Circulation
Cardiovascular Genetics
JOURNAL OF THE AMERICAN HEART ASSOCIATION
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Circ Cardiovasc Genet 2012;5;630-638; originally published online November 8, 2012;
DOI: 10.1161/CIRCGENETICS.112.962852
Circulation: Cardiovascular Genetics is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 1942-325X. Online ISSN: 1942-3268

Integration of Genetics into a Systems Model of Electrocardiographic Traits Using HumanCVD BeadChip

Tom R. Gaunt, PhD*; Sonia Shah, MSc*; Christopher P. Nelson, PhD*; Fotios Drenos, PhD; Peter S. Braund, MSc; Ismail Adeniran, MSc; Lasse Folkersen, PhD; Debbie A. Lawlor, PhD; Juan-Pablo Casas, PhD; Antoinette Amuzu, MA; Mika Kivimaki, PhD; John Whittaker, PhD; Per Eriksson, PhD; Henggui Zhang, PhD; Jules C. Hancox, FSB; Maciej Tomaszewski, MD; Paul R. Burton, FFPH; Martin D. Tobin, FFPH; Steve E. Humphries, FRCPath; Philippa J. Talmud, PhD; Peter W. Macfarlane, DSc*; Aroon D. Hingorani, PhD*; Nilesh J. Samani, FRCP*; Meena Kumari, PhD*; Ian N.M. Day, FRCPath*

Background—Electrocardiographic traits are important, substantially heritable determinants of risk of arrhythmias and sudden cardiac death.

Methods and Results—In this study, 3 population-based cohorts (n=10 526) genotyped with the Illumina HumanCVD Beadchip and 4 quantitative electrocardiographic traits (PR interval, QRS axis, QRS duration, and QTc interval) were evaluated for single-nucleotide polymorphism associations. Six gene regions contained single nucleotide polymorphisms associated with these traits at $P<10^{-6}$, including *SCN5A* (PR interval and QRS duration), *CAV1-CAV2* locus (PR interval), *CDKN1A* (QRS duration), *NOS1AP*, *KCNH2,* and *KCNQ1* (QTc interval). Expression quantitative trait loci analyses of top associated single-nucleotide polymorphisms were undertaken in human heart and aortic tissues. *NOS1AP*, *SCN5A, IGFBP3, CYP2C9,* and *CAV1* showed evidence of differential allelic expression. We modeled the effects of ion channel activity on electrocardiographic parameters, estimating the change in gene expression that would account for our observed associations, thus relating epidemiological observations and expression quantitative trait loci data to a systems model of the ECG.

Conclusions—These association results replicate and refine the mapping of previous genome-wide association study findings for electrocardiographic traits, while the expression analysis and modeling approaches offer supporting evidence for a functional role of some of these loci in cardiac excitation/conduction. **(*Circ Cardiovasc Genet*. 2012;5:630-638.)**

Key Words: arrhythmia ■ conduction ■ electrocardiography ■ genetic association ■ QT interval electrocardiography

Standard measurement methods from the resting ECG trace define quantitative ECG measurements PR interval, QRS axis, QRS duration, and QTc interval (QT interval corrected for heart rate) that predict cardiovascular morbidity and mortality.[1–3] These traits predict risk of arrhythmias and sudden death, particularly where there is prolongation of QTc or of QRS durations.[4] PR interval, QRS axis, QRS duration, and QTc interval reflect a system of anatomically compartmented electrophysiological events and characteristics underpinned by voltage-gated ion channels, together acting in a cyclical fashion. Cardiac P wave represents atrial depolarization, QRS complex represents ventricular depolarization, and QTc interval represents ventricular repolarization. Some of these electrocardiographic traits have a significant genetic basis,[5] with a wide diversity of rare mutations in more than 20 genes known to have monogenic effects on these traits and predispose to arrhythmia (online-only Data Supplement Table I).

Clinical Perspective on p 638

Received February 8, 2012; accepted October 22, 2012.

From the MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine (T.R.G., D.A.L., I.N.M.D.) and Cardiovascular Research Laboratories, School of Physiology and Pharmacology (J.C.H.), University of Bristol, Bristol, UK; UCL Genetics Institute, Department of Genetics, Environment and Evolution (S.S., S.E.H.), Centre for Cardiovascular Genetics, Institute of Cardiovascular Science (F.D., S.E.H., P.J.T.), Department of Epidemiology and Public Health (J.-P.C.), Genetic Epidemiology Group, Department of Epidemiology and Public Health (M.K., A.D.H., M.K.), and Centre for Clinical Pharmacology, Department of Medicine (A.D.H.), University College London, London, UK; Department of Cardiovascular Sciences (C.P.N., P.S.B., M.T., N.J.S.) and Department of Health Sciences (P.R.B., M.D.T.), University of Leicester, Glenfield Hospital, Leicester, UK; NIHR Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, UK (C.P.N., N.J.S.); Biological Physics Group, School of Physics and Astronomy, University of Manchester, Manchester, UK (I.A., H.Z.); Atherosclerosis Research Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden (L.F., P.E.); Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London (J.-P.C., A.A., J.W.); Quantitative Sciences, GlaxoSmithKline, Stevenage, UK (J.W.); and Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK (P.W.M.).

The online-only Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.112.962852/-/DC1.

*These authors contributed equally to this work.

Correspondence to Ian N.M. Day, FRCPath, MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Bristol, UK. E-mail: ian.day@bristol.ac.uk

Less is understood of the contributions of common genetic variations that underpin population variation in these electrocardiographic traits. Recent genome-wide association studies (GWAS) have identified associated genomic regions, although the causal variants (or even genes) usually remain unknown from these analyses. The most widely replicated gene from these studies is NO synthase 1 (neuronal) adaptor protein (*NOS1AP*),[6–10] but the identity and number of functional single-nucleotide polymorphisms (SNPs) in this gene remain unresolved. Following GWAS with SNP replication, it is proposed that gene-centric SNP panels that more densely capture the genetic architecture for specific loci, as well as representing important candidate genes, may help to refine association signals within candidate genes and also permit follow-up in a wider variety of cohorts and traits.[11] For cardiovascular risk genes, the Illumina HumanCVD BeadChip was constructed for this purpose.[11] In this study, we describe a meta-analysis of PR interval, QRS axis, QRS duration, and QTc interval using HumanCVD BeadChip data for 3 cohorts (n=10526). For each of the main loci identified, we model the genotypic difference in gene expression that would be consistent with the phenotypic effects observed in our analysis. Ultimately, a systems model should be able to integrate genotypic, expression, conductance, electrophysiological, and clinical data.

Materials and Methods

Study Cohorts

Association analyses were carried out using 10526 participants from 3 population-based cohorts: British Women's Heart and Health Study (BWHHS), Genetic Regulation of Arterial Pressure of Humans in the Community (GRAPHIC), and the Whitehall II Study. Full details of these cohorts and collection of ECG data and blood samples for genetic analyses are presented in the online-only Data Supplement Materials and Methods.

