Genome-wide association analyses identify new Brugada syndrome risk loci and highlight a new mechanism of sodium channel regulation in disease susceptibility

Genome-wide association meta-analysis identifies novel Brugada syndrome susceptibility loci and highlights a new mechanism of sodium channel regulation in disease susceptibility

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Brugada syndrome is a cardiac arrhythmia disorder associated with sudden death in young adults. With the exception of *SCN5A*, encoding the cardiac sodium channel $Na_V1.5$, susceptibility genes remain largely unknown. Here we performed a genome-wide association meta-analysis comprising 2,820 unrelated cases with Brugada syndrome and 10,001 controls and identified 21 association signals at 12 loci (10 novel). SNP-heritability estimates indicate a strong polygenic influence. Polygenic risk score analyses based on the 21 susceptibility variants demonstrate varying cumulative contribution of common risk alleles among different patient sub-groups, as well as genetic associations with cardiac electrical traits and disorders in the general population. The predominance of cardiac transcription factor loci indicates that transcriptional regulation is a key feature of Brugada syndrome pathogenesis. Furthermore, functional studies conducted on *MAPRE2*, encoding the microtubule plus-end-binding protein EB2, point to microtubule-related trafficking effects on $Na_V1.5$ expression as a novel underlying molecular mechanism. Taken together, these findings broaden our understanding of the genetic architecture of Brugada syndrome and provide new insights into its molecular underpinnings.

Brugada syndrome (BrS) is a cardiac disorder characterized by hallmark ST-segment elevation in the right precordial leads of the electrocardiogram (ECG) and increased risk of sudden death in young adults[1,2]. Rare coding variants in *SCN5A*, encoding the cardiac sodium channel $Na_V1.5$ which underlies the sodium current (I_{Na}), are reported in approximately 20% of cases[3,4]. Other susceptibility genes contributing to the disorder remain largely unknown. In a genome-wide association study (GWAS) conducted in 312 patients with BrS, we previously identified 3 common susceptibility variants and provided evidence for a complex genetic architecture[5]. Here we extended this original association scan to a large meta-analysis comprising 2,820 unrelated cases and 10,001 controls of European ancestry (**Supplementary Table 1**, **Supplementary Table 2**), testing 6,990,521 variants with a minor allele frequency (MAF) ≥0.01 (**Figure 1, Supplementary Fig. 1, Supplementary Fig. 2**). A total of 12 loci (10 novel) reached the genome-wide statistical significance threshold of $P<5x10^{-8}$ (**Table 1; Supplementary Fig. 3, panels a-l**). Conditional analysis uncovered 7 additional association signals at genome-wide significance at the chromosome 3 locus, and an additional signal at the chromosome 6 and the chromosome 7 loci (**Table 1; Supplementary Fig. 3, panels m-u**). Analysis of SNP-based heritability (h^2_{SNP}) demonstrated that a substantial portion of susceptibility to BrS is attributable to common genetic variation. h^2_{SNP} estimates ranged from 0.17 (standard error, SE, 0.035) using LDSC[6] to 0.34 (SE 0.02) using GREML[7], assuming a disease prevalence of 0.05%[8], with 24% of the total SNP-based heritability being explained by the 12 loci reaching genome-wide significance (**Supplementary Table 4**).

Seven association signals (defined by the lead SNP and SNPs with $r^2 \geq 0.6$) at the chromosome 3 locus overlapped *SCN5A* and one overlapped the neighboring *SCN10A* gene encoding the sodium channel isoform $Na_V1.8$ (**Supplementary Fig. 4, panels a-h**). While previous work[9] proposed that the latter signal may act through regulation of *SCN5A* expression, a possible involvement of *SCN10A* itself is suggested by a significant eQTL in left ventricular tissue ($P=5.29x10^{-6}$, colocalization posterior probability (CLPP) = 0.16) (**Supplementary Fig. 4, panel h, Supplementary Table 3**), whereas no eQTL was detected for *SCN5A* (P=0.27). Notably, 6 association signals overlapped genes encoding cardiac

