp fragment encompassing the T-1062C SNP were F3 and R3 (Table 1). Genotyping for the other 4 polymorphisms (T-847G, 835insGC, G-354C, and C287T) was done by DNA resequencing of both strands. PCR primers used to amplify the 638-bp fragment encompassing the T-847G, 835insGC and G-354C polymorphisms were F4 and R4, while those used to amplify the 599-bp fragment encompassing the C287T polymorphism were F5 and R5.

Statistical analysis

Using the individual genotypes for the 6 polymorphisms, haplotype frequencies were estimated using an E-M algorithm.[9] The haplotype frequencies were used to calculate the probabilities of the haplotype pairs compatible with the genotype combinations of the multiple heterozygous patients using Bayes' theorem. Observed haplotype pair frequencies were compared with those expected under Hardy-Weinberg equilibrium in the Brugada syndrome population and control population separately using a chi-squared test. To compare haplotype pair frequencies among Brugada syndrome patients and controls, a Fisher exact test was used.

All quantitative phenotypes were normally distributed and data are expressed as mean $\pm$ standard deviation (SD). Continuous ECG phenotypes were compared between *SCN5A* mutation-negative Brugada syndrome patients, *SCN5A* mutation-positive Brugada syndrome patients and controls using analysis of variance (ANOVA), adjusted for age and gender followed by a post-hoc test for pairwise comparisons. Student's t-tests were used to compare the after drug

challenge continuous ECG phenotypes between *SCN5A* mutation-negative and positive Brugada syndrome patients. Correlations between quantitative phenotypes before and after sodium channel blockade are expressed as Pearson correlation coefficients (*r*). For comparison of the proportion of males a Fisher exact test was used.

The effect of haplotype pairs on the continuous ECG phenotypes was tested in the Brugada syndrome patients and controls separately by ANOVA with adjustment for age and gender. The nine *SCN5A* mutation-positive Brugada syndrome patients were treated as a separate category (7 HapA/HapA homozygotes, 2 HapA/HapB heterozygotes, pooled). The two individuals with the rare HapC variant (one patient from each group) were excluded from the analyses. In all analyses, the proportion of variance attributable to the haplotype pair (R^2) was calculated, corrected for effects of age and gender.

Difference in reporter gene expression activity between Haplotype A and Haplotype B were examined for statistical significance using Student *t*-test. Throughout, P-values < 0.05 were interpreted significant. All statistical analyses were done with SAS software (version 9, SAS Institute, Cary, NC).

Multiple testing

When a Bonferroni correction for the 24 statistical models is used to compare the continuous ECG phenotypes, the significance level for the overall P-values is 0.002. Similarly, the Bonferroni corrected significance levels for the pairwise comparisons between three or four groups is 0.017 and 0.008, respectively.

4.3 Results

Haplotypes

The 6 polymorphisms were in near-complete linkage disequilibrium, with only 2 (similar) discordant haplotypes (out of 364; <1%), each occurring in 1 subject from each population. We designated Haplotype A (HapA) as containing all common alleles and Haplotype B (HapB) containing all minor alleles (Figure 1). The discordant haplotype was designated Haplotype C (HapC). The estimated frequencies of HapA, HapB and HapC were 0.755, 0.240 and 0.005 in the controls and 0.782, 0.211 and 0.007 in the *SCN5A* mutation-negative Brugada syndrome patients, respectively. Haplotype distributions were in Hardy-Weinberg equilibrium (P>0.05) in both populations. No significant difference in haplotype frequencies was observed between the Brugada syndrome group and the controls. The haplotypes were absent in Caucasian and African-American samples.

Functional analysis

In cardiomyocytes, reporter activity of HapB was markedly reduced, by 62%, compared to HapA: 5.5±0.4 (mean±SE) vs. 14.5±2.8 (normalized activity units; n=9 each, P=0.006, Figure 2). A similar trend was seen in the non-cardiac cells: 2.7±0.3 vs. 3.6±0.3 (n=13 each, P=0.04, Figure 2).

