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CLINICAL RESEARCH
Arrhythmia/electrophysiology

Predicting cardiac electrical response to sodium-channel blockade and Brugada syndrome using polygenic risk scores

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Aims Sodium-channel blockers (SCBs) are associated with arrhythmia, but variability of cardiac electrical response remains unexplained. We sought to identify predictors of ajmaline-induced PR and QRS changes and Type I Brugada syndrome (BrS) electrocardiogram (ECG).

Methods and results In 1368 patients that underwent ajmaline infusion for suspected BrS, we performed measurements of 26 721 ECGs, dose–response mixed modelling and genotyping. We calculated polygenic risk scores (PRS) for PR interval (PRS_{PR}), QRS duration (PRS_{QRS}), and Brugada syndrome (PRS_{BrS}) derived from published genome-wide association studies and used regression analysis to identify predictors of ajmaline dose related PR change (slope) and QRS slope. We derived and validated using bootstrapping a predictive model for ajmaline-induced Type I BrS ECG. Higher PRS_{PR}, baseline PR, and female sex are associated with more pronounced PR slope, while PRS_{QRS} and age are positively associated with QRS slope ($P < 0.01$ for all). PRS_{BrS}, baseline QRS duration, presence of Type II or III BrS ECG at baseline, and family history of BrS are independently associated with the occurrence of a Type I BrS ECG, with good predictive accuracy (optimism-corrected C-statistic 0.74).

Conclusion We show for the first time that genetic factors underlie the variability of cardiac electrical response to SCB. PRS_{BrS}, family history, and a baseline ECG can predict the development of a diagnostic drug-induced Type I BrS ECG with clinically relevant accuracy. These findings could lead to the use of PRS in the diagnosis of BrS and, if confirmed in population studies, to identify patients at risk for toxicity when given SCB.

Keywords Polygenic risk score • Brugada syndrome • Ajmaline • QRS • PR

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Introduction

Cardiac arrhythmia as a consequence of the use of cardiac and non-cardiac drugs is a long-recognized clinical problem, notably QT prolongation and *torsades de pointes*. Rare monogenic variants causing drug-induced *torsades de pointes* are uncommon, and mostly reported in patients with unrecognized congenital long QT syndrome. Recently, a polygenic risk score derived from a QT interval study in ~100 000 subjects[1]—i.e. based on common genetic variants that modulate the QT in the general population—has been shown to predict drug-induced QT prolongation and *torsades de pointes*.[2] These findings support a vision of pre-prescription genotyping to reduce adverse events.

Impaired cardiac depolarization predisposes to cardiac arrhythmias, through conduction block and re-entry. Sodium-channel blocking drugs inhibiting cardiomyocyte depolarization are associated with major adverse cardiovascular events, both in the general population[3–6] and in specific patient subgroups.[7–9] These studies demonstrate the potential for cardiac adverse events using sodium-channel blockers (SCBs) prescribed both for cardiac arrhythmia[7,8,10] as well as for non-cardiac conditions.[3–6] The presence of structural heart disease and myocardial ischaemia are well-recognized risk factors, yet, SCB proarrhythmia can also be observed in patients with apparently normal hearts, such as in the Brugada syndrome (BrS).[3,11]

Brugada syndrome is an inherited electrical disease associated with sudden cardiac death (SCD) and characterized by ST-segment elevation and T-wave inversion in the right precordial electrocardiogram (ECG) leads (Type I ECG).[12] Brugada syndrome involves impaired sodium-channel function, through loss of function mutations in the underlying *SCN5A* gene and/or decreased expression mediated by common genetic variants.[13,14] Patients with suspected BrS often do not manifest the diagnostic Type I ECG at baseline. An infusion of a SCB, such as ajmaline, is performed in these cases to unmask the diagnostic Type I ECG. Although the prevalence of BrS has been traditionally considered to be low, recent data show that the prevalence of ajmaline-induced Type I BrS ECG is ~5% in the general population[15] and up to 28% in families of SCD cases with normal autopsy.[16,17]

As in drug-induced *torsades de pointes*, sporadic cases harbouring rare pathogenic variants in *SCN5A* point to the genetic basis of proarrhythmia risk in the setting of SCB use.[3,18] Such mutations may explain some cases of drug toxicity. Whether more common genetic variants predict inter-individual variability in the cardiac electrical response to sodium-channel blockade, akin to drug-induced QTc prolongation,[2] remains entirely unexplored.

Recent genome-wide association studies (GWAS) of PR interval[19] and QRS duration[20] have identified multiple loci harbouring common genetic variants that impact on these conduction ECG parameters. A GWAS of BrS[13] also identified three common single-nucleotide polymorphisms (SNPs) associated with BrS with moderate effect sizes. We now test the hypothesis that a weighted combination of such common genetic variants predicts the individual response to sodium-channel blockade. In a large set of 1400 consecutive patients who underwent ajmaline testing, we demonstrate that polygenic risk scores (PRS) based on SNPs modulating QRS duration and risk of BrS, are independent predictors of the response to sodium-channel blockade. Findings from this proof of concept study establish a framework for individualized risk prediction of SCB cardiac toxicity. We also developed and internally validated a prediction model of ajmaline-induced Type I BrS ECG that could be used in the diagnostic strategy when suspecting BrS.

Methods

Patient inclusion and ajmaline testing

The study included 1400 consecutive consenting patients that underwent ajmaline testing in the Amsterdam University Medical Centre, location Academic Medical Center (AMC; Amsterdam) from December 2004 to September 2016 for suspected BrS. Ajmaline testing was performed as recently described[17] in a reproducible manner by one physician (H.L.T.). Intravenous ajmaline was administered at consecutive boluses of 10 mg/min. A 10-s ECG was recorded ~1 min after each bolus using a GE Healthcare electrocardiograph. The test was stopped when the target dose of 1 mg/kg rounded up to the next 10 mg was reached, if ventricular arrhythmia occurred, or at the manifestation of a Type I BrS pattern, defined as an ST elevation >2 mm with a coved morphology in any lead among V1–V2 in the 2nd to 4th intercostal spaces.[12]

Electrocardiogram processing and dose–response modelling

PR intervals and QRS durations of 26 721 ECGs recorded during ajmaline testing of included individuals were measured with the Modular ECG Analysis System (MEANS),[21] an extensively evaluated computer programme often used to analyse ECGs from large population datasets, including recent GWAS.[19,20] MEANS determines common waveform markers (i.e. beginning of P-wave and QRS complex, and end of QRS) for all 12 leads together on one representative averaged beat (see two examples in *Figure 1A*). Measurements corresponding to identical sample-dose pairs were averaged and individuals with less than 4 PR or QRS data points or with a baseline QRS >120 ms were removed. The presence of a Type II or III BrS patterns at baseline was assessed by manually reviewing the ECG of all participants recorded immediately prior to drug infusion with V1 and V2 recorded at the 4th (normal) and 3rd (high) intercostal spaces.