Electrocardiographic Measures

Standard 12 lead ECGs were recorded on either Siemens 460 electrocardiographs (Camberley, Surrey, UK) or Burdick Eclipse (Manchester, UK) or Atria (Manchester, UK) models. Digital ECG data were transferred to the University of Glasgow ECG Core Lab at Glasgow Royal Infirmary and analyzed by the University of Glasgow ECG analysis program.[12,13] This software meets all of the required specifications in terms of measurement accuracy and is used widely in various commercial products. All ECGs were reviewed manually, and technically unsatisfactory ECGs were excluded (eg, reversed limb lead connections, excessive artifact, etc). The measurements used in this study were: PR interval, QRS axis, QRS duration, and QTc interval (corrected for heart rate using the Hodges equation: QTc=QT+1.75×[RATE−60] ms).[14] Outliers >3 SDs from the mean in each study were excluded from the analysis.

Genotyping and Quality Control

All 3 cohorts used the Illumina HumanCVD BeadArray (Illumina Inc, San Diego, CA), which comprises ≈50000 SNPs in ≈2100 genes systematically selected as cardiovascular disease candidates by an international consortium.[11] Genotypes were called using Illumina BeadStudio (v3) Genotyping Module (based on GenCall application, which incorporates the GenTrain clustering algorithm). Samples with genotype call rate <90% were removed from the analysis. SNPs were included in the association analysis if they satisfied the following criteria: SNP call rate ≥0.95, minor allele frequency >1%, and Hardy-Weinberg Equilibrium $P>1\times10^{-4}$. Based on HumanCVD principal components analysis, there is no evidence for significant population stratification in these 3 cohorts (this finding is consistent with self-reported ancestry).

SNP Association Analyses

Separate within-cohort linear regression analyses (n=3 cohorts) were performed for each trait using an additive genetic model relating the trait to genotype dosage (0–2 copies of the minor allele) for each SNP, adjusting for age, sex, body mass index, and systolic blood pressure (after correction for antihypertensive medication).[15] Covariates were selected based on prior knowledge of physiological measurements affecting the ECG. In GRAPHIC, additional adjustment for age (using age^2 because of the 2 generational structure of the cohort) and familial correlation (using Generalized Estimating Equations with an exchangeable correlation structure) was made to take into account the family structure. After verifying strand alignment, a meta-analysis of the results from the 3 studies was conducted using a fixed-effects model. A comparison with a random-effects model (DerSimonian-Laird)[16] was also performed and showed similar results. Heterogeneity (measured using I^2 [17]) was variable between SNPs. We adopted a P value threshold of 1×10^{-5} for the reporting of putative associations but only discuss those with a P value below 1×10^{-6} in detail. The 1×10^{-5} threshold represents a trade-off between maintaining a low false-positive rate and taking into account the higher prior odds of association that a selected, gene-centric approach offers over unbiased GWAS,[11] as SNPs represented on the array were selected based on prior knowledge of cardiovascular disease loci. Based on a linkage disequilibrium (LD) cut-off of r^2=0.2 in Whitehall II, there are ≈19000 independent SNPs in the HumanCVD array. A Bonferroni correction based on 19000 SNPs gives a P=0.05 equivalent of $P=2.6\times10^{-6}$. Association results between these 2 thresholds ($1\times10^{-6}<P<1\times10^{-5}$) should be interpreted with caution but are included for information. At each locus with >1 significantly associated SNP, an adjusted association analysis was performed to identify independent effects from the lead SNPs. For each locus identified, the lead SNP was added to the regression model as a covariate to identify additional SNPs that passed our significance threshold.

Expression Quantitative Trait Loci Analysis

The Advanced Study of Aortic Pathology (ASAP) consisted of patients undergoing aortic valve and aorta replacement surgery at the Karolinska University Hospital, Stockholm, Sweden.[18] In this study, samples from heart (needle biopsy of left ventricle), aorta intima-media, and aorta adventitia were investigated. Altogether 399 samples from 215 different patients were included. RNA was extracted as described by Folkersen et al[18] and hybridized onto Affymetrix GeneChip Human Exon 1.0 ST expression arrays to determine gene expression levels. Cel files were preprocessed in Affymetrix Power Tools (1.10.2) and normalized using Robust Multichip Average Normalization[19] (with measurements $\log_2$ transformed as part of this process). The preprocessing and analysis are described in reference 18. DNA samples from ASAP patients were genotyped using Illumina Human 610 W-Quad Beadarrays and imputed using the MACH algorithm[20] and data from the 1000 genomes project.

All SNPs associated with electrocardiographic traits at $P<1\times10^{-5}$ were investigated for expression quantitative trait loci (eQTL) effects with the closest gene and fold-change of gene expression per minor allele estimated using an additive linear model, that is, values >1 indicate increased expression with the minor allele of an SNP, and values <1 indicate decreased expression with the minor allele of an SNP. A false discovery rate of 5% for the 21 reported tests corresponds to an uncorrected $P\leq0.00532$.

Integrative Modeling

We applied an established model of electrical action potentials of human ventricular cells (see reference 21 for description and validation of this model) to simulate conduction of excitation wave across the transmural strand of human ventricle.[22] The algorithm of Shaw and Rudy[23] was used to simulate the effects of changes of ion channel expression or activity on electrocardiographic parameters. We used our data except for *SCN10A*, *KCNE1,* and *KCNJ,* which were not represented in HumanCVD BeadChip; for these we used the replication datasets as in references[24] and [9]. We modeled the effects of changes of ion channel expression on QRS duration and QTc interval but did not model PR interval as the model lacks consideration of atria. Eight distinct ion currents influencing the cardiac action potential[25] and hence electrocardiographic traits

Table 1. Demographic and Clinical Characteristics of the Cohorts

	Whitehall II		BWHHS		GRAPHIC	
	Males (n=3721)	Females (n=1338)	Males (n=0)	Females (n=3443)	Males (n=1021)	Females (n=1003)
Age, y	60.8 (5.9)	61.2 (6.1)	-	68.9 (5.5)	39.4 (15.1)	39.2 (13.9)
BMI, kg/m^2	26.6 (3.8)	27.0 (5.5)	-	27.8 (4.9)	26.4 (4.3)	25.8 (4.9)
DBP, mm Hg	75.1 (10.4)	73.2 (10.7)	-	79.4 (11.9)	73.5 (8.1)	69.9 (6.8)
SBP, mm Hg	128.7 (16.1)	126.5 (18.2)	-	147.0 (25.1)	122.5 (10.1)	114.9 (9.8)
% on BP-lowering drugs	23.1	22.1	-	30.0	7.9	5.4
Corrected SBP, mm Hg	132.2 (18.1)	129.7 (20.6)	-	151.6 (27.3)	123.4 (10.9)	115.5 (10.6)
PR interval	0.17 (0.02)	0.16 (0.02)	-	0.16 (0.02)	0.16 (0.02)	0.15 (0.02)
QRS duration	0.10 (0.01)	0.09 (0.01)	-	0.09 (0.01)	0.10 (0.01)	0.09 (0.01)
QT interval (corrected for heart rate)	0.41 (0.02)	0.42 (0.02)	-	0.42 (0.02)	0.40 (0.02)	0.41 (0.02)
QRS axis	13.45 (35.00)	22.61 (29.72)	-	10.59 (28.87)	36.97 (35.34)	44.13 (29.45)

have been considered. These currents are summarized in relation to the 4 phases of the cardiac action potential in Shaw and Rudy's Figure 1.[23]

We assumed that most genotypic effects would be through an effect on expression level because there are no explanatory protein variants in most instances. Using the observed magnitude of genotypic effects to estimate likely size of molecular effect, we can read off from the model what the percentage change in current should be. Assuming a quantitative linear correspondence, this enables induction of the predicted magnitude of expression difference according to genotype.