developmental transcription factors (*HEY2, TBX20, ZFPM2, GATA4, WT1, TBX5*) and 4 were <300kb from such genes (*TBX20*, *IRX3/IRX5, HEY2*)[10]. In support for the involvement of transcription factor genes, an enrichment in genes encoding DNA binding proteins was found at BrS GWAS loci by permutation testing (one-tailed permutation $P = 1 \times 10^{-4}$; **Supplementary Fig. 5**). The transcription factors HEY2, TBX20, GATA4, TBX5 and IRX3/IRX5 are established regulators of ion channel expression in the adult heart, including that of $Na_V1.5$[11–15], suggesting that modulation of ion channel expression is an important mechanism in BrS. Potential regulatory effects of the transcription factors WT1 and ZFPM2 on ion channel expression have not yet been investigated. One association signal overlapped *PRKCA* (supported by a co-localizing eQTL (P=4.63 $\times 10^{-28}$, CLPP = 0.99); (**Supplementary Fig. 4, panel s, Supplementary Table 3**), which encodes protein kinase C alpha involved in contractility and calcium handling in cardiomyocytes[16]. Lastly, two association signals overlapped genes encoding microtubule or myofiber associated proteins, namely *MAPRE2*[17] and *MYO18B*[18]. A full annotation of the association signals (see Online Methods) is presented in **Supplementary Table 3 and Supplementary Fig. 4**.

We performed a transcriptome-wide analysis (TWAS)[19] based on predicted gene expression in cardiac tissues[20] and identified 24 associations corresponding to 20 unique genes at the Bonferroni-corrected threshold of P<5.2x10^{-6} (**Supplementary Table 5**). Eighteen of these genes are within ≈0.5 Mb of GWAS signals while two point to additional loci (**Supplementary Table 5**). MAGMA gene property analysis for tissue specificity[21] as well as enrichment analysis using LDSC-SEQ[22] and GARFIELD[23] identified left ventricle, right ventricle and fetal heart, respectively, as significantly associated with BrS (**Supplementary Fig. 6 and 7, Supplementary Tables 6 and 7**). MAGMA gene-set analysis[21] identified, amongst others, gene sets related to heart development and regulation of heart growth (**Supplementary Table 8**), which may point to a broader role of transcriptional dysregulation in the pathogenesis of BrS, beyond regulation of ion channel expression.

MAPRE2 overlaps the association signal tagged by rs476348 and its causal role is supported by chromatin interaction between its promoter region and the association signal and by a significant eQTL (*P*=2.9x10^{-5}, CLPP=0.10) **Supplementary Fig. 4, panel t, Supplementary Table 3**), where the BrS risk allele is associated with lower *MAPRE2* expression in left ventricular tissue compared to the non-risk allele. *MAPRE2* encodes the microtubule plus-end binding protein EB2, a regulator of microtubule organization[17]. While effects on transcription factor expression and ion-channel patterning are established molecular mechanisms associated with BrS susceptibility[5,13], mechanisms involving microtubule function and ion channel trafficking, as suggested by the association signal near *MAPRE2*, have not yet been explored. We therefore generated loss-of-function mutants (KO) using CRISPR/Cas9 in both zebrafish (**Supplementary Fig. 8**) and human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) (**Supplementary Fig. 9**) to study the role of *MAPRE2* in cardiac electrophysiology. Using optical mapping, we observed a significantly lower conduction velocity and action potential upstroke velocity (V_{max}) in zebrafish hearts isolated from *mapre2* KO compared to control (CTRL) larvae (**Fig. 2a,b**). Similarly, V_{max} observed in single *MAPRE2* KO hiPSC-CMs was lower than isogenic control hiPSC-CMs measured using manual patch clamp (**Fig. 2d,e**). The lower V_{max} observed in both mutant zebrafish and hiPSC-CMs suggested lower I_{Na}. This was confirmed by automated patch-clamp measurements which demonstrated ≈50% less I_{Na} density in *MAPRE2* KO compared to

control hiPSC-CMs (**Fig. 2f**, left panel). Additionally, a small positive shift in voltage dependency of activation was observed, while voltage dependency of inactivation and recovery from inactivation were not different between control and KO cells (**Supplementary Fig. 10a,b,c**). Whereas no repolarization abnormalities were observed in intact *mapre2* KO zebrafish hearts (**Fig. 2c**), significant action potential duration (APD) prolongation was observed in single *MAPRE2* KO hiPSC-CMs (**Fig. 2d and e**). This APD prolongation may be explained by the significantly lower repolarizing outward current ($I_{outward}$) amplitude in the KO hiPSC-CMs (**Fig. 2f**, right panel), although the voltage-dependency of activation was unchanged (**Supplementary Fig. 10d,e**). Together with the multiple levels of evidence that implicate conduction slowing and decreased I_{Na} in the pathogenesis of BrS, and previous work linking end-binding proteins to ion channel targeting to the plasma membrane[24], our data suggest that modulation of microtubule function and subsequent alterations in ion channel trafficking may be a novel molecular mechanism contributing to BrS. Future work is needed to address the underlying molecular mechanisms and provide insight into the ion channels that underlie the observed abnormalities in repolarization, although a role for prolonged repolarization is not reconcilable with current hypotheses on BrS pathogenesis [25].