The relations of PR and QRS with weight-adjusted ajmaline dose (in mg/kg) were fit to a linear mixed model using restricted maximum likelihood, with fixed and random effects for both intercept and slope (*Figure 1B*). Individuals having poor dose–response fits (defined as having ≥1 data point with a residual absolute value greater than 3 standard deviations, SDs) were identified and the waveform markers in all their ECGs were manually checked and adjusted if necessary, with the assumption that poor fit may reflect improper automated detection of complex waveforms.

While inter-individual variability in baseline PR and QRS (i.e. intercept) has been the subject of recent GWAS,[19,20] the variability of the response to sodium-channel blockade (i.e. slope) has not yet been explored (*Figure 1*). In the present study, we sought to identify clinical and genetic predictors of PR and QRS dose–response slopes (referred to as 'PR slope' and 'QRS slope').

Genome-wide array genotyping, quality control and imputation

We performed genome-wide array genotyping for all study subjects on the Illumina Global Screening Array at the Genome analysis centre at Helmholtz Zentrum München. All downstream analyses were performed at the AMC. Single-nucleotide polymorphism-level and sample-level

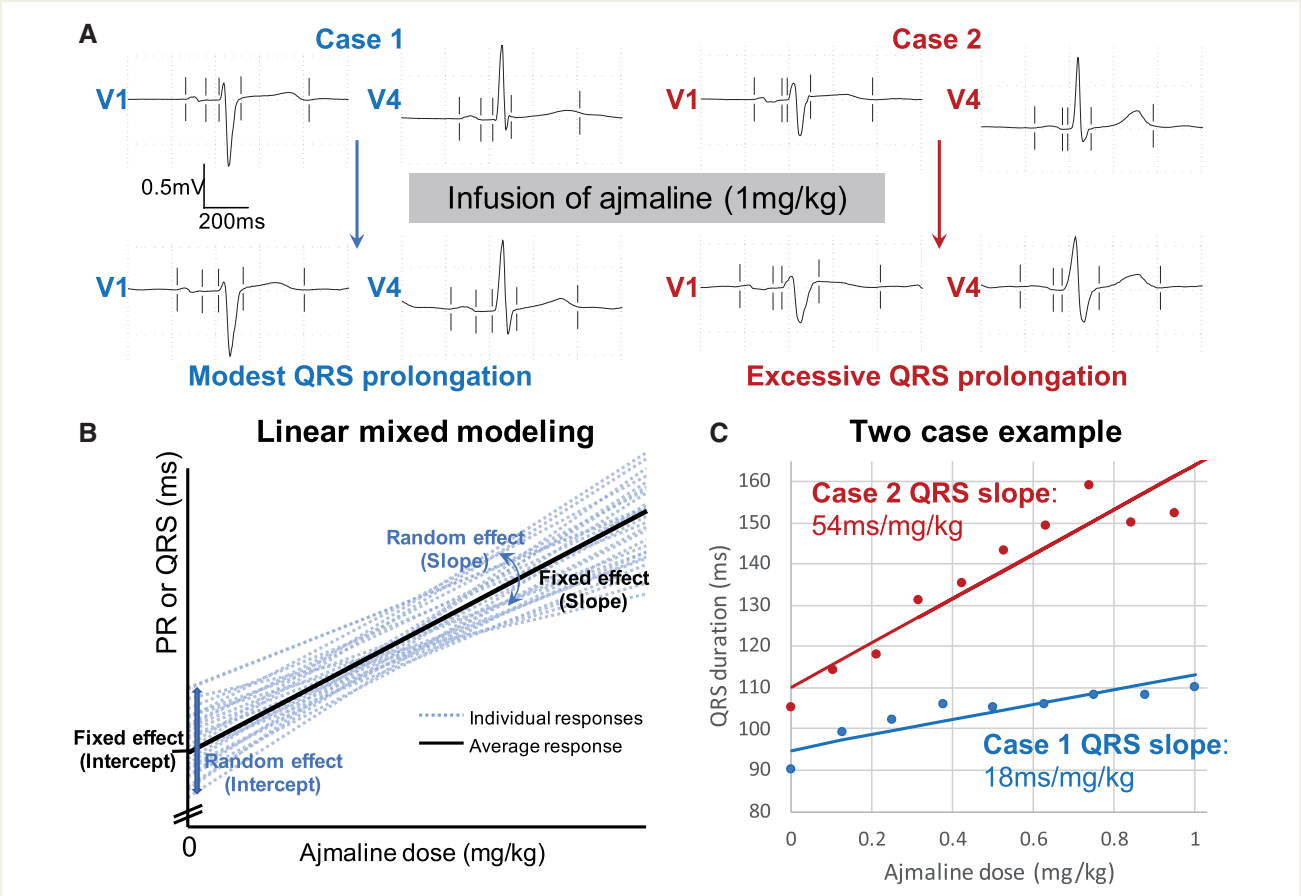


Figure 1 Variability in ajmaline response and linear mixed modelling. (*A*) Electrocardiograms (leads V1 and V4) at baseline (top) and peak ajmaline infusion (bottom) of two representative cases. Automatic waveform markers are overlaid on the electrocardiograms. Electrocardiogram scale (0.5 mV/200 ms) on the left. (*B*) Schematic representation of linear mixed modelling of ajmaline dose–response on PR and QRS, illustrating the fixed and random effects on intercept and slope, where fixed effects are average responses, while random effects are individual differences from the average. (*C*) Automatic measurements (points) and linear mixed model fit (line) of QRS vs. weight-adjusted ajmaline dose for the two cases shown in (*A*).

quality control (QC) was performed using PLINKv1.9 and in-house scripts. We excluded ambiguous SNPs (A/T or C/G) and those with missingness >0.05, Hardy–Weinberg equilibrium test $P < 10^{-6}$, minor allele frequency (MAF) <0.001. Samples with missingness >0.03, inbreeding coefficient |F|>0.1, as well as those with sex mismatch were excluded. Related samples were not excluded. Samples with divergent ancestry were excluded from PRS analysis (see below).

Genome-wide imputation was performed using Eagle2 phasing, Minimac3, and the Haplotype reference consortium (HRCr1.1) panel implemented on the Michigan Imputation Server.[22] After imputation, only SNPs with MAF > 0.05 and a Minimac3 $R^2 > 0.5$ were included.