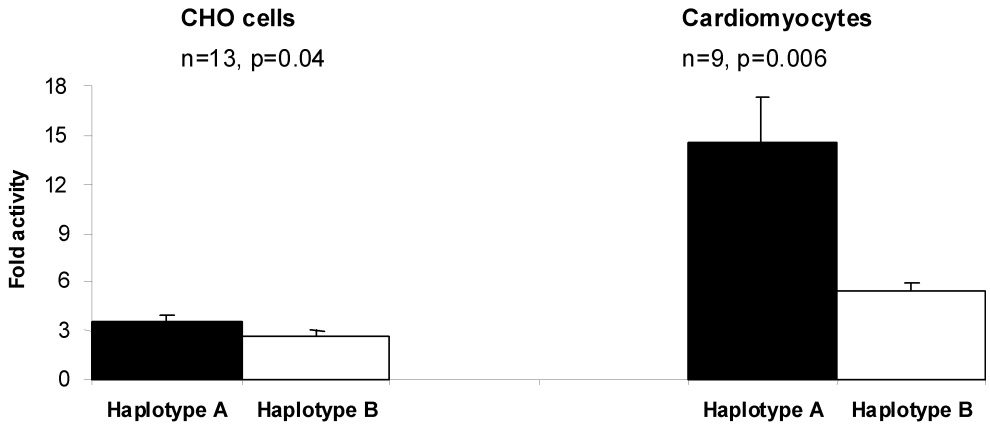

Figure 2: Reporter activity of *SCN5A* promoter haplotypes A and B. Firefly luciferase expression levels, which report the activities of the inserted *SCN5A* sequence were divided by co-expressed Renilla luciferase activities and expressed as relative luciferase units.[7] Data are presented as mean±1SE (vs empty vector). CHO indicates Chinese hamster ovary.

Phenotypic characteristics of the control and Brugada syndrome patient populations

The decreased reporter activity for HapB suggested that individuals carrying this promoter haplotype would display electrocardiographically-detectable conduction slowing. Accordingly, the relationships between genotype and ECG intervals were evaluated in the control and Brugada syndrome populations.

ECG data are shown in Table 2. As expected, Brugada syndrome patients had significantly longer conduction intervals (PR_{II}, QRS_{V1}, QRS_{V6}) and greater ST segment elevation (ST_J, ST_{80}) compared to controls. Heart rate was not significantly different between the two populations. In addition, we found differences between *SCN5A* mutation-positive and *SCN5A* mutation-negative Brugada syndrome patients similar to those previously reported[10]: mutation-positive subjects had significantly longer baseline PR and QRS intervals and longer RR intervals. Data on the subset of Brugada syndrome patients who underwent drug challenge are presented in Table 3.

Table 1. Sequence of Oligonucleotide Primers Used

Primer name	Oligonucleotide sequence, 5' - 3'
F1	TAGGAAGTGCCTGTCTCCAGACACCTGTTG
R1	CGCTCTCTGGAACCACATTCATGGCG
F2	CCCTGATGGCCTGTTTTGTTT
R2	ACTCAGAGACATGGTCACAGGCA
F3	ACCTAAGGCGTCCAACGAAGC
R3	CCAGGGTCTCAGAGGGCACAG
F4	AGGCTCTGCATGTGTCAAG
R4	GACGCGGACAGGCTCACA
F5	GTAGGATGCAGGGATCGCT
R5	CGCTCTCTGGAACCACATTC

Table 2: Baseline ECG characteristics of the control and Brugada syndrome patient populations.