Results

Association analyses were performed on 10 526 participants; 3443 from the BWHHS, 2024 from GRAPHIC, and 5059 from the Whitehall II study. The cohort characteristics are presented in Table 1, and the correlations of phenotypes and covariates are presented in online-only Data Supplement Table II. The phenotypes were all approximately normally distributed (online-only Data Supplement Figure I). Quantile–quantile plots with Genomic Inflation Factor for each trait in each cohort are presented in online-only Data Supplement Figure II.

Association With Electrocardiographic Traits

In a fixed-effects meta-analysis across 4 phenotypes, 6 independent genomic regions were identified containing associated SNPs at a threshold of $P<10^{-6}$ (top hits at each locus in Table 2, regional association plots in Figure 1, and full details of associated SNPs in online-only Data Supplement Table IV). In these data, the regression coefficient indicates change in phenotype (seconds) per minor allele of an SNP. SNPs in the gene for sodium channel, voltage-gated, type V, alpha subunit (*SCN5A*) were associated with both PR interval (top hit rs7372712, $P=8.08\times10^{-12}$) and QRS duration (top hit rs7374540, $P=5.87\times10^{-9}$). These 2 SNPs are essentially

Table 2. SNPs With the Strongest Evidence of Association at Each Locus Reaching Arraywide Significance in a Meta-Analysis of 10 526 Individuals

SNP	CHR	BP	Minor (Coded) Allele	Gene	I^2 *	Fixed-Effects β (SE)	Fixed-Effects *P*	Random-Effects β (SE)	Random-Effects *P*
PR interval									
rs7372712	3	38661196	T	*SCN5A*	0.00	0.00305 (0.00045)	8.08E-12	0.00302 (0.00049)	6.21E-10
rs3807989	7	1.16E+08	A	*CAV1*	67.89	0.00199 (0.00036)	1.99E-08	0.00199 (0.00036)	1.99E-08
QRS duration									
rs7374540	3	38609146	C	*SCN5A*	0.00	0.00104 (0.00018)	5.87E-09	0.00104 (0.00018)	5.87E-09
rs3176326	6	36755267	A	*CDKN1A*	0.00	0.00115 (0.00022)	1.41E-07	0.00115 (0.00022)	1.41E-07
rs10988728	9	1.01E+08	G	*TGFBR1*	0.00	0.00352 (0.00079)	7.90E-06	0.00352 (0.00079)	7.90E-06
QTc interval									
rs12039600	1	1.6E+08	A	*NOS1AP*	0.00	0.00476 (0.00048)	7.12E-23	0.00476 (0.00048)	7.12E-23
rs12271931	11	2435095	G	*KCNQ1*	36.93	−0.00292 (0.00044)	3.17E-11	−0.00301 (0.00074)	4.36E-05
rs3815459	7	1.5E+08	A	*KCNH2*	0.00	0.00219 (0.00038)	9.44E-09	0.00219 (0.00038)	9.44E-09
rs2267368	22	36895155	A	*PLA2G6*	72.98	−0.00499 (0.00112)	7.80E-06	−0.00499 (0.00112)	7.80E-06
QRS axis									
rs2132570	7	45928988	A	*IGFBP3*	0	2.41 (0.51)	2.89E-06	2.41 (0.51)	2.89E-06
rs1934968	10	96731807	A	*CYP2C9*	0	−3.17 (0.68)	3.43E-06	−3.17 (0.68)	3.43E-06

The regression coefficient indicates change in magnitude of phenotype (seconds) per minor allele of an SNP. Minor allele frequencies and missingness are detailed in online-only Data Supplement Table III.

*I^2 is a measure of heterogeneity in meta-analyses.[17]

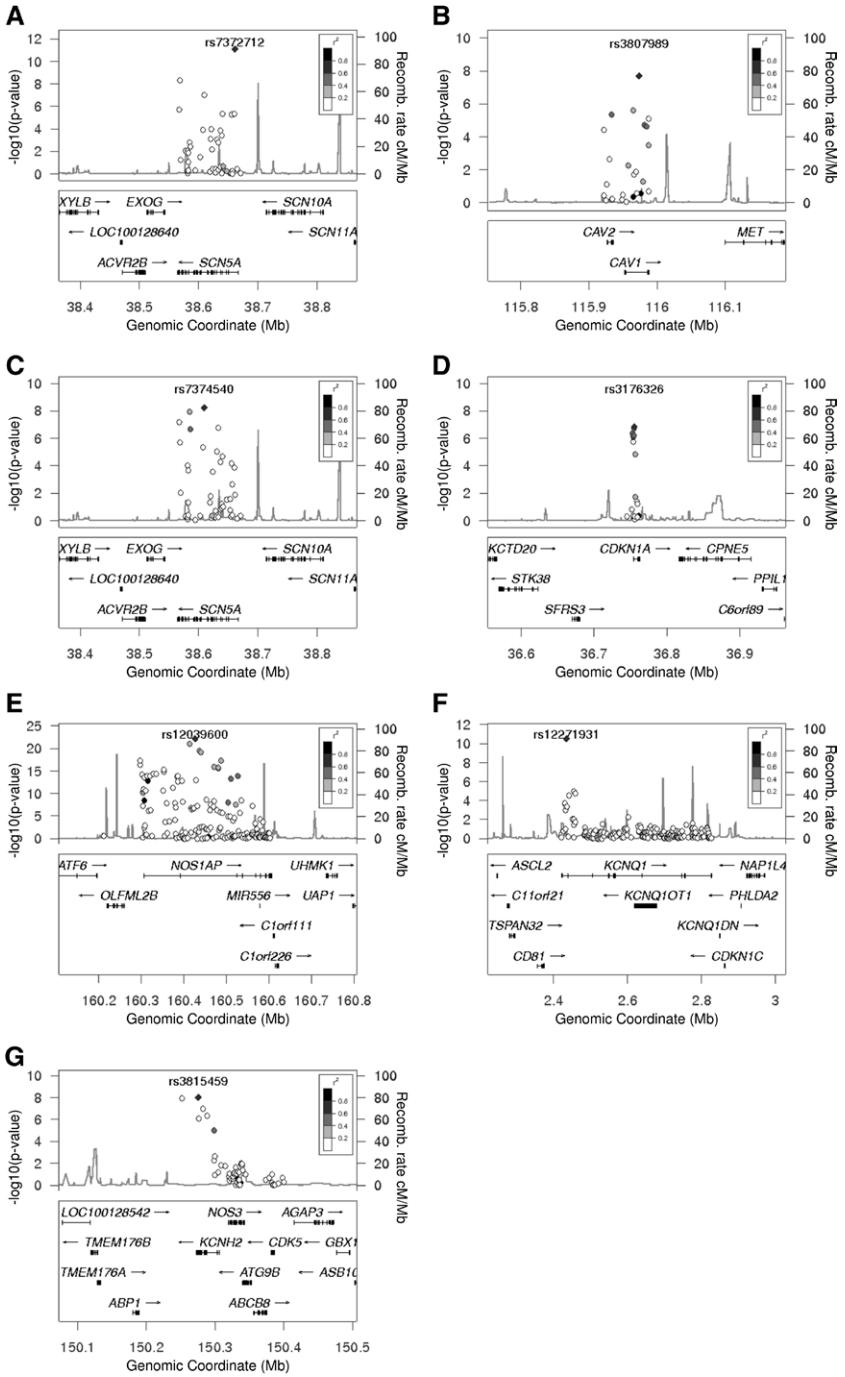

Figure 1. Regional association plots illustrating genomic location (x-axis), $-\log_{10}$ (*P* value) (y-axis), and linkage disequilibrium (r^2 on grayscale from white: r^2=0–0.2 to black: r^2=0.8–1) for each of the main associated regions. **A**, *SCN5A* (PR interval), (**B**) *CAV1-CAV2* locus, (**C**) *SCN5A* (QRS duration), (**D**) *CDKN1A*, (**E**) *NOS1AP*, (**F**) *KCNQ1*, (**G**) *KCNH2*.

not in LD (r^2=0.02 in HapMap Europeans). PR interval SNP rs7372712 is located in an LD block in the 5' region of *SCN5A*.