PRS analyses

Association data from previously published GWAS on PR interval,[19] QRS duration,[20] and BrS[13] were used to calculate weighted (PRS) for PR (PRS_{PR}), QRS (PRS_{QRS}), and BrS (PRS_{BrS}), respectively. Each PRS was calculated for each individual as the sum of [alternate allele dosage × published regression coefficient (β) for that allele] for each independent SNP reaching genome-wide significance in the published study (Supplementary material online, *Table S1*): 44, 26, and 3 SNPs for PRS_{PR}, PRS_{QRS}, and PRS_{BrS}, respectively. Since the reported regression coefficients are primarily derived from European populations, we used genotypic principal component analysis to exclude non-European samples for PRS analyses.

The association of PRS and clinical parameters with the PR and QRS slopes was performed using univariable linear regression followed by multivariable analysis, with only variables with a $P < 0.05$ in the univariable analyses included in the model. A linear mixed effect model was used to account for genetic relatedness using a kinship matrix (R *lmekin* function in the *coxme* package). The genetic relatedness matrix was constructed using GCTA.[23] The association of PRS and clinical parameters with the appearance of a Type I BrS ECG was performed using univariable and multivariable logistic regression.

Development and validation of a Brugada syndrome risk prediction model

The discriminative value of the PRS_{BrS} with or without clinical variables in predicting ajmaline-induced Type I BrS ECG was assessed using a receiver-operating characteristic curve, C-statistic, and sensitivity, specificity, and positive/negative predictive values at different thresholds, using

the *pROC* package in R. Internal validation of the predictive model was performed using bootstrapping by fitting the model to 1000 bootstrap datasets of identical size as the study population, using the *rms* package. Optimism-corrected C-statistic and R^2 as well as calibration slope were calculated.

As an alternative strategy to bootstrapping, we derived a prediction model from patients that had ajmaline testing prior to 31 December 2011, and validated it in those that had the test in and following 2012.

General statistics

We systematically assessed normal distribution using the Shapiro–Wilk test. Normally distributed variables are presented as mean ± standard deviation and compared using a Student's *t*-test. Non-normally distributed variables are presented as median (interquartile range) and compared using the Wilcoxon rank-sum test. Categorical variables are presented as *N* (%) and compared using the Pearson χ^2 test. The statistical significance level was set to $P < 5 \times 10^{-8}$ for GWAS. The primary objectives were to test the association of (i) PRS_{PR} with PR slope, (ii) PRS_{QRS} with QRS slope, and (iii) PRS_{BrS} with ajmaline-induced Type I pattern, corrected for other associated variables. Significance threshold for these primary analyses was set to $P < 0.05/3$ (0.017; Bonferroni correction) and $P < 0.05$ for other secondary analyses and variable selection.

Results

Study population and sample quality control

A total of 1400 individuals were included and underwent genome-wide array genotyping. During QC, 32 were excluded (25 with high genotype missingness and 7 with sex mismatch). Basic characteristics of the remaining 1368 individuals are presented in Supplementary material online, *Table S2*. Of these, 530 were singletons and 838 belonged to one of 249 families.

Electrocardiogram processing and dose–response modelling

In total, 10 824 PR and 10 966 QRS data points (Supplementary material online, *Figure S1*) were used for dose–response linear mixed modelling. Supplementary material online, *Figure S2* shows the residuals vs. fitted values and measured vs. fitted values for PR and QRS in this initial modelling. We manually inspected and readjusted ECG waveform markers, as necessary, for individuals with any outlier data point, as prespecified. Linear mixed modelling was again performed in the corrected dataset. This resulted in an improved fit (Supplementary material online, *Figure S3*), with a coefficient of determination (R^2) of 0.83 for PR and 0.78 for QRS. The average baseline PR was 162 ms (fixed effect intercept) and the average PR change with ajmaline was 51 ms/mg/kg (fixed effect slope). The corresponding values for QRS were 101 ms and 36 ms/mg/kg, respectively. Intercepts and slopes showed large variability for both PR and QRS (Supplementary material online, *Figure S4*).

Array genotyping analysis

After QC, 523 549 SNPs were retained and 4.1M common SNPs were well imputed using the Haplotype Reference Consortium panel. A single SNP genome-wide association analysis was performed as described in the Supplementary material online, *Data Supplement* and shown in Supplementary material online, *Figures S5* and *S6*, *Table S3*. The *SCN5A–SCN10A* locus (lead SNP rs10428132) was significantly associated with ajmaline-induced Type I BrS ECG ($P = 8.6 \times 10^{-19}$).

Of the 1368 samples passing QC, 111 were excluded from PRS-based analyses because of non-European ancestry. The distributions of PRS across the cohort are presented in Supplementary material online, *Figure S7*.

Predictors of baseline PR and QRS

Baseline PR and QRS were significantly higher in *SCN5A* mutation carriers vs. non-carriers (PR: 194 ± 37 vs. 160 ± 25 ms, $P = 3 \times 10^{-9}$; QRS: 114 ± 19 vs. 101 ± 13 ms, $P = 4 \times 10^{-6}$) and were positively correlated with PRS_{PR} [correlation coefficient (r) = 0.23; $P = 3 \times 10^{-15}$] and PRS_{QRS} ($r = 0.15$; $P = 6 \times 10^{-7}$), respectively, in mutation non-carriers (*Figure 2A and B*).

Predictors of PR slope

As for baseline PR, PR slope was also higher in *SCN5A* mutation carriers and was positively correlated with PRS_{PR} (*Figure 2C*). We assessed the association of the PR slope with clinical and genetic parameters using a linear mixed model (*Table 1*). In univariable analysis, sex, PRS_{PR}, baseline PR, and the presence of *SCN5A* pathogenic variant were associated with PR slope. In multivariable analysis, sex and baseline PR were significantly associated with PR slope, while the association of PRS_{PR} was not statistically significant ($P = 0.062$). Because only ~30% of patients underwent *SCN5A* sequencing as per clinical indications, the presence of a mutation was not integrated in the predefined multivariable model (*Table 1*). Results from a multivariable model including *SCN5A* mutation status, when available, appear in Supplementary material online, *Table S4*. Considering the correlation between PR and PRS_{PR}, we assessed for collinearity in the model by calculating the variance inflation factors. Variance inflation factors were 1.09 and 1.05 for PR and PRS_{PR}, respectively, suggesting negligible collinearity.

When excluding patients known to have an *SCN5A* mutation, sex, PRS_{PR}, and baseline PR were all associated with PR slope. When also excluding patients with a Type I BrS ECG, only sex and baseline PR remained significantly associated with PR slope (*Table 1*).