	Controls	Brugada Syndrome patients		Overall P-value	Pairwise comparison P-values	
		SCN5A^{-ve}	*SCN5A*$^{+ve}$		*SCN5A*$^{-ve}$ vs. *SCN5A*$^{+ve}$	*SCN5A*$^{-ve}$ vs. controls
N	102	71	9			
Males	67 (66%)	67 (94%)	9 (100%)	<0.0001	1.000	<0.0001
Age	40.0 ± 14.2	46.5 ± 16.3	51.1 ± 8.4	0.005	0.376	0.005
RR (ms)	925.3 ± 130.0	913.7 ± 134.3	1055.6 ± 154.2	0.012	0.003	0.572
PR_{II} (,ms)	162.3 ± 21.8	180.4 ± 20.4	238.9 ± 26.7	<0.0001	<0.0001	<0.0001
QRS_{V1} (ms)	93.8 ± 11.8	104.9 ± 19.3	142.2 ± 19.1	<0.0001	<0.0001	<0.0001
QRS_{V6} (ms)	87.4 ± 12.4	100.2 ± 19.1	139.4 ± 21.6	<0.0001	<0.0001	<0.0001
ST_J (mV)	0.10 ± 0.05	0.30 ± 0.14	0.34 ± 0.18	<0.0001	0.249	<0.0001
ST_{80} (mV)	0.18 ± 0.10	0.25 ± 0.12	0.24 ± 0.13	0.001	0.778	0.001

Values are given as mean ± standard deviation. Underlined P-values are below the Bonferroni corrected overall or pairwise significance levels (see: Multiple testing)

For all ECG parameters investigated highly significant (P<0.0001) correlations were present between measures before and after drug challenge (Table 3). As previously reported, *SCN5A* mutation-positive patients displayed longer PR and QRS intervals after challenge with sodium channel blockers, compared to *SCN5A* mutation-negative patients.

Haplotype pair effects

PR and QRS durations were significantly longer in HapB individuals in both study populations (Brugada syndrome, controls: P≤0.002 for PR_{II}; P<0.0001 for QRS_{V1} and QRS_{V6}, Figure 3). In the control population PR_{II}, QRS_{V1} and QRS_{V6} intervals showed a gene-dose effect, being longest in HapB homozygotes, intermediate in HapA/HapB heterozygotes and shortest in HapA homozygotes. A similar pattern was observed in the *SCN5A* mutation-negative Brugada syndrome patient group. As discussed in the Methods section, these analyses excluded data in the two individuals with HapC. PR_{II}, QRS_{V1} and QRS_{V6} means (± SD) per haplotype group for the two populations are listed in Table 4. Both the overall and pairwise P-values were highly statistically significant even after correction for multiple testing.

The amount of variance (R^2) in PR and QRS intervals explained by the haplotype pair after correction for age and gender is shown in Table 5. As can be seen, a significant proportion of variance in PR and QRS intervals, both at baseline (both groups) as well as after drug challenge (Brugada syndrome group) was attributable to the haplotype. No significant association was found between haplotype and RR, ST_J and ST_{80}, in either population (data not shown).