To further explore the genetic architecture of BrS in specific patient subgroups as well as the association of common variants in aggregate with disease severity, we calculated a polygenic risk score (PRS_{BrS}) per individual based on the 21 risk alleles and their corresponding effect sizes. Of the 2,469 study participants tested, 454 (18.4%) carried a rare pathogenic or likely pathogenic variant in *SCN5A* (*SCN5A*$^+$). *SCN5A*$^+$ cases had a lower mean PRS_{BrS} compared to cases without such variants (*SCN5A*$^-$) (8.8±1.1 vs. 9.3±1.0; P=2.1x10^{-17}; **Fig. 3a**), suggesting a higher burden of BrS-associated common variants in *SCN5A*$^-$ patients, as similarly shown in other heritable diseases[26,27]. Using LDSC, we observed a strong genome-wide correlation between the genetic contributors in *SCN5A*$^+$ and *SCN5A*$^-$ patient subgroups (r_g=0.82; SE=0.2), suggesting the involvement of the same risk alleles. Out of 2,367 BrS cases with complete data, 228 had a life-threatening arrhythmic event (LAE) at diagnosis or during follow-up (median age at last follow-up was 50.0 years, interquartile range 39.5 - 60.7). Although *SCN5A*$^+$ cases had a higher risk for LAE compared to *SCN5A*$^-$ cases (HR 1.87; 95% CI 1.37-2.55; P=8.1x10^{-5}; **Supplementary Table 9**), PRS_{BrS} was not significantly associated with LAE in BrS cases (P=0.30, **Supplementary Fig. 11**). On the other hand, PRS_{BrS} was significantly higher in BrS cases that presented with a spontaneous type 1 BrS ECG compared to those with a type 1 BrS ECG after sodium channel blocker challenge (9.3±1.1 vs. 9.1±1.1 P=1.7x10^{-5}; **Fig. 3b**), an effect that seemed more pronounced in the subgroup of *SCN5A*$^-$ cases (9.2±1.0 vs. 9.5±1.1; P=3.5x10^{-8}; **Supplementary Fig. 12**). These data support the concept that disease susceptibility in different individuals relies upon varying contributions of multiple factors including both rare and common genetic variations and exposure to sodium channel blockade.

To explore the genetic relationship of BrS with other traits, we performed a phenome-wide association study (PheWAS) in the UK Biobank using PRS_{BrS}, applying Bonferroni correction ($P<7x10^{-4}$) to define statistical significance (**Supplementary Tables 10-12** and **Fig. 4A**). PRS_{BrS} was associated with greater risk for atrioventricular conduction disorders (P=1.5x10^{-9}; OR=1.16 [1.10-1.21] per SD increase), as well as longer ECG activation/conduction times reflected in the P-wave duration (P=5.3x10^{-9}; β=0.76 ms, SE=0.13), PQ interval duration (P=1.9x10^{-45}; β=2.70 ms, SE=0.19), and QRS duration (P=4.2x10^{-55}; β=1.23 ms, SE=0.08). This

underscores the important role of conduction slowing in the pathogenesis of BrS, and is further supported by a significant positive genome-wide correlation between BrS and QRS duration[28] (r_g=0.44, P=1x10^{-8}; **Supplementary Table 13**). In contrast, PRS_{BrS} was negatively associated with the QT interval duration (P=4.8x10^{-16}; β=-1.56 ms, SE=0.19), consistent with suggestions of higher cardiomyocyte phase 1 repolarizing drive in BrS[13,25]. PRS_{BrS} was also negatively associated with the occurrence of atrial fibrillation (AF) or flutter (P=6.2x10^{-13}; OR=0.94 [0.92-0.95]). The effects of each of the 21 BrS risk alleles in previously published GWAS of PQ[29], QRS[28], QT[30] and AF[31] are generally concordant with the aggregate effect of those alleles (PRS_{BrS}) in the PheWAS (**Fig. 4B**, **Supplementary Table 14-17, Supplementary Fig. 13**). One exception is the BrS risk allele near *MYO18B* (rs133902-T) which was also associated with greater risk for AF (P=9x10^{-10} in Nielsen et al[32], and P=1x10^{-7} in Roselli et al[31]; **Supplementary Fig. 13**). This suggests that although changes in conduction velocity through sodium channel expression effects modulate risk for AF and BrS in opposite directions, some disease mechanisms such as those involving structural proteins (e.g. MYO18B) may be shared in both arrhythmias, with concordant effects. We also observed novel associations of PRS_{BrS} with non-electrical phenotypes namely body mass index (log-transformed; P=6.2x10^{-6}; β= 0.0012, SE=0.0003) and systolic blood pressure (P=4.3x10^{-5}; β=0.12 mmHg, SE=0.03; **Supplementary Table 12**). Of note, a recent study identified a modulatory effect of hypertension in cardiac sodium channel disease[33]. Lastly, a lookup of loci previously associated with ECG traits and AF identified 9 additional novel loci associated with BrS at a Bonferroni-corrected P<1.9x10^{-4} (**Supplementary Table 18**).