Predictors of QRS slope

QRS slope was higher in *SCN5A* mutation carriers and was positively correlated with PRS_{QRS} (*Figure 2*). The results of linear mixed modelling of QRS slope are shown in *Table 2*. In univariable analysis, age, PRS_{QRS}, and the presence of an *SCN5A* pathogenic variant were associated with the QRS slope. In multivariable analysis combining age and PRS_{QRS}, both variables were independently and significantly associated with QRS slope. Both age and PRS_{QRS} remained independently associated with QRS slope in the subgroup of patients without a pathogenic *SCN5A* variant, as well as those without a BrS Type I ECG (*Table 2*). Results from a multivariable model including *SCN5A* mutation status appear in Supplementary material online, *Table S4*. The association of PRS_{QRS} with QRS slope was not significant when *SCN5A* mutation status was included in the model. This may reflect lower statistical power (sample size 295 vs. 1097) but also a higher proportion of *SCN5A* carriers in whom the effect of common variants is

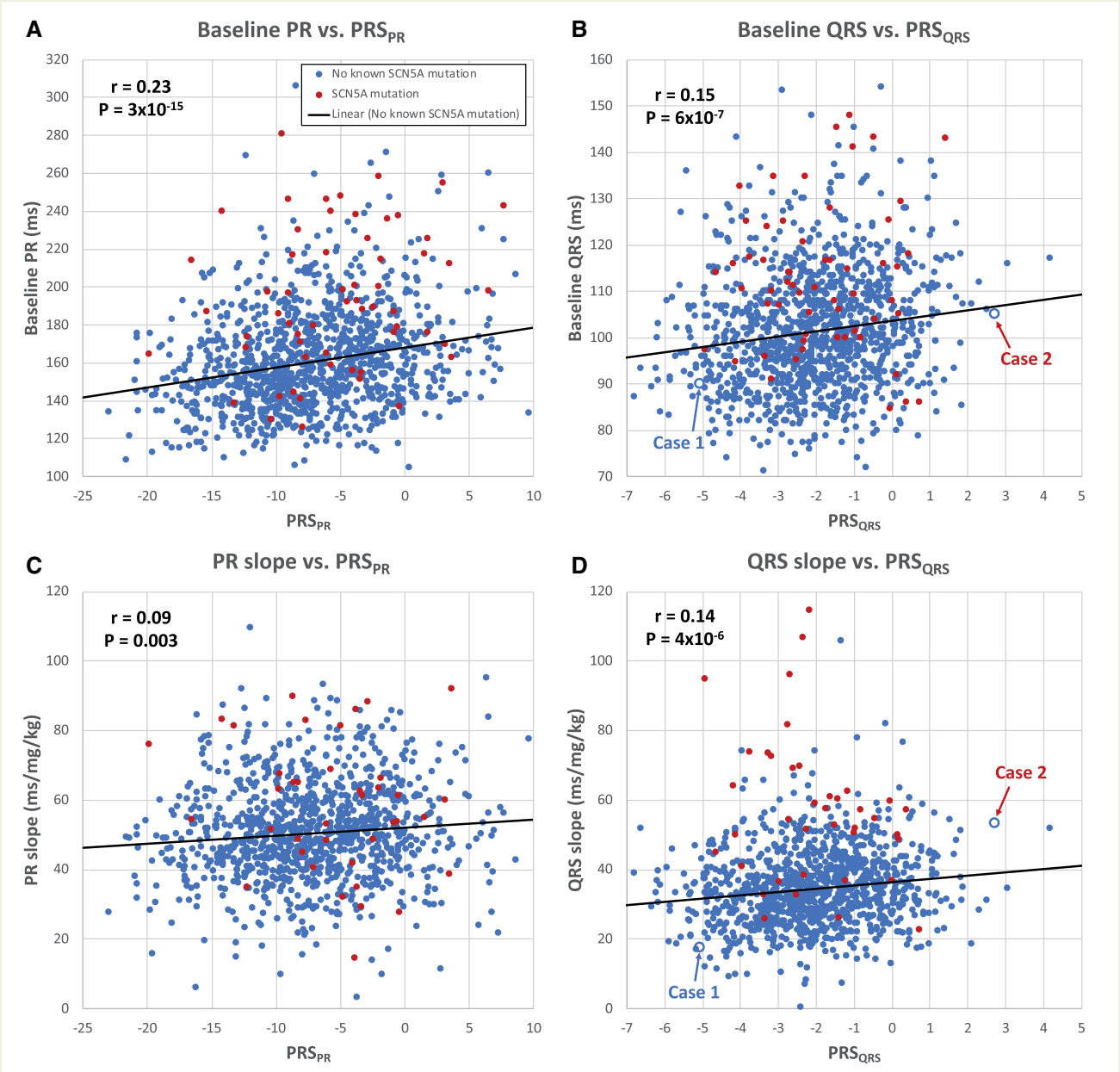


Figure 2 Correlation plots of baseline PR and QRS vs. PRS_{PR} (*A*) and PRS_{QRS} (*B*), respectively and PR and QRS slopes vs. PRS_{PR} (*C*) and PRS_{QRS} (*D*), respectively. Red and blue markers represent *SCN5A* mutation carriers and those without a known *SCN5A* mutation, respectively. The line represents the linear regression between correlated variables in cases without a known *SCN5A* mutation, with the correlation coefficient (*r*) and Pearson's correlation test *P*-value (*P*) on the top left corner. Legend applies to all panels. Arrows in panels *B* and *D* highlight the two cases shown in *Figure 1*.

modest (see red markers in *Figure 2D*; *SCN5A*-PRS_{QRS} interaction effect $P = 0.004$).

Predictors of Type I Brugada syndrome electrocardiogram and ventricular arrhythmia

The results of univariable and multivariable logistic regression for the development of a Type I BrS ECG are shown in *Table 3*. The 3-SNP PRS_{BrS} was strongly associated with ajmaline-induced Type I BrS ECG. Baseline QRS, presence of a Type II or III pattern on baseline ECG, and family history of BrS were also independent predictors of the BrS Type I ECG, both in the overall cohort and when excluding *SCN5A* mutation carriers. PRS_{QRS} and PRS_{PR} were associated with a Type I ECG in univariable but not multivariable analyses, likely because of their strong correlation with PRS_{BrS} ($r = 0.49$ for PRS_{PR} and 0.28 for PRS_{QRS}; $P < 10^{-15}$ for both) reflecting the important contribution of the *SCN5A–SCN10A* locus in all three PRS (see Supplementary material online, *Table S1*). *Figure 3A* represents the number of individuals with and without an ajmaline-induced Type I