SNP rs3807989 in the *CAV1*/*CAV2* genomic region was also associated with PR interval (P=1.99×10^{-8}), with all other significant associations in this region attributable to LD with rs3807989. In contrast with SNPs in *SCN5A*, SNPs in the *CAV1*/*CAV2* locus did not exhibit any significant association with QRS duration. Conversely, in the *CDKN1A* gene region, SNP rs3176326 displayed evidence of association with QRS duration (P=1.41×10^{-7}) but little evidence of association with PR interval (P=0.069).

For QTc interval, we observed 56 SNPs in *NOS1AP* associated at P<1×10^{-6}, with adjusted analyses (see below) suggesting there may be >1 independent effect.

Nonsynonymous SNP rs1805123 (K899T) in *KCNH2* was significantly associated with QTc interval (P=8.68×10^{-7}, beta coefficient=–0.00174). However, the most significantly associated SNP in this region was rs3815459, an intronic SNP (P=9.44×10^{-9}, beta coefficient=0.00219).

In *KCNQ1*, SNP rs12271931 was significantly associated with QTc interval (P=3.17×10^{-11}, beta coefficient=–0.00292).

Adjusted Analyses

Analysis adjusted for the lead SNP effect at each locus (Figure 2) showed no strong evidence of additional independent SNP effects at the *CDKN1A* locus. For QRS duration, we observed

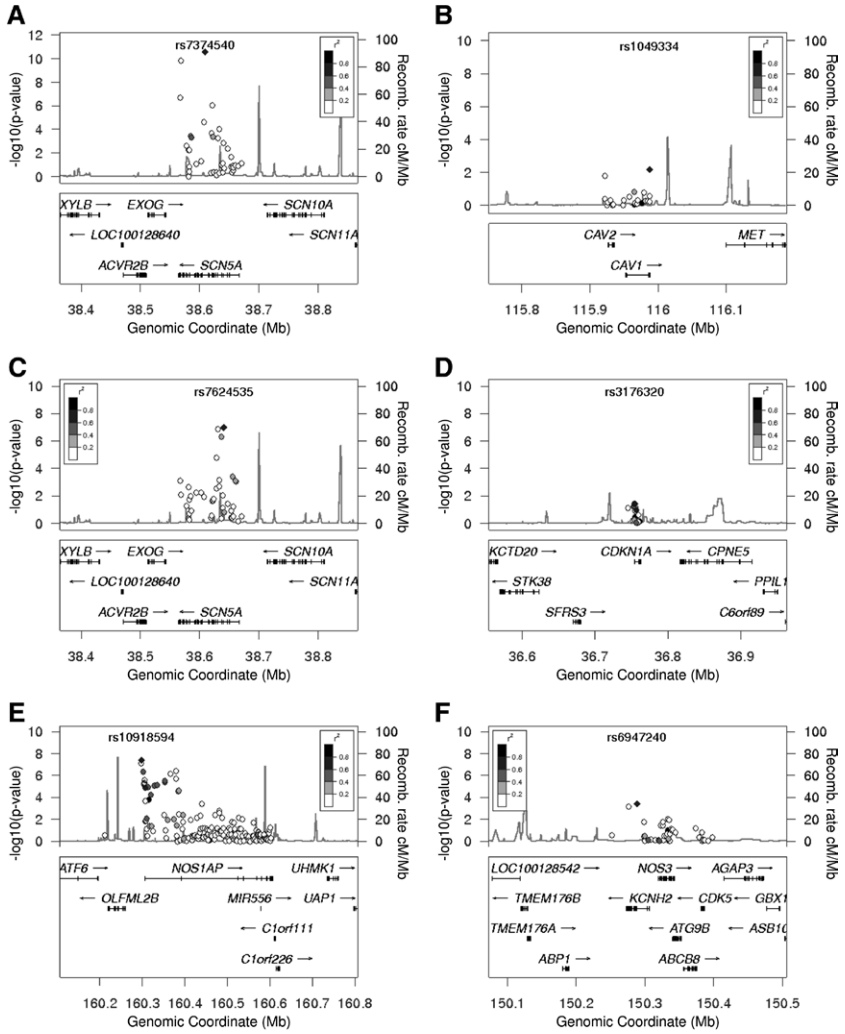

Figure 2. Regional association plots adjusted for the most associated single-nucleotide polymorphisms illustrating genomic location (x-axis), $-\log_{10}$ (*P* value) (y-axis), and linkage disequilibrium (r^2 on grayscale from white: r^2=0–0.2 to black: r^2=0.8–1) for each of the main associated regions. (**A**) *SCN5A* (PR interval), (**B**) *CAV1-CAV2* locus, (**C**) *SCN5A* (QRS duration), (**D**) *CDKN1A*, (**E**) *NOS1AP*, (**F**) *KCNH2*.

independent signals for rs6797133 (P=1.42×10^{-7}), rs7624535 (P=1.02×10^{-7}), rs11710077 (P=6.75×10^{-4}), and rs1805126 (P=7.59×10^{-4}) adjusted for the top hit rs7374540 in *SCN5A*. For PR interval, we analyzed SNPs in *SCN5A* adjusted for the top hit rs7372712 and observed evidence of independence for rs7374540 (P=2.65×10^{-11}), rs12053903 (P=1.52×10^{-10}), and rs1805126 (P=2.00×10^{-7}). For QTc interval, the strongest independent signal came from rs10918594 (P=4.00×10^{-8}), with a number of other SNPs showing evidence of association in the analysis adjusted for rs12039600 in *NOS1AP*. We analyzed the SNPs in *KCNH2* adjusted for our top hit rs3815459 and observed some evidence of independent effects from rs6947240 (P=3.83×10^{-4}) and rs1805123 (P=6.74×10^{-4}).

eQTL Analyses

SNPs with strong evidence of association with electrocardiographic traits in our meta-analysis were investigated for their impact on expression of genes in cardiac tissue (Table 3). The most associated SNPs for each trait (rs12039600 in *NOS1AP* for QTc interval; rs1934968 in *CYP2C9*, and rs2132570 in *IGFBP3* for QRS axis; rs7372712 in *SCN5A* and rs3807989 in *CAV1* for PR interval) show evidence of association with expression levels (Table 3 and online-only Data Supplement Figure III).

Comparison with Mendelian Conduction Disorders

We compared our results with those reported for single-gene arrhythmia disorders and GWAS results, and we present a review of rare and common variation influencing electrocardiographic traits in online-only Data Supplement Table I. There is no evidence of a role of common variation at the population level for a number of genes in which rare mutations have reported pathology (within the limitations of HumanCVD coverage). We confirm previously reported findings from a number of GWAS for both Mendelian disorder genes (eg, *SCN5A*) and genes for which no Mendelian disorder is known (eg, *NOS1AP*).