In conclusion, several important findings emerge from this work: (1) We identified a total of 12 loci, of which 10 novel, associated with BrS, a rare disease and a significant cause of sudden cardiac death in young adults. Of these loci, 3 harbour multiple association signals. (2) The 8 independent association signals at the *SCN5A-SCN10A* locus highlight the primacy of reduced sodium channel function in BrS susceptibility, whereas the 8 loci harboring cardiac transcription factor genes point to transcriptional regulation as a key feature of BrS pathogenesis. (3) Functional studies of *MAPRE2* support a novel mechanism of $Na_V1.5$ modulation via the microtubule network in BrS pathogenesis. (4) Analyses using the UK Biobank highlight a genetic overlap between the BrS and cardiac electrical traits and common disorders in the general population. (5) Polygenic risk score analyses support the concept that disease threshold in different individuals with BrS is reached by varying contributions of rare *SCN5A* variants, common risk alleles and sodium channel blockade. Taken together, these findings broaden our understanding of the genetic architecture of BrS and provide new insights into its molecular underpinnings.

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548 **Table 1: Lead SNPs and effect estimates for genome-wide significant association signals ($P<5x10^{-8}$) in the BrS GWAS meta-analysis**

Locus	Lead SNP	Genomic position (hg19)	Risk allele	Other allele	Risk allele frequency in cases	Risk allele frequency in controls	OR [95% CI]	P value	Nearest gene
1	rs7638909*	3:38594973	G	T	0.32	0.24	1.28 [1.17 - 1.40]	2.79E-08	*SCN5A*
	rs62241190*	3:38607468	G	A	0.06	0.03	1.96[1.63 - 2.32]	8.56E-14	*SCN5A*
	rs7374540*	3:38634142	C	A	0.51	0.39	1.72 [1.61 - 1.81]	3.56E-57	*SCN5A*
	rs7433206*	3:38657708	A	T	0.45	0.42	1.48 [1.37 - 1.60]	9.52E-24	*SCN5A*
	rs34760424*	3:38683018	G	T	0.98	0.94	2.32 [1.96 - 2.70]	3.03E-23	*SCN5A*
	rs41310232*	3:38689242	A	G	0.16	0.09	1.56 [1.40 - 1.74]	1.19E-15	*SCN5A*
	rs6782237*	3:38696553	C	G	0.78	0.68	1.74 [1.61 - 1.87]	1.05E-47	*SCN5A*
	rs6801957	3:38767315	T	C	0.65	0.42	2.49 [2.34 - 2.65]	1.30E-180	*SCN10A*
2	rs6913204*	6:125664540	C	T	0.51	0.47	1.22 [1.13 - 1.29]	1.30E-08	*HDDC2*
	rs9398791	6:126115821	C	T	0.61	0.51	1.53 [1.44 - 1.63]	1.49E-39	*HEY2, NCOA7*
3	rs11765936	7:35349146	G	T	0.18	0.15	1.37 [1.25 - 1.49]	4.30E-11	*TBX20*
	rs340398*	7:35413788	C	T	0.42	0.38	1.22 [1.15 - 1.30]	1.76E-09	*TBX20*
4	rs804281	8:11611865	G	A	0.63	0.58	1.22 [1.15 - 1.30]	1.22E-09	*GATA4*
5	rs72671655	8:106347897	T	A	0.97	0.95	1.85 [1.59 - 2.22]	2.51E-13	*ZFPM2*
6	rs72905083	11:32474374	A	G	0.1	0.08	1.43 [1.27 - 1.60]	2.09E-09	*WT1*
7	rs883079	12:114793240	C	T	0.34	0.28	1.25 [1.16 - 1.33]	1.59E-10	*TBX5*
8	rs11645463	16:54456353	A	G	0.59	0.54	1.22 [1.15 - 1.30]	1.27E-09	*IRX3*
9	rs72622262	16:54662944	C	G	0.87	0.83	1.36 [1.25 - 1.49]	1.37E-11	*CRNDE, IRX5*
10	rs12945884	17:64300281	T	C	0.58	0.53	1.2 [1.12 - 1.28]	3.31E-08	*PRKCA*
11	rs476348	18:32670021	C	T	0.73	0.69	1.25 [1.16 - 1.33]	2.64E-09	*MAPRE2*
12	rs133902	22:26164079	T	C	0.48	0.43	1.21 [1.13 - 1.29]	7.73E-09	*MYO18B*