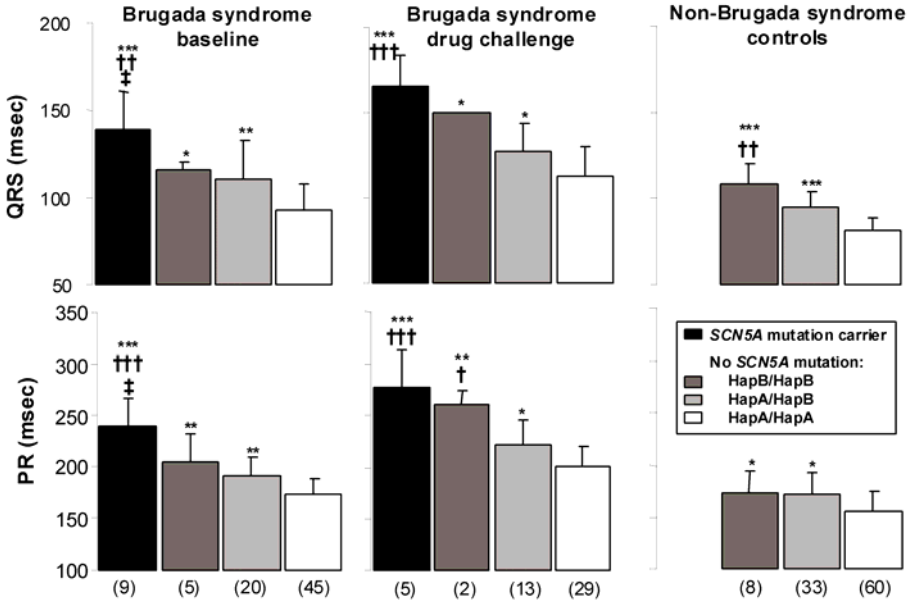

Figure 3: *SCN5A* promoter haplotype effects on durations of QRS_{V6} and PR_{II} in Brugada syndrome patients at baseline and after challenge with sodium channel blocking agents, and non-Brugada syndrome controls. Patient numbers are indicated between parentheses. Genotype effects on QRS_{V1} were similar to those on QRS_{V6} due to a high correlation between these two parameters (Pearson's coefficient, r=0.96). Data are presented as mean±SD. For Bonferroni corrected significance levels for pairwise comparisons, refer to Multiple Testing section in Materials and Methods.

Drug challenge and haplotype

The haplotype pairs were also highly associated with conduction intervals (PR_{II}, QRS_{V1}, QRS_{V6}) after sodium channel blockade in 44 *SCN5A* mutation-negative Brugada syndrome patients who underwent drug challenge (for PR_{II}, QRS_{V1}, QRS_{V6}, P<0.0001, Figure 3). PR_{II}, QRS_{V1} and QRS_{V6} means (± SD) per haplotype group are listed in Table 4. Also here, overall and pairwise P-values were highly statistically significant even after correction for multiple testing.

In addition, the extent of QRS widening (ΔQRS) following drug challenge was genotype-dependent and a gene dose effect was also observed (ΔQRS_{V6}: HapB/HapB=30 ms [mean±SD]; HapA/HapB=24.2±7.9; HapA/HapA=17.8±7.2; P=0.002, Figure 4). A similar trend was seen for extent of PR widening (ΔPR) following drug challenge (ΔPR_{II}: HapB/HapB=40 ms; HapA/HapB=33.8±13.2; HapA/HapA=28.6±8.3; P=0.05).

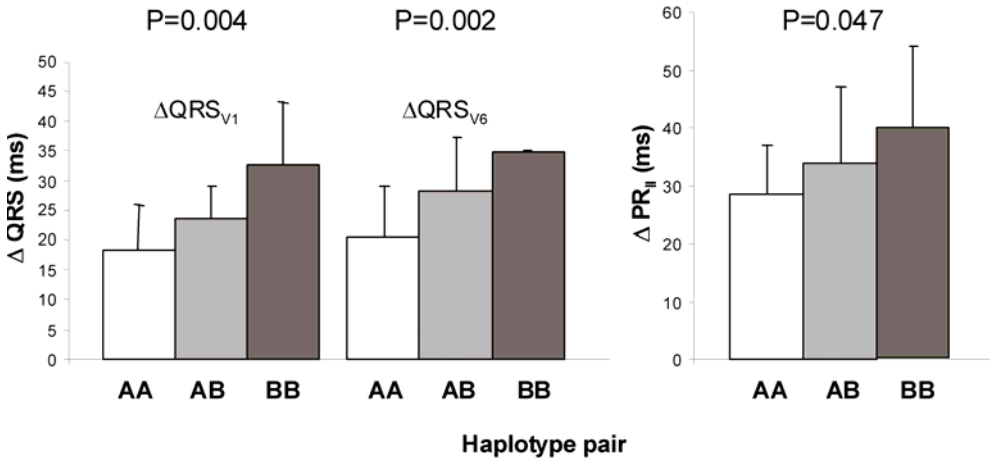

Figure 4: *SCN5A* promoter haplotype effects on extent of QRS (ΔQRS_{V1} and ΔQRS_{V6}) and PR (ΔPR_{II}) widening following sodium channel blockade. AA, n=29; AB, n=13; BB, n=2. Data are presented as mean±SD. The Bonferroni corrected significance level is 0.002.

4.4 Discussion

We demonstrate that a set of six *SCN5A* promoter polymorphisms found in Asian subjects are in near-complete linkage disequilibrium, have a significant impact on sodium channel expression *in vitro*, account for a large proportion of variance in ECG conduction parameters in two independent Japanese populations, and represent pharmacogenetic markers predicting variable drug response.