Integrative Modeling

We modeled the effects of changes of ion channel expression or activity on electrocardiographic parameters for comparison with the observed genotypic effects on these traits. Figure 3 illustrates the channels and genes considered, with magnitude of phenotypic effect shown in milliseconds/allele. In Table 4, we show estimates of percentage changes in expression, which would be necessary to achieve the percentage changes in each specific current consistent with the observed phenotypic effects of SNPs in the relevant current-influencing gene. This table is based on an explicit assumption that links changes in expression level to current magnitude (note that for *NOS1AP* other

Table 3. Difference in Gene Expression (Expressed as Difference Per Allele) Among eQTL Genotypes

	Left Ventricle		Aorta Intima/Media		Aorta Adventitia	
	P	Per Allele Effect (95% CI)	*P*	Per Allele Effect (95% CI)	*P*	Per Allele Effect (95% CI)
NOS1AP rs12039600	0.295	0.98 (0.95–1.02)	0.93	1 (0.96–1.04)	0.0217	1.04 (1.01–1.08)
CYP2C9 rs1934968	0.719	1.01 (0.96–1.06)	0.00117	0.95 (0.91–0.98)	0.555	0.99 (0.95–1.03)
KCNQ1 rs12271931	0.124	0.96 (0.91–1.01)	0.718	1.01 (0.96–1.07)	0.161	0.97 (0.92–1.01)
SCN5A rs7374540	0.137	1.07 (0.98–1.17)	0.371	1.01 (0.98–1.04)	0.478	0.99 (0.96–1.02)
SCN5A rs7372712	0.346	1.06 (0.93–1.21)	0.0129	0.95 (0.91–0.99)	0.513	0.98 (0.94–1.03)
CDKN1A rs3176326	0.116	1.05 (0.99–1.11)	0.846	1.01 (0.96–1.06)	0.847	0.99 (0.94–1.05)
IGFBP3 rs2132570	0.971	1 (0.93–1.07)	0.0423	0.86 (0.75–0.99)	0.00532	0.86 (0.78–0.95)
CAV1 rs3807989	0.00391	0.91 (0.86–0.97)	0.689	0.99 (0.95–1.03)	0.256	0.97 (0.93–1.02)
KCNH2 rs3815459	0.669	1.01 (0.96–1.07)	0.835	1.01 (0.96–1.05)	0.3	0.98 (0.93–1.02)

Values above 1 indicate increased expression with the minor allele of the SNP. Values below 1 indicate decreased expression with the minor allele of the SNP. SNPs from Table 2 were tested against the closest gene. FDR 5% corresponds to $P \leq 0.00532$ in this set of tests. CI indicates confidence interval.

channels may also be influenced by NO).[26] Online-only Data Supplement Figure IV provides illustrative data on the predicted effect of I_{Na} change on QRS duration. The genotypic effects we observe on QRS duration (*SCN5A* and *SCN10A*) are consistent with an 11% to 12% predicted difference in expression per allele, whereas for QTc interval the genotypic effects are consistent with anywhere from a 1% per allele (*KCNE1*) to a 10% per allele (*NOS1AP*) difference in expression.

Discussion

Association Analyses

Using the HumanCVD BeadChip we identified 6 regions containing SNPs with evidence of association with electrocardiographic traits in cohorts representative of the general population, broadly consistent with previous reports.[9,10,27] The high density of SNPs in HumanCVD BeadChip for some of these regions offers additional insight into possible causal sites or haploblocks around these genes.

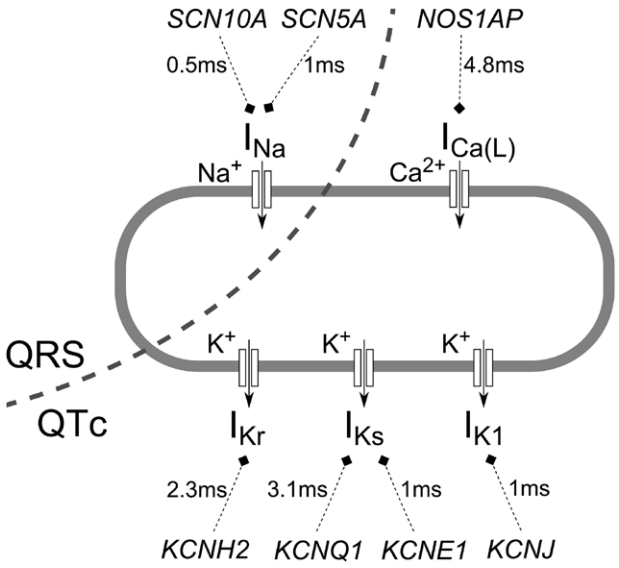

Figure 3. Schematic of the modeling approach used. Variants in labeled genes are assumed to influence the channel indicated. The schematic is partitioned into channels affecting QRS duration, and channels affecting QTc interval. The per-allele effect of the most significant allele in each gene on either QRS or QTc is indicated in milliseconds.

SNPs in the gene for the voltage-gated, sodium channel type V, alpha subunit (*SCN5A*) gave highly significant signals for both PR interval and QRS duration. The most significant SNPs, which were not in LD (r^2=0.02 in HapMap Europeans), differed for each trait, respectively, rs7372712 and rs7374540 (online-only Data Supplement Figure V). SNP rs7372712, located in an LD block in the 5' region of *SCN5A*, appears to mark a novel independent effect poorly represented in earlier chip-based analyses. Additionally, rs7372712 is essentially in linkage equilibrium with the coding SNPs in *SCN5A* claimed to influence electrocardiographic traits, specifically rs1805126 (D1819) and rs1805124 (H558R) in Europeans (HapMap r^2=0.003 and r^2=0.011, respectively). In a separate gene-focused study, Newton-Cheh et al[10] reported *SCN5A* rs11720524, an intron 1 SNP, as the highest ranking marker for association of *SCN5A* genotype with sudden cardiac death. There is weak LD (r^2=0.112) between rs7372712 and rs11720524, and these 2 SNPs colocalize at the 5' end of *SCN5A* (intron 1, 10.8kb apart). This finding indicates that variation in the 5' region of *SCN5A* may exert considerable influence on both electrocardiographic traits, being our leading SNP for PR duration, and clinical outcomes.

SNP rs12053903 in *SCN5A* was the second most significant SNP for PR interval and corresponds with previously reported SNP effects.[27,28] These SNPs in the 3' region of *SCN5A* are in 1 LD block and may represent the same functional mechanism or (unknown) causal SNP. Our data suggest independent effects from variants in the functionally related *SCN10A*.[27,29] *SCN5A* SNPs are also reportedly associated with QTc interval; the same SNP and 3' *SCN5A* block that we also observed to affect PR duration has been associated with QTc interval.[10]

We replicated a PR interval specific association of rs3807989[27] located in the genomic region of *CAV1* and *CAV2* (P=1.99×10^{-8}). All other significant SNP associations in this region were explicable by their linkage disequilibrium with this SNP. Caveolins act as scaffolds in caveolae, cell membrane pits found in almost all myocardial cell types. Receptors, ion channels, and endothelial nitric oxide synthase localize at caveolae, thus influencing signaling and excitation–contraction

Table 4. Estimates of Percentage Changes in Expression Predicted to be Necessary to Achieve the Model Predicted Change in Each Specific Current

ECG Feature	Gene	Current	Observed Effect (ms/allele)	Predicted % Difference of Expression Per Allele
QRS	*SCN5A*	I_{Na}	1	12
QRS	*SCN10A*	I_{Na}	0.5	11
QTc	*NOS1AP*	$I_{Ca(L)}$*	4.8	10
QTc	*KCNH2*	I_{Kr}	2.3	5
QTc	*KCNQ1*	I_{Ks}	3.1	3
QTc	*KCNE1*	I_{Ks}	1.0	1
QTc	*KCNJ*	I_{K1}	1	2.5

Observed effect was used in electrophysiological models of QRS and QTc (see Methods) to estimate % change of current which would generate that effect. It was then assumed that % change of current would directly correspond with % change of gene expression.