549 *Variants associated with BrS in conditional analyses. **Abbreviations:** 95% CI, 95% confidence interval; OR, odds ratio referring to each unit increase in the risk
550 allele. Confidence intervals are given for a nominal p-value of 0.05 in order to allow comparability with other studies and reports.

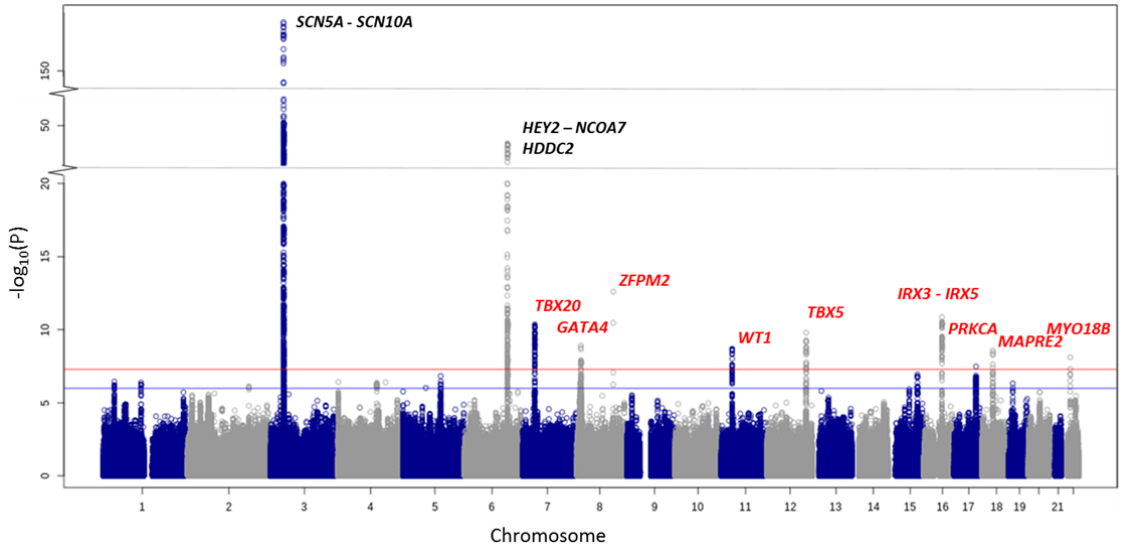

Fig 1: Manhattan plot of genome-wide association meta-analysis comprising 2820 unrelated Brugada Syndrome cases and 10001 controls. The association P values were derived from a meta-analysis of the 10 GWAS strata using a fixed effects model with an inverse-variance weighted approach. The y-axis has breaks to emphasize the novel loci. The red and blue lines indicate the genome-wide significance ($P<5x10^{-8}$) and suggestive significance ($P<1x10^{-6}$) thresholds, respectively. Genes at novel loci are depicted in red.