Table 1 Regression analysis for PR slope in patients of European ancestry

Variables	All individuals				No *SCN5A* mutation		No *SCN5A* mutation and no Type I BrS ECG	
	Univariable		Multivariable					
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Sex (female)	2.5 (0.9)	4.0×10^{-3}	3.1 (0.9)	2.3×10^{-4}	3.1 (0.9)	2.5×10^{-4}	3.0 (1.0)	2.7×10^{-3}
Age (years)	-0.02 (0.03)	0.41	NA	NA	NA	NA	NA	NA
PRS_{PR}	0.22 (0.08)	3.4×10^{-3}	0.14 (0.08)	6.2×10^{-2}	0.16 (0.08)	3.5×10^{-2}	0.18 (0.09)	5.1×10^{-2}
Baseline PR (ms)	0.08 (0.02)	2.7×10^{-6}	0.08 (0.02)	1.7×10^{-6}	0.07 (0.02)	1.3×10^{-4}	0.07 (0.02)	3.3×10^{-4}
SCN5A mutation	7.9 (2.5)	1.7×10^{-3}	NA	NA	NA	NA	NA	NA

SCN5A mutation status is not included in the multivariable models because of high missing data related to the fact that *SCN5A* sequencing was clinically-driven. Only multivariable analysis results are shown for the subgroups 'no SCN5A mutation' and 'no SCN5A mutation and no Type I BrS'.
β, regression coefficient; NA, not applicable; SE, standard error of the β.

Table 2 Regression analysis for QRS slope in patients of European ancestry

Variables	All individuals				No *SCN5A* mutation		No *SCN5A* mutation and no Type I BrS ECG	
	Univariable		Multivariable					
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Sex (female)	0.69 (0.72)	0.34	NA	NA	NA	NA	NA	NA
Age (years)	0.12 (0.02)	3.9×10^{-7}	0.12 (0.02)	3.2×10^{-7}	0.10 (0.02)	2.2×10^{-6}	0.09 (0.02)	1.8×10^{-4}
PRS_{QRS}	0.80 (0.22)	3.0×10^{-4}	0.80 (0.22)	2.5×10^{-4}	0.93 (0.20)	2.5×10^{-6}	0.58 (0.21)	6.5×10^{-3}
Baseline QRS (ms)	0.05 (0.04)	0.13	NA	NA	NA	NA	NA	NA
SCN5A mutation	21.3 (2.2)	1.6×10^{-18}	NA	NA	NA	NA	NA	NA

SCN5A mutation status is not included in the multivariable models because of high missing data related to the fact that *SCN5A* sequencing was clinically-driven. Only multivariable analysis results are shown for the subgroups 'no *SCN5A* mutation' and 'no *SCN5A* mutation and no Type I BrS'.
β, regression coefficient; NA, not applicable; SE, standard error of the β.

Table 3 Regression analysis for an ajmaline-induced Type I BrS ECG in patients of European ancestry

Variables	All individuals				No *SCN5A* mutation	
	Univariable		Multivariable			
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sex (female)	1.014 (0.963–1.067)	0.58	NA	NA	NA	NA
Age (per 10-year increase)	1.018 (1.001–1.036)	4.8×10^{-2}	1.005 (0.988–1.023)	0.57	NA	NA
PRS_{PR}	1.017 (1.013–1.022)	3.6×10^{-13}	1.002 (0.997–1.007)	0.42	NA	NA
Baseline PR (per 10-ms increase)	1.015 (1.005–1.026)	3.4×10^{-3}	1.005 (0.995–1.016)	0.33	NA	NA
PRS_{QRS}	1.047 (1.031–1.063)	9.3×10^{-9}	1.012 (0.995–1.028)	0.16	NA	NA
Baseline QRS (per 10-ms increase)	1.062 (1.035–1.090)	7.7×10^{-6}	1.032 (1.006–1.059)	1.6×10^{-2}	1.003 (1.000–1.005)	4.3×10^{-2}
PRS_{BrS}	1.174 (1.138–1.210)	3.0×10^{-24}	1.141 (1.101–1.183)	1.3×10^{-12}	1.159 (1.124–1.195)	4.1×10^{-20}
Baseline Type II or III pattern	1.388 (1.289–1.494)	1.2×10^{-17}	1.270 (1.172–1.376)	6.3×10^{-9}	1.296 (1.197–1.403)	2.6×10^{-10}
FHx BrS	1.116 (1.061–1.175)	4.8×10^{-5}	1.113 (1.058–1.171)	3.4×10^{-5}	1.100 (1.046–1.157)	2.3×10^{-4}
SCN5A mutation	1.221 (1.064–1.402)	4.3×10^{-3}	NA	NA	NA	NA

SCN5A mutation status is not included in the multivariable models because of high missing data related to the fact that *SCN5A* sequencing was clinically-driven. Only multivariable analysis results are shown for the subgroup 'no SCN5A mutation'.
FHx BrS, family history of BrS; NA, not applicable; OR (95% CI), odds ratio and 95% confidence interval.