*This appears to be the primary mode of action of *NOS1AP*,[26] but other channels may also be influenced by NO.[26]

coupling. Altered caveolin activity alters NO modulation of membrane excitability.[30] This genetic effect, as for *NOS1AP* on QTc interval (see below), may therefore involve NO, but equally plausibly could involve ryanodine receptors and calcium or sodium channels.[31] A systems view of the NO system is presented in the online-only Data Supplement.

SNPs in *CDKN1A* were uniquely associated with QRS duration consistent with previous reports.[24,32] It is likely that our lead SNP marks the same effect as previously reported. *CDKN1A* encodes cyclin-dependent kinase inhibitor 1A, which is involved in cell cycle regulation.[33] The mechanism by which a cell cycle gene could differentially influence QRS duration relative to other electrocardiographic traits remains obscure but may reflect an influence on ventricular development or remodeling, given that the QRS complex represents ventricular depolarization. Alternatively, it could influence ventricular depolarization through an effect on peripheral circulatory resistance.

Numerous studies have reported association of common variation in *NOS1AP* with QTc interval, and our results are in broad accord with data that suggest 3 independent effects.[10] Other SNPs in our data and reported in the literature do not appear to be independent of these. *NOS1AP* encodes carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein, a protein that binds neuronal NO synthase (NOS1) via a C-terminal binding domain and other proteins such as Dexras1 and synapsins via an N-terminal phosphotyrosine-binding domain. In the heart and ventricular myocytes, carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein interacts with NOS1 to accelerate cardiac repolarization by inhibition of L-type calcium channels, which generate a slow depolarizing current.[26] *NOS1AP* association plots for all 4 traits are presented in online-only Data Supplement Figure VI.

At the *KCNH2* locus, we replicated findings for a nonsynonymous SNP, rs1805123 (K899T) widely reported for association with QTc and with electrophysiological effects in vitro.[34–38] We also replicated findings for SNPs emergent from published GWAS,[9,10,24,29] where our best proxy was rs6972137 (r^2=0.083 with rs1805123 in HapMap Europeans). Our lead SNP effect, rs3815459 (intronic), is inconsistent with rs1805123 being the only functional site in *KCNH2*. Our data support reports of independent effects in *KCNH2*.[10] We note that 1 overarching LD block spans both *KCNH2* and *NOS3* (online-only Data Supplement Figure VI), separated by ≈10 kb, and speculate that intronic or intergenic SNPs ascribed to *KCNH2* could alternatively mark effects of *NOS3* (also a plausible candidate for effects on QTc, particularly considering the recent discovery of unexpected *NOS1AP* association with QTc).[10]

Our results also broadly replicate earlier observations for *KCNQ1*.[9,24] Numerous reports have implicated *KCNQ1* variation in type 2 diabetes mellitus risk[39–42] and also in association with adrenaline, adenosine diphosphate,[43] and alpha2-macroglobulin levels.[44] The diabetes mellitus SNP is not represented in our data; however rs179429, reportedly[43] associated with adrenaline levels, is present but showed no association with QTc. It seems unlikely that *KCNQ1* effects on QTc could be explained by indirect effects through adrenaline level rather than by direct effects on cardiac outward potassium current.

Integrative Modeling

It is notable that common variants are observed affecting 5 of the 8 or so ionic currents (Figure 3). We observe a strong correspondence between the leading genotypic effects on PR, QRS, and QTc intervals and the major genes encoding products influencing those traits (eg, I_{Na}: *SCN5A* on PR and QRS duration, I_{Kr}: *KCNH2* on QTc, and I_{Ks}: *KCNQ1* on QTc). *CACNA1C* was represented by 193 SNPs in the HumanCVD array, but none showed any evidence of association (all P>0.05). However, *NOS1AP*, which influences $I_{Ca(L)}$, showed strong evidence of association with QTc interval. From a modeling perspective (online-only Data Supplement Figure IV), percentage changes in QRS duration are an order of magnitude greater per percent change in I_{Na} (eg, through possible differential level of expression according to genotype). In Table 4 it is evident that remarkably small (down to 1%) effects can be detected for QTc interval compared with PR and QRS duration, possibly reflecting the relatively smaller SD of QTc. There is no evidence of effect on QTc for *SCN5A* SNPs with prominent effects on QRS and PR duration. From the predicted QRS duration based on percentage change in I_{Na}, the observed ≈1 ms/allele effect of *SCN5A* rs7374540 on QRS duration would correspond with 12% change in I_{Na}, possibly represented as a 12% difference of *SCN5A* allelic expression were I_{Na} to directly correlate with *SCN5A* expression. Such a difference would be predicted to exert a small effect on QTc, creating a 0.3% difference per allele. Mean QTc was ≈0.42 seconds, which predicts a beta of ≈0.001; however, we did not observe this finding in our association results.

Generally, the various potassium and calcium channel currents have little impact on QRS duration, thus the genotypes

influencing QTc are unlikely to show detectable effects on QRS duration. We also cannot readily integrate *CAV1/2* or *CDKN1A* to this model except to note CAV1/2 interaction with channels, receptors, and NO signaling. Finally, our assumption of a quantitative linear correspondence between expression and phenotypic effect is a potential limitation of the approach.

eQTL Analyses

We tested the top associated SNPs for each gene (Table 2) for differential expression by genotype. rs12039600 in *NOS1AP*, rs7372712 in *SCN5A,* rs2132570 in *IGFBP3*, rs1934968 in *CYP2C9,* and rs3807989 in *CAV1* all showed evidence of eQTL effects (Table 3), with per-allele effects of 4%, 5%, 14%, 5%, and 9%, respectively. Because the SNP associations with electrocardiographic traits do not appear to reflect coding variants, the ECG effects may operate through expression levels. Our findings support this hypothesis for these SNPs. rs12039600 is in intron 1 of *NOS1AP*, and rs7372712 is in the 5' region of *SCN5A*. These may therefore mark a haplotypic effect on allelic expression in the promoter regions of these genes. In our eQTL analyses for *NOS1AP* rs12039600, a 4% difference of allelic expression was observed. The G allele leads to lower expression, which would imply less inhibition of the depolarizing L-type calcium channel, leading to a longer QTc interval. This finding is consistent with the observed G allele association with QTc. The *IGFBP3*, *CYP2C9,* and *CAV1* eQTL effects were greater than might be anticipated from their regression coefficients (eg, 9% effect per allele on expression vs. 1% per allele for rs3807989 on PR interval); however, their mechanisms of effect are complex and cannot readily be modeled. It should be noted that we did not find complete consistency of allelic imbalance by SNP across different vascular tissues. Also, eQTL analysis for the ideal tissue (eg, ventricular myocardium for QRS) was not available.