Twin studies have identified strong genetic effects for ECG parameters including PR and QRS durations.[11-14] Indeed, associations have been reported between ECG parameters and single coding region nonsynonymous (amino acid-changing) SNPs in ion channel genes.[15,16] However, common functional variants in regulatory regions that strongly modulate basal ECG intervals have not been previously identified; one preliminary report has suggested an association between a potassium channel promoter polymorphism and QRS axis in women only.[17]

Table 3: Clinical characteristics of the Brugada syndrome patients after sodium channel blocker challenge.

	SCN5A^{-ve}	*SCN5A*$^{+ve}$	P-value	*r* before and after sodium channel blockade
N	44	5		
Males	42 (95%)	5 (100%)	1.000	
Age	46.3 ± 14.8	52.0 ± 5.4	0.397	
aRR (ms)	892.3 ± 113.1	956.0 ± 99.4	0.234	0.94
aPR$_{II}$ (,ms)	209.6 ± 25.1	278.0 ± 35.6	<0.0001	0.95
aQRS$_{V1}$ (ms)	124.1 ± 16.1	166.0 ± 17.8	<0.0001	0.92
aQRS$_{V6}$ (ms)	119.2 ± 17.1	166.0 ± 17.8	<0.0001	0.92
aST$_{J}$ (mV)	0.51 ± 0.21	0.78 ± 0.25	0.013	0.84
aST$_{80}$ (mV)	0.41 ± 0.17	0.70 ± 0.31	0.109	0.63

Values are given as mean ± standard deviation. Pearson correlation coefficients (*r*) observed between measures before and after sodium channel blocker challenge (P<0.0001). Mean baseline ECG parameters for the 44 *SCN5A*$^{-ve}$ and 5 *SCN5A*$^{+ve}$ patients (not shown) were very similar to those for the total patient group given in Table 2. Underlined P-values are below the Bonferroni corrected overall significance levels (see: Multiple testing).

Table 4: Haplotype pair effects on QRS and PR intervals in the control population and the *SCN5A* mutation-negative (*SCN5A*-ve) Brugada syndrome population at baseline and after challenge with sodium channel blockers. Per haplotype pair, mean ± SD values are given.

	HapA / HapA homozygotes	HapA / HapB heterozygotes	HapB / HapB homozygotes
Controls	n=60	n=33	n=8
QRS_{V1} (ms)	87.42 ± 8.36	100.45 ± 8.60	112.50 ± 10.35
QRS_{V6} (ms)	80.58 ± 8.44	94.39 ± 8.91	107.50 ± 11.65
PR_{II} (ms)	155.33 ± 20.21	172.42 ± 20.62	173.75 ± 20.66
SCN5A-ve Brugada Syndrome, baseline	n=45	n=20	n=5
QRS_{V1} (ms)	97.67 ± 13.59	115.75 ± 23.97	124.00 ± 8.94
QRS_{V6} (ms)	93.44 ± 15.03	110.50 ± 22.59	116.00 ± 5.48
PR_{II} (ms)	172.89 ± 16.46	191.58 ± 18.34	204.00 ± 27.02
SCN5A-ve Brugada Syndrome, drug challenge	n=29	n=13	n=2
QRS_{V1} (ms)	118.10 ± 14.78	133.08 ± 9.90	152.50 ± 10.61
QRS_{V6} (ms)	113.10 ± 16.98	128.08 ± 5.96	150.00 ± 0.0
PR_{II} (ms)	200.69 ± 20.17	221.54 ± 22.67	260.00 ± 14.14

Only recently has the concept of tightly linked polymorphisms (constituting a haplotype block) been applied to understanding variability in cardiac electrophysiology. In one study, a small degree of variance (<1%) in QT-interval in a central European population could be attributed to single SNPs and haplotype blocks in four potassium channel genes.[18] By contrast, the *SCN5A* promoter haplotype we report here explained a remarkable proportion of variance in conduction parameters in the Japanese subjects studied (Table 5). Such associations could arise because the haplotypes studied are, in turn, in linkage disequilibrium with other functionally-important variants in regulatory or other regions of the gene. However, in this case, the *in vitro* functional studies indicate that the effect is attributable to a variant within the haplotype block; at this point, the specific variant mediating this effect has not been identified.