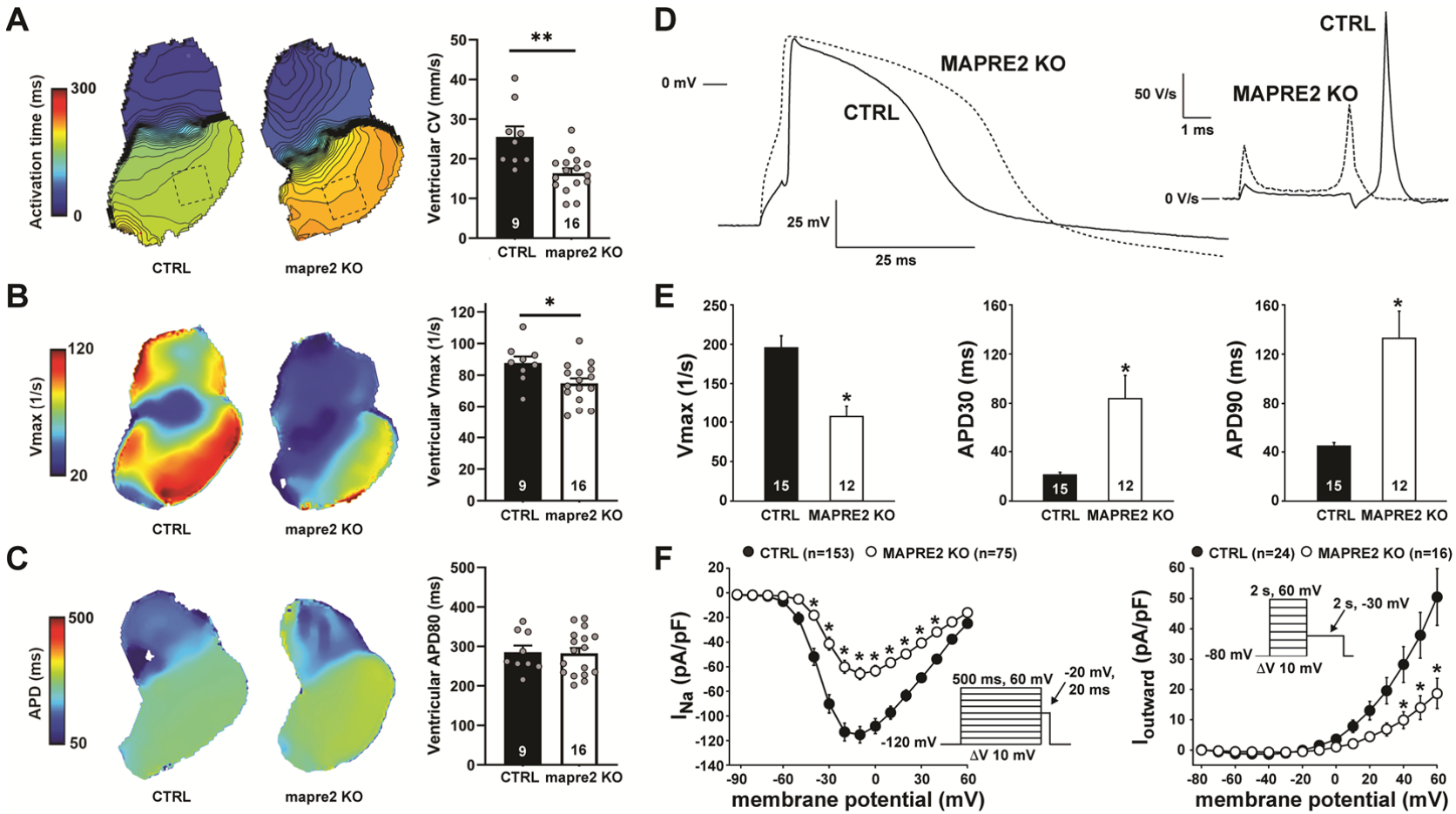

Fig 2: Loss of *MAPRE2* leads to lower conduction velocity, action potential upstroke velocity and sodium current. (a) Left panel. Representative isochrone maps of hearts isolated from 5 day post-fertilization zebrafish larvae injected with tracrRNA/Cas9 and multiple gRNAs targeting *mapre2* (mapre2 KO) or tracrRNA/Cas9 without gRNA (CTRL). The dotted squares reflect the main ventricular area in the hearts from which the various parameters are measured. **Right panel**. Average ventricular conduction velocity (CV) in CTRL and *mapre2* KO hearts. **(b) Left panel.** Representative maximum action potential (AP) upstroke velocity (V_{max}) maps from zebrafish hearts. **Right panel.**

Average V_{max} in CTRL and *mapre2* KO hearts. **(c) Left panel.** Representative maps of AP duration at 80% repolarization (APD80) in isolated hearts paced at 100 bpm. **Right panel.** Average APD80 in CTRL and mapre2 KO hearts. **(d)** Representative APs at 1 Hz pacing from single human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) with CRISPR/Cas9–mediated *MAPRE2* knockout and isogenic control (CTRL) hiPSC-CMs. A constant ohmic current was injected to set the membrane potential just before the APs at approximately -80 mV to overcome the depolarized state of the hiPSC-CMs (see Online Methods). **Inset**. First derivative of the AP upstroke velocity (Vmax). **(e)** Average Vmax and APD at 30 and 90% repolarization (APD_{30} and APD_{90}. respectively) in CTRL and MAPRE2 KO hiPSC-CMs. Maximal diastolic potential and AP amplitude did not differ significantly between CTRL and MAPRE2 KO hiPSC-CMs (data not shown) **(f) Left panel.** Average current-voltage relationships of the sodium current (I_{Na}). **Right panel.** Average repolarizing outward current ($I_{outward}$) in CTRL and MAPRE2 KO hiPSC-CMs. **Insets.** Voltage protocol used. Results are expressed as mean ± s.e.m. Numbers in the bar graph refer to the number of hearts or cells studied. * P <0.05, ** P <0.01 vs. CTRL.