Conclusions

We have replicated across the range of electrocardiographic traits a number of previously reported SNPs. Our data point to a previously unrecognized allele and region effect in *SCN5A* on PR and QRS durations and confirm multiple allelic effects of *NOS1AP* on QTc. We also identify relevant eQTL effects in *NOS1AP*, *SCN5A, CYP2C9, IGFBP3,* and *CAV1*. We consider the genetic findings in the light of both biological knowledge and a general systems model of electrocardiographic traits, representing a first step toward an integrative genetic model of the ECG as a complex trait. The impact of specific allelic differences in this model may be of value in predicting the effects of drugs targeting specific gene products influencing electrocardiographic traits.

Acknowledgments

We acknowledge the work of Shahid Latif, Louise Inglis, Kathryn McLaren, and Jean Watts at the University of Glasgow ECG Core Lab for the processing and analysis of ECGs.

Sources of Funding

The British Women's Heart and Health Study is supported by funding from the British Heart Foundation (BHF) and the Department of Health Policy Research Programme (England), with HumanCVD genotyping funded by the BHF [PG/07/131/24254]. Recruitment and genotyping of the Genetic Regulation of Arterial Pressure of Humans in the Community (GRAPHIC) Study was funded by the BHF. The GRAPHIC Study is part of the research portfolio supported by the Leicester National Institute for Health and Research Biomedical Research Unit in Cardiovascular Disease. The Whitehall II study has been supported by grants from the Medical Research Council (MRC); BHF; Health and Safety Executive; Department of Health; National Heart Lung and Blood Institute [grant number NHLBI: HL36310] and National Institute on Aging (AG13196), US, National Institutes of Health; Agency for Health Care Policy Research [grant number HS06516]; and the John D and Catherine T MacArthur Foundation Research Networks on Successful Midlife Development and Socioeconomic Status and Health. The Advanced Study of Aortic Pathology study was supported by the Swedish Research Council [grant number 12660]; the Swedish Heart-Lung Foundation [grant number 20090541]; the European Commission [FAD, Health F2, 2008 grant number 200647]; and a donation by Fredrik Lundberg. N. Samani and S. Humphries hold Chairs funded by the BHF. Dr Hingorani, S. Humphries, Dr Kivimaki, and Dr Talmud were supported by the BHF [PG07/133/24260, BHFPG08/008] and Dr Whittaker by the BHF [PG07/133/24260]. I. Adeniran was funded by a BHF studentship to Drs Zhang and Casas [FS/08/021]. M. Tobin has been supported by MRC fellowships [G0501942, G0902313]. Dr Gaunt, Dr Lawlor, and I. Day work in a centre funded by the UK MRC [G0600705] and University of Bristol. The views in the article are those of the authors and not necessarily any funding body. All data collection, analyses, and interpretation of results were done independently of any funding body.

Disclosures

Dr Whittaker is employed by GlaxoSmithKline, owns GlaxoSmithKline shares, and holds a UK MRC project grant (G0801414). Dr Kivimaki is principal investigator of National Heart Lung and Blood Institute grant R01HL036310. S. Humphries has 5 grants from the British Heart Foundation and European Union Seventh Framework Programme, has received payment for speaking at the Genzyme Meeting (Amsterdam, November 2011), and is a consultant for StoreGene. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Electrocardiographic traits are substantially heritable determinants of risk of arrhythmias and sudden cardiac death. We describe a genetic association analysis of PR interval, QRS axis, QRS duration, and QTc interval in 3 population cohorts. Genotyping was performed using the HumanCVD high-density array, and electrocardiographic traits were derived from digital electrocardiographic data using the Glasgow ECG analysis program. Our analyses confirmed a number of previously reported genetic associations and identified a novel independent genetic locus near the voltage-gated sodium channel gene *SCN5A*. Genetic associations were followed up by analysis of the effects of these loci on gene expression in heart tissue and by modeling the expected effects on electrocardiographic parameters using an established model of electrical action potentials of human ventricular cells. These analyses offered additional insight into the molecular mechanisms underlying variability in electrocardiographic traits. The findings contribute toward the development of an integrated description of the molecular system and common human variation underpinning electrocardiographic pathophysiology.

SUPPLEMENTAL MATERIAL

Integration of genetics into a systems model of cardiac conduction traits using HumanCVD BeadChip [CIRCCVG/2012/962852]

Journal: Circulation: Cardiovascular Genetics

Authors: Tom R. Gaunt[1]*, Sonia Shah[2]*, Christopher P Nelson[3,4]*, Fotios Drenos[5], Peter S Braund[3], Ismail Adeniran[6], Lasse Folkersen[7], Debbie A Lawlor[1], Juan-Pablo Casas[8,9], Antoinette Amuzu[8], Mika Kivimaki[10], John Whittaker[11], Per Eriksson[7], Henggui Zhang[6], Jules C. Hancox[12], Maciej Tomaszewski[3], Paul R Burton[13], Martin D Tobin[13], Steve E Humphries[2,5], Philippa J Talmud[5], Peter W Macfarlane[14]*, Aroon D Hingorani[10,15]*, Nilesh J Samani[3,4]*, Meena Kumari[10]* and Ian N M Day[1]* [* The authors wish it to be known that, in their opinion, these authors should be regarded as equal contributors]

Corresponding author: Prof Ian NM Day, MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Bristol, UK. Tel: +44-117-3310097. Fax: +44-117-3310123. Email: ian.day@bristol.ac.uk

Contents

Supplementary Materials and Methods

Study Cohorts

British Women's Heart and Health Study (BWHHS): The BWHHS is a prospective cohort study of British women aged 60 to 79 years at baseline assessment (1999-2001). The study comprises 4286 women randomly selected from 23 British towns. Baseline measurements are described in [1] and follow-ups in [2]. Illumina HumanCVD genotype data are available on 3443 women (European-British ancestry) from the cohort. ECG measurements were carried out in the baseline assessment. Informed consent was obtained from all participants and the study was approved by UK NHS local and multicentre ethics committees.

Genetic Regulation of Arterial Pressure of Humans in the Community (GRAPHIC) Study: The GRAPHIC Study comprises 2024 individuals from 520 nuclear families of White-European origin recruited from the general population for the purpose of investigating the genetic determinants of blood pressure and related cardiovascular traits. Families were recruited through participating family practitioners in Leicestershire, UK, between 2003 and 2005 and included if both parents aged 40-60 years and two offspring ≥18 years wished to participate. There was no preferential selection based on history of hypertension, but subjects were excluded if they had known renal disease. A detailed medical history was obtained from study subjects by standardized questionnaires and clinical examination was performed by research nurses following standard operating procedures. Measurements included height, weight, waist-hip ratio, clinic and ambulatory blood pressure and 12-lead ECGs. Blood samples were obtained for laboratory analysis. Analysis used generalized estimating equations (GEE). We have shown (in-house) that the results from a GEE in GRAPHIC are broadly similar to the results we obtain from Merlin (http://www.sph.umich.edu/csg/abecasis/Merlin/index.html) giving us confidence in their ability to adequately adjust for the familial correlation. The study

was approved by the Leicestershire Research Ethics Committee, and all subjects provided written informed consent. The study conforms to the principles outlined in the Declaration of Helsinki, and all procedures followed were in accordance with institutional guidelines. Further details are provided elsewhere[3].