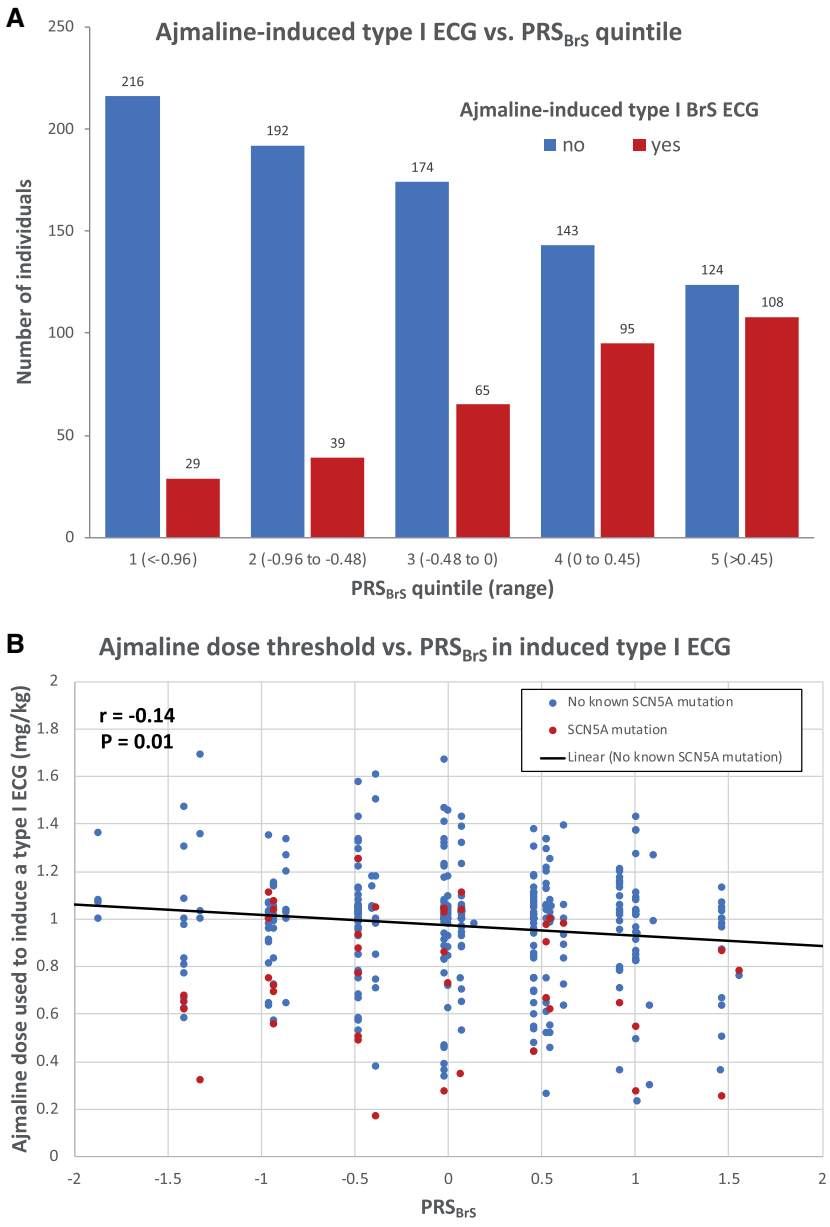


Figure 3 (*A*) Bar plot representing number of individuals per PRS$_{BrS}$ quintile in the cohort without a known *SCN5A* mutation, with (red bars) and without (blue bars) ajmaline-induced Type I Brugada syndrome electrocardiogram. (*B*) Correlation plot of ajmaline dose required to induce a Type I Brugada syndrome electrocardiogram and PRS$_{BrS}$ for *SCN5A* mutation carriers (red markers) and non-carriers (blue markers). Line represents the linear regression in cases without a known *SCN5A* mutation, with the correlation coefficient (*r*) and test *P*-value (*P*) at the top left corner.

BrS ECG per PRS$_{BrS}$ quintile. As for PR and QRS slopes, results from a multivariable model including *SCN5A* mutation status, when available, appear in Supplementary material online, *Table S4*. Of note, in a bivariable interaction model including both *SCN5A* mutation status and PRS$_{BrS}$, both variables were independently associated with Type I BrS ECG with a significant interaction effect ($P = 0.049$), where the BrS risk increasing effect of PRS$_{BrS}$ was non-significant in *SCN5A* mutation carriers.

Ajmaline infusion was associated with the appearance of ventricular ectopy in ~4% of patients. The presence of ajmaline-induced ventricular arrhythmias was significantly associated with the presence of an *SCN5A* pathogenic variant ($P = 0.003$). Furthermore, in the primary analysis of patients with European ancestry, those with induced ventricular arrhythmias tended to have a higher PRS$_{BrS}$ than those without arrhythmias (-0.1 ± 0.7 vs. -0.31 ± 0.8; $P = 0.056$). In a subsequent (non-predefined) analysis where we also included cases of East-Asian ancestry, who have similar effect sizes in the BrS GWAS,[13] the association became significant ($P = 0.049$).

The weight-adjusted dose of ajmaline required to induce a Type I BrS ECG was significantly lower in *SCN5A* mutation carriers

Table 4 PRS_{BrS} diagnostic performance for predicting BrS at different thresholds and at optimal Youden's index

PRS_{BrS} threshold (percentile)	−1.4 (10th)	−0.9 (30th)	−0.4 (50th)	−0.02 (Youden)	0.1 (70th)	0.9 (90th)
Specificity	0.06	0.36	0.57	0.64	0.76	0.95
Sensitivity	0.99	0.85	0.70	0.64	0.49	0.12
Negative predictive value	0.93	0.84	0.81	0.81	0.77	0.71
Positive predictive value	0.32	0.37	0.41	0.42	0.47	0.53