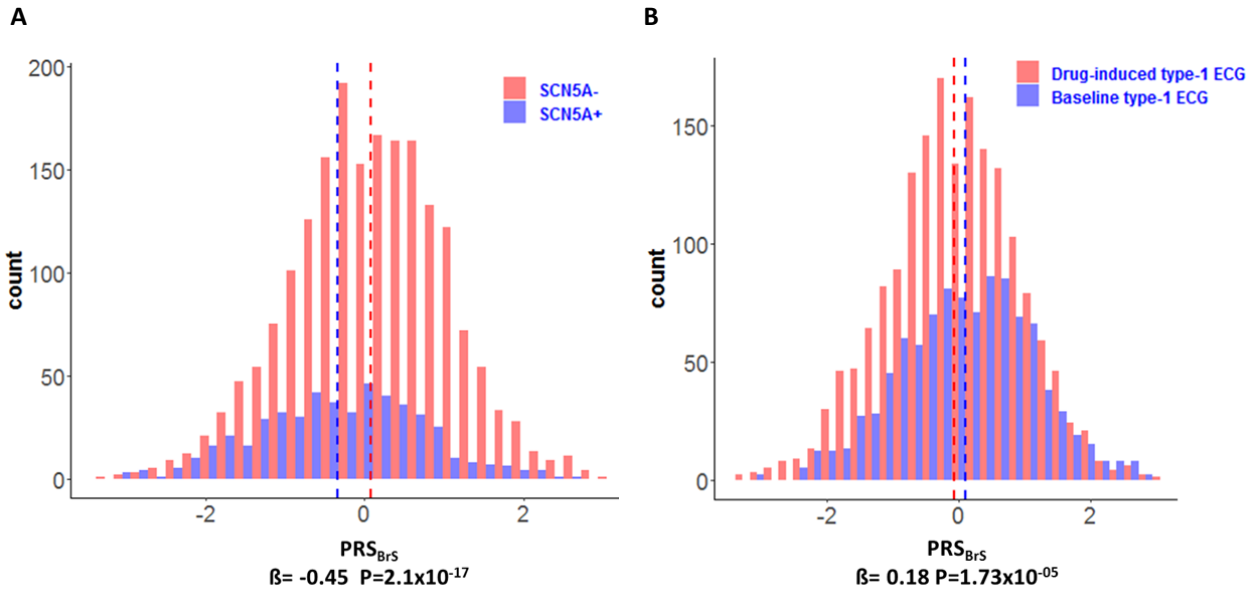

Fig 3: Distribution of PRS_{BrS} in specific patient sub-groups. (A) Histograms displaying PRS_{BrS} distribution in BrS cases carrying a rare pathogenic or likely-pathogenic variant in *SCN5A* (*SCN5A*$^{+}$; blue) compared to BrS cases without such variants (*SCN5A*$^{-}$; red). **(B)** Histograms displaying PRS_{BrS} distribution in BrS cases presenting with a spontaneous type 1 BrS ECG (blue) compared with those presenting with a type 1 BrS ECG only after sodium channel blocker challenge (drug-induced; red). PRS_{BrS} was calculated per individual based on the 21 BrS risk alleles and their corresponding effect sizes. Reported *P* values refer to the difference in PRS_{BrS} units between two groups. Dashed lines showing the mean PRS_{BrS} for each group.