A principal determinant of cardiac conduction in atrial and ventricular muscle is the sodium current; sodium channel blockers prolong PR and QRS durations, an effect also seen with loss of function mutations in *SCN5A*.[3] Critical degrees of conduction slowing represent a final common pathway to VF,[19] so dissection of the genetic determinants of cardiac conduction in the general population is a key step to understanding variable susceptibility to common arrhythmias due to conduction slowing, as in myocardial ischemia or heart failure.[19] Thus the data we present here implicates the *SCN5A* promoter variant HapB, which slowed conduction in normal subjects, and exacerbated conduction slowing in those with Brugada syndrome, as a candidate modulator of variability in risk of SCD. Importantly, imposition of further depression of sodium channel function by administration of sodium channel blocking drugs further exacerbated conduction slowing, in a gene-dose-dependent fashion. Studies in large numbers of subjects at risk for SCD will be required to further establish the role of this and other regulatory region polymorphisms in modulating that risk.

Differences in disease penetrance and expression have been widely reported in the cardiac sodium and other channelopathies.[20-23] Relatives carrying *SCN5A* mutation identical to that of the proband may be clinically unaffected,[23] and family members may display different phenotypes, for instance Brugada syndrome or conduction disease.[21] Genetic variants like the one presented here are obvious candidate modulators of this variability in phenotypic expression. Inter-individual variability has also been noted in response to pharmacological challenge with sodium channel blockers in Brugada syndrome patients.[23,24] In some patients, some even carrying an *SCN5A* mutation, drug challenge fails to unmask a Brugada syndrome ECG. The significantly greater increases in PR and QRS durations with sodium channel block in HapB carriers thus identify variability in expression of the drug target, the sodium channel, as key mediator of this variable drug effect. It is thus possible, that other sodium channel blocker response phenotypes, such as the increased mortality with sodium channel blockers in the Cardiac Arrhythmia Suppression Trial,[2] was determined by variable sodium channel expression. DNA samples from that important clinical trial were not archived so this will remain an open question. More generally, the data indicate that sodium channel function is additively suppressed by drug challenge, Brugada syndrome mutations, and the HapB regulatory variant.

Brugada syndrome is endemic in Asia, where the disorder is also known as sudden unexplained nocturnal death syndrome (SUNDS)[25], and in fact the incidence is higher in Asia than in the United States and Europe.[26] Because HapB is common in Asians and absent in Caucasians, and has a large negative impact on cardiac conduction, a long-recognized feature of Brugada syndrome,[27] it may logically contribute to differences in Brugada syndrome incidence as a function of ethnicity. In this study, PR and QRS durations in individuals matched for haplotype were consistently longer in the Brugada syndrome group compared to controls; thus, the greatest conduction slowing was in those subjects with Brugada syndrome and the HapB/HapB genotype. Indeed, control HapB/HapB subjects had longer QRS durations than did those with manifest Brugada syndrome and the commoner HapA/HapA genotype. Thus, although the minor allele is quite common, it alone may give rise to one part of the spectrum of loss of sodium channel function that constitutes the Brugada syndrome. More generally the data fit nicely the concept that individuals vary in their ability to maintain sodium channel function to protect against the arrhythmia-prone substrate, and identify HapB as a variant that contributes to such variable "antifibrillatory reserve".[10,28]

Table 5: Variance explained by the haplotype pair (R^2, %)

	R^2 (%) Controls	Brugada Syndrome baseline	Brugada Syndrome drug challenge
PR_{II}	12.2	28.4	33.0
QRS_{V1}	47.6	26.4	33.0
QRS_{V6}	48.5	24.9	36.2

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Conflict of interest statement

W Shimizu and Y Miyamato are applying for Japanese domestic patent based on this work.

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