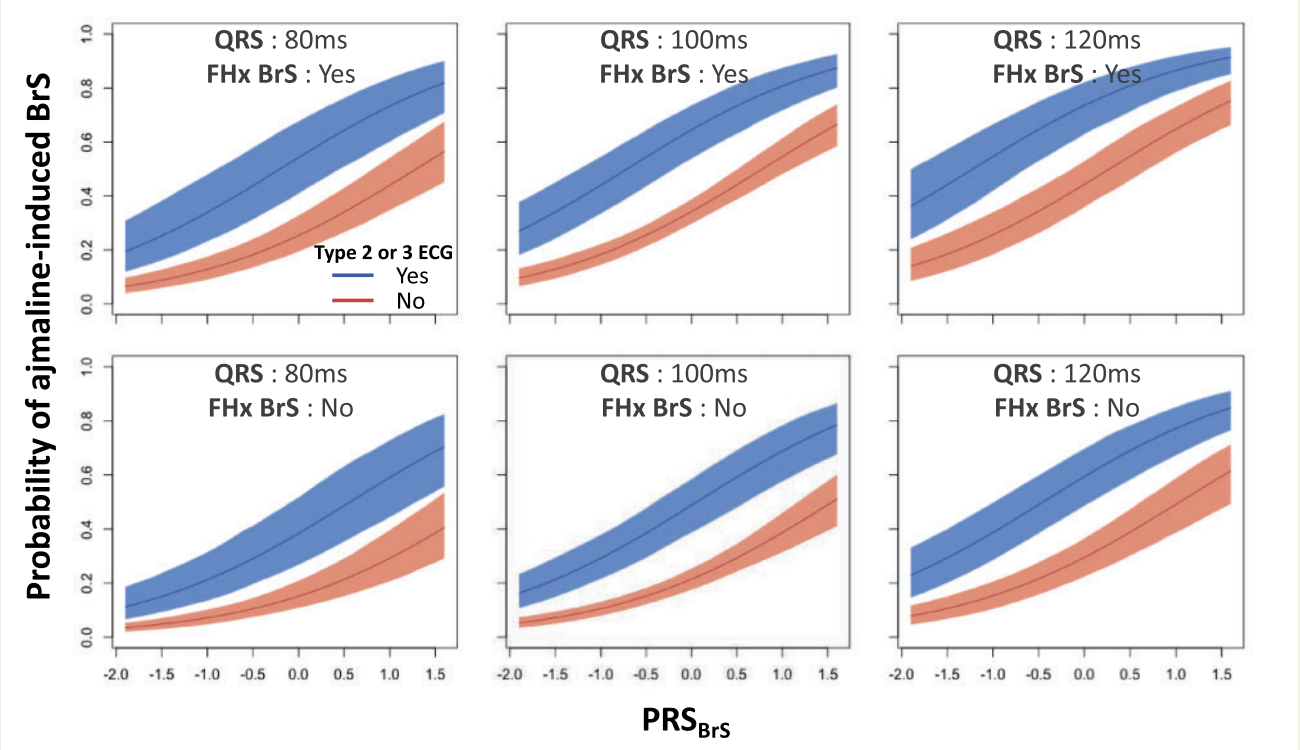


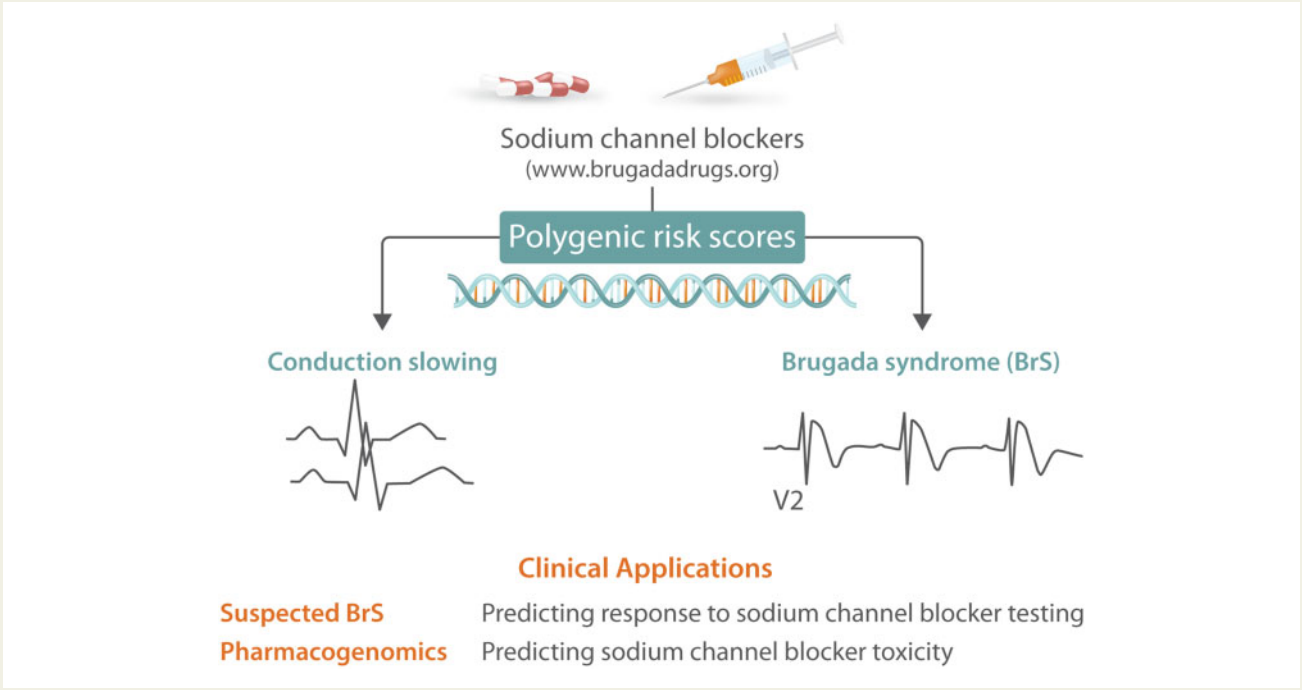
Figure 4 Probability estimate of ajmaline-induced Type I Brugada syndrome electrocardiogram in patients with suspected Brugada syndrome, depending on QRS duration and presence of Type II or III Brugada syndrome electrocardiogram at baseline, family history of Brugada syndrome, as well as PRS_{BrS}. Shaded area represent the 95% confidence interval. $PRS_{BrS} = 0.55 \times$ #rs11708996_C - $0.94 \times$ #rs10428132_G + $0.46 \times$ #rs9388451_C, where #rs11708996_C, #rs10428132_G, and #rs9388451_C indicate the number of respective alleles an individual carries.

(0.76 ± 0.28 mg/kg) than in the others (0.97 ± 0.25 mg/kg, $P < 10^{-5}$). In non-mutation carriers, the PRS_{BrS} was negatively correlated with the weight-adjusted dose of ajmaline required to induce a Type I ECG ($r = -0.14$; $P = 0.01$; *Figure 3B*). This suggests an allelic dose-response where the higher number of BrS associated alleles an individual carries, the more sensitive he is to sodium-channel blockade.

Development and validation of a drug-induced Brugada syndrome risk prediction model

We assessed the predictive value of PRS in ajmaline-induced BrS. Using PRS_{BrS} as a sole predictor (Supplementary material online, *Figure S8A*), the C-statistic was 0.68 [95% confidence interval (CI) 0.65–0.71]. Using the Youden's index, the optimal PRS_{BrS} threshold for predicting an ajmaline-induced Type I BrS ECG was -0.02, corresponding to 64% sensitivity and specificity. Test performance using other PRS_{BrS} thresholds and percentiles is shown in *Table 4*. A PRS_{BrS} threshold at the 90th percentile (+0.91) had 95% specificity for an ajmaline-induced BrS, while a threshold at the 10th percentile (-1.41) provided a sensitivity of 99% to exclude BrS, potentially alleviating the need to perform ajmaline testing in this population, representing ~10% of the studied cohort.

Adding family history of BrS, baseline QRS duration and Type II or III ECG to PRS_{BrS} resulted in a significantly better prediction model [C-statistic 0.741 (95% CI 0.710–0.773); R^2 0.197; likelihood ratio test



Take home figure Figure summarizing the proof of concept that polygenic scores may be used to predict response to sodium-channel blockers in the context of suspected Brugada syndrome and conduction slowing.

$P < 10^{-5}$; Supplementary material online, *Figure S8B*]. Validation of the four-variable model using bootstrapping suggests minimal optimism/overfitting: the optimism-corrected C-statistic is 0.737 and R^2 is 0.188 with a calibration slope of 0.98. As an alternative to bootstrapping, we also derived a prediction model in cases tested prior to 2012 ($N = 380$) and validated it in those tested in and after 2012. Model performance was good, with a C-statistic 0.732, R^2 0.09 and calibration slope 0.86.

To facilitate clinical implementation, *Figure 4* provides the probability estimates of drug-induced BrS based on the validated four-variable prediction model. PRS_{BrS} can be calculated following genotyping of three SNPs (equation in legend of *Figure 4*).