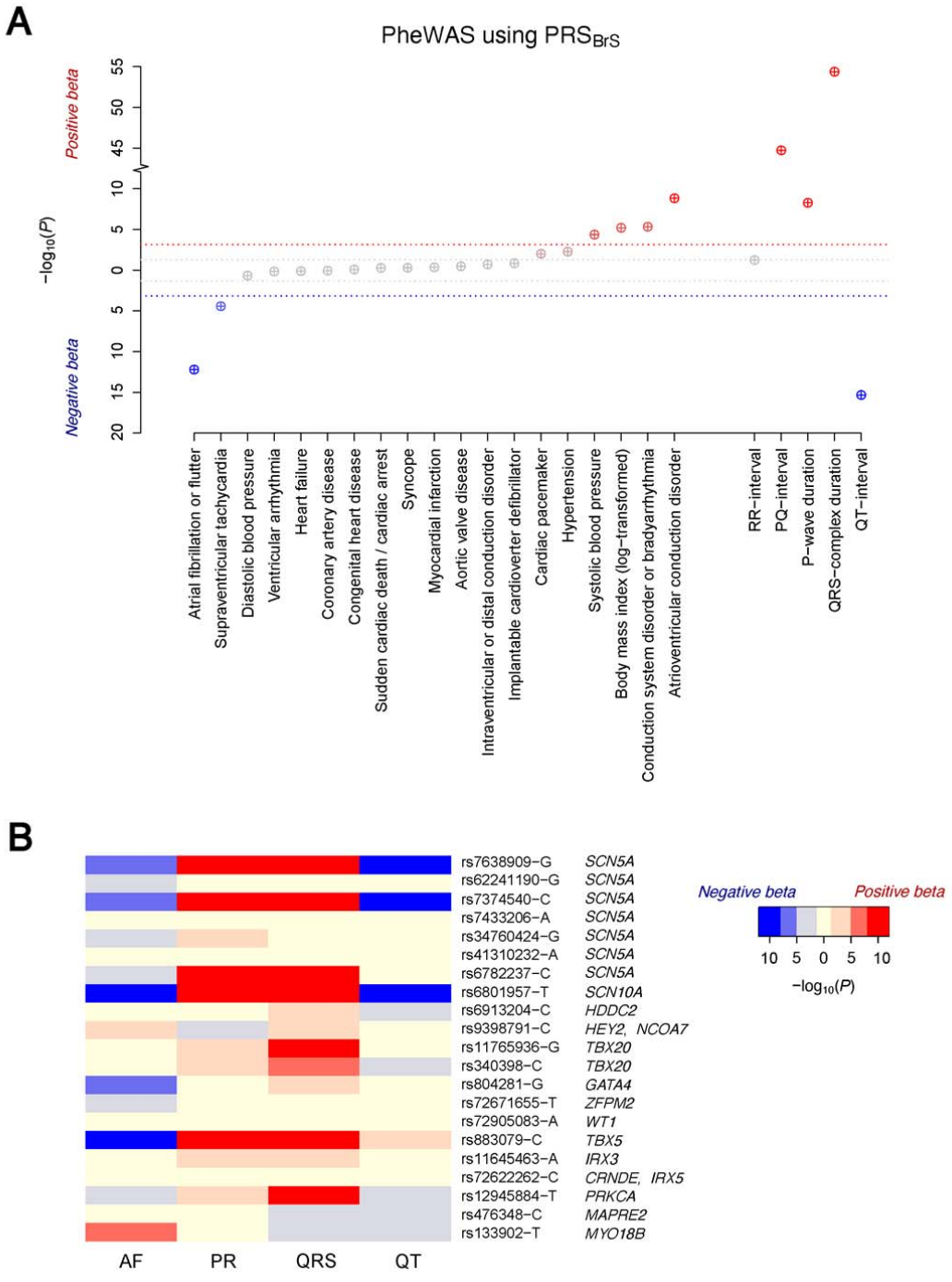

Fig 4. Associations between polygenic susceptibility to Brugada syndrome and common cardiovascular diseases and traits. Panel A shows the results of the phenome-wide association analysis (PheWAS) for the Brugada syndrome (BrS) polygenic risk score (PRS_{BrS}) among individuals of European ancestry from the UK Biobank. Phenotypes significantly associated with PRS_{BrS} and phenotypes relevant to the heart are shown on the x-axis (5 electrocardiographic traits are depicted on the right of the plot); the P values from multiple regression are depicted on the y-axis. Red circles indicate that polygenic predisposition to BrS is associated with a positive beta (e.g. increased risk of the condition or higher value for continuous traits), whereas blue circles indicate that polygenic predisposition to BrS is associated with a negative beta (e.g. decreased risk of the condition or lower

value). We set the significance threshold to $P < 0.0007$ after Bonferroni correction ($P < 0.05/70$), shown as dotted colored lines. The grey dotted lines indicate the nominal significance threshold ($P < 0.05$). The complete PheWAS results are shown in **Supplementary Tables 11 and 12** for dichotomous and continuous traits, respectively. **Panel B** depicts a heat-map of associations between BrS risk alleles and atrial fibrillation/flutter (AF), PR-interval (PR), QRS-complex duration (QRS) and QT interval duration (QT) from previously published GWAS[28–31]. Each row represents an independent BrS risk allele, while each column represents a phenotype. Red indicates that the BrS risk allele (or a proxy with $R^2 > 0.8$) is associated with higher risk of AF or prolongation of the electrocardiographic interval; blue indicates that the BrS risk increasing allele is associated with lower risk of AF or shortening of the interval. The darkest red and blue colors represent conventional genome-wide significance in the published GWAS ($P < 5 \times 10^{-8}$).