Discussion

Drugs with cardiac sodium-channel blocking properties such as Class I antiarrhythmic drugs, anti-epileptics, and tricyclic antidepressants have been associated with major cardiac adverse events in diverse populations.[3–10] Prediction of response could result in increased use of effective drugs in lower risk patients, while decreasing adverse events through better surveillance and withdrawal in high-risk patients. The standard ECG is used to monitor SCB toxicity by examining conduction parameters, mainly the QRS duration. Cardiovascular societies recommend withdrawal of Class I anti-arrhythmic drugs in the presence of QRS prolongation exceeding ≥25% of the baseline value[24] and avoidance of all SCBs in patients with the Type I BrS ECG, either spontaneously or drug-induced.[12]

The genetic determinants of PR, QRS, and QT intervals have been extensively studied through large-scale GWAS in the general population.[1,19,20] Single-nucleotide polymorphisms associated with QT in the general population are also associated with drug-induced QT prolongation.[2,25] In contrast to drug-induced QT prolongation, the genetic determinants of cardiac response to sodium-channel blockade have not yet been studied. The present study is, to the best of our knowledge, the first to address this question.

Summary of study findings

Novel findings can be summarized as follows: (i) ajmaline-induced PR and QRS changes accurately fit a linear model (Supplementary material online, *Figure S3*); (ii) PRS combining 44 common variants associated with PR in the general population[19] (PRS_{PR}) is associated with ajmaline-induced PR prolongation in addition to *but not independently of* baseline PR and female sex (*Table 1* and *Figure 2C*); (iii) PRS combining 26 common variants associated with QRS in the general population[20] (PRS_{QRS}) as well as age are independently associated with ajmaline-induced QRS prolongation (*Table 2* and *Figure 2D*); (iv) family history of BrS, baseline QRS, presence of a Type II or III BrS at baseline ECG, and a 3-SNP PRS derived from a case–control BrS GWAS[13] (PRS_{BrS}) are independently associated with ajmaline-induced Type I BrS ECG (*Table 3* and *Figure 3A*); (v) a prediction model integrating PRS_{BrS}, baseline ECG, and family history of BrS

performs well to predict the occurrence of ajmaline-induced Type I ECG (*Figure 4*, *Table 4* and Supplementary material online, *Figure S8*).

Mechanistic insights: central role of *SCN5A* in sodium-channel blockade response

SCN5A codes for the α-subunit of the cardiac sodium-channel $Na_v1.5$, the target of Class I antiarrhythmic drugs such as ajmaline. $Na_v1.5$ is also blocked through non-specific binding by drugs directed to other pharmacologic targets.[3,26] *Rare coding* variants in *SCN5A* that alter the amino acid sequence can result in impaired $Na_v1.5$ function or decreased membrane expression, resulting in higher sensitivity to sodium-channel blockade.[3,18,27] *Common non-coding* variants in the *SCN5A–SCN10A* locus are unequivocally associated with BrS as well as electrocardiographic traits in the general population (Supplementary material online, *Table S1*). These variants map to gene regulatory elements and modify cardiac electrophysiology by affecting expression of *SCN5A*.[14] In the present study, we now show that these same variants not only affect the resting ECG but also cardiac electrical response to sodium-channel blockade (Supplementary material online, *Figure S5* and *Table S3*). Although a polygenic score only including the *SCN5A–SCN10A* locus also predicts QRS slope and BrS, SNPs in other loci have an added predictive value (data not shown), suggesting that other loci also affect sodium-blocker sensitivity, perhaps in part by modulating transcription factors (e.g. *TBX5*, *HEY2*).

Limitations

The study subjects were not randomly selected from the general population but had ajmaline infusion for suspected BrS. This cohort was used because of large sample size, availability of raw ECG data and DNA, as well as consistency in drug infusion performed by an experienced physician. Validation of the findings in a general population cohort using other SCBs is desirable. The reproducibility of our QRS slope association results in the subgroup of patients with neither an *SCN5A* mutation nor a Type I BrS ECG is reassuring regarding applicability of the findings to the general population.

In contrast to drug-induced QT prolongation,[2] the proportion of explained variability in ajmaline-induced PR and QRS slopes is low (*Figure 2C and D*). Although this may reflect differences in the genetic component of drug-induced QT prolongation vs. conduction slowing, it may also reflect inter-individual pharmacokinetic variability (e.g. distribution volumes) that are not accounted for in the present study. Although not logistically possible, if drug concentrations were used instead of infused drug dose, PRS may have possibly explained a larger portion of the variability. The statistically robust associations provide a strong proof of concept on which to base future pharmacogenomic studies.

Sequencing of *SCN5A* was performed as clinically relevant (mostly because a Type I BrS ECG occurred during ajmaline testing). As such, *SCN5A* mutation status was known for less than a third of the study population. Extrapolation of PRS associations to the subgroup of patients with *SCN5A* mutations should be made with caution (see Supplementary material online, *Table S4*). Patients with *SCN5A* mutations are sensitive to sodium-channel blockade regardless of their polygenic risk.

Potential clinical applications

The current findings may translate into clinical applications in two settings. First, SNP genotyping may be performed to assess pre-test probability when considering drug testing *in suspected BrS* (*Figure 4*). A BrS diagnostic algorithm integrating SNP genotyping could have several potential advantages compared with current practice: (i) Reduction of test-related adverse events, such as life-threatening arrhythmia (~2% in Conte *et al.*[28]) and ajmaline-induced cholestatic liver injury;[29–31] (ii) Reduction of cost considering the higher expenses of SCB testing (performed in a hospital setting) compared with SNP genotyping; and (iii) Identification of family members at risk of BrS in centres with limited access to drug testing or no access to ajmaline (other drugs have limited sensitivity[32,33]). Prospective studies are needed to assess the predictive values and cost-effectiveness in a real-world setting. It is worth mentioning that sensitivity and specificity of PRS_{BrS} are within the same range as those of some commonly used diagnostic tests, such as exercise electrocardiography to diagnose coronary artery disease.

A second potential application of study findings is pre-emptive genotyping prior to prescription of drugs with cardiac sodium-channel blocking activity. PRS may be used to identify patients at risk of drug toxicity. Although high PRS_{QRS} and PRS_{BrS} may not be sufficiently predictive of adverse events to contraindicate those drugs upstream, it may justify closer patient follow-up using electrocardiography, with drug withdrawal in patients who show evidence of toxicity. The current effect sizes for PRS_{PR} and PRS_{QRS} are modest and it is expected that further understanding of the genetic determinants of response to sodium-channel blockade would improve risk assessment.

Conclusions

PRS are associated with ajmaline-induced cardiac conduction slowing and BrS. The current study provides a strong proof-of-concept in support of an innovative strategy using genotyping of common SNPs in the diagnostic strategy for BrS and possibly in predicting SCB toxicity.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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