Whitehall II Study: The Whitehall II study recruited 10,308 participants (70% men) between 1985 and 1989 from 20 London-based Civil service departments. Clinical measurements are taken every 5 years. Blood samples for DNA were collected in phase 7 (2002–2004) from over 6000 white-European participants. ECG measurements and other phenotype information from phase 7 were used in this study since this phase had the largest number of ECG measurements. Informed consent was obtained from all participants and the study was approved by UK NHS local ethics committees.

The Nitric Oxide system

The NO system is widely represented in the heart and vasculature, with NOS1 (neuronal NOS) expressed in conduction tissue, intracardiac neurons and sarcoplasmic reticulum of myocytes; NOS3 (endothelial NOS) expressed in coronary endothelium, endocardium and sarcolemma and T-tubules of myocytes; and NOS2 (inducible NOS) induced by inflammatory cytokines. In the peripheral vascular system, vascular smooth muscle relaxation through NO action via cGMP can alter cardiac workload and hence ECG. By direct action in the heart, much influence is on the calcium signalling components of the ECG, with NOS1 localised near ryanodine receptors and Ca2+ ATPase (SERCA2a) in the sarcoplasmic reticulum, and NOS3 localised in sarcolemmal caveolae with receptors and L-type calcium channels[4]. *NOS1, 2* and *3* genes were respectively represented by 117, 48 and 23 SNPs in the HumanCVD array but no variation reached chip-wide significance for any conduction trait. Our high density of coverage, and the fact that these genes have not emerged in GWAS of

any disease, suggests that there may not be much functionally meaningful common variation in these important genes, in contrast with *NOS1AP*. However, for *NOS3*, we cannot rule out the possibility that effects apparently from the directly adjacent *KCNH2* gene, might actually represent SNPs influencing gene regulation of *NOS3*. *NOS1AP* and *NOS1* also interact with the second PDZ domain of DLG4 (discs large homolog 4 or post-synaptic density protein 95), respectively through their C terminus and PDZ domain; DLG4 is a member of the membrane-associated guanylate kinase family and with DLG2 is recruited into neuronal potassium channel clusters. *DLG4, 3* and *2* are represented in the HumanCVD array. In *DLG4*, an intronic SNP showed weak evidence of association with QTc duration (p = 0.018) but numerous SNPs in *DLG3* (on the X chromosome) showed p values in the range 0.001-0.0001. While this does not reach our pre-defined significance threshold, it suggests that there might be variation in additional functional products centred around *NOS1AP* influencing cardiac repolarization that could be identified with large collections.

Supplementary Tables

Supplementary Table 1: Reported Mendelian and common genetic variants related to cardiac conduction traits.

Gene	Single gene arrhythmia disorder known (based on data in OMIM extracted May 2011 [5])	Mechanism	Complex trait ECG effect observed	SNP coverage in our data	Reported SNPs in other studies	Most significant SNP in our study and r^2 with other reported SNPs
ABCC9	Yes 3-BP DEL, 4-BP INS, EX38 and ALA1513THR (rs121909304) with dilated cardiomyopathy[6]	Sulfonylurea receptor; sulfonylureas inhibit activite of K_{ATP} channels.	No	25	None	rs4148674: p=0.015266994 with T axis

AKAP9	Yes SER1570LEU (rs121908566) with long QT[7]	Part of the I_{Ks} PKA complex binding KCNQ1 C terminus and PKA. Likely regulatory function.	No	5	None	rs6960867: p=0.008763673 with QRS duration rs6960867: p=0.039303174 with QTc interval
ANK2	Yes GLU1425GLY (rs72544141) with cardiac arrhythmia[8] THR1626ASN, LEU1622ILE (rs35530544), GLU1813LYS (rs45454496) with cardiac arrhythmia[9] ARG1788TRP with long QT[9]	Involved in distribution of ion channels	No	3	None	rs28377576: p=0.088422556 with QTc interval
CACNA1C	Yes GLY406ARG (rs79891110) with Timothy Syndrome[10] GLY402SER (rs80315385) with Timothy Syndrome[11] GLY490ARG and ALA39VAL with Brugada Syndrome[12]	Calcium channel mediating calcium ion influx	No	194	None	rs11832738: p=0.00067468 with QRS axis
CAV3	Yes 21 reported mutations in OMIM causing long QT, cardiomyopathy, rippling muscle disease, limb-girdle muscular dystrophy Long QT mutations: SER141ARG (rs104893713), PHE97CYS (rs104893714), THR78MET (rs72546668), ALA85THR (rs104893715) with long QT[13]	Muscle-specific caveolin, a scaffold protein in caveolae.	No	10	None	rs237872: p=0.060216592 with QTc interval

	VAL14LEU (rs121909281), LEU79ARG (rs121909282) with long QT[14]					
CASQ2	Yes ASP307HIS with ventricular tachycardia[15] 16-BP DEL, NT339, LEU167HIS with ventricular tachycardia[16]	Main calcium ion reservoir in the cardiac myocyte sarcoplasmic reticulum	Yes	14	rs4074536 with QRS duration ($p=2.36x10^{-8}$)[17]	rs10754355: p=0.030020001 with T axis (Hapmap CEU r^2 = 0.015 with rs4074536). rs4074536: p=0.279696876 with QRS duration
DMPK 3'UTR	Yes (CTG)n EXPANSION with myotonic dystrophy[18]	A protein kinase with a functional role in myotonic dystrophy.	No	3	None	rs16939: p=0.034070514 with PR interval
GJA5	Yes ALA96SER (rs121434557), PRO88SER (rs121434558) with atrial fibrillaion[19]	A connexin protein with a structural role in gap junctions between cardiac myocytes.	No	9	None	rs6703824: p=0.004763167 with QTc interval
GPD1L	Yes ALA280VAL (rs72552291) with Brugada syndrome[20] GLU83LYS (rs72552292), ILE124VAL (rs72552293), ARG273CYS (rs72552294) with Brugada syndrome[21]	A gene involved in Brugada syndrome, possibly influencing sodium transport.	No	0	None	N/A
HCN4	Yes	A nucleotide-gated ion channel with a	No	14	None	rs11635910: p=0.003807708

	SER672ARG (rs104894488) with sick sinus syndrome[22] 1-BP DEL, 1631C with sick sinus syndrome[23] ASP553ASN (rs104894485) with sick sinus syndrome[24] GLY480ARG (rs121908411) with sick sinus syndrome[25] 4-BP INS, GTGA with Brudgada syndrome[26]	role in cardiac pacemaking				with QRS duration
KCNA5	Yes GLU375TER (rs121908590) with familial atrial fibrillation[27] THR527MET (rs121908591), ALA576VAL (rs121908592), GLU610LYS (rs121908593) with familial atrial fibrillation[28]	Voltage-gated potassium channel expressed in heart.	No	7	None	rs9788217: p=0.126869271 with T axis
KCNE1	Yes THR59PRO AND LEU60PRO with Jerfell and Lange-Nielsen syndrome[29] THR7ILE (rs28933384), ASP76ASN (rs74315445) with Jerfell and Lange-Nielsen syndrome[30] ASP76ASN (rs74315445), SER74LEU (rs74315446) with long QT[31] ASP85ASN (rs1805128) with long QT[32]	Voltage-gated potassium channel expressed in heart.	No	24	None	rs1012945: p=0.034395743 with QTc interval
KCNE2	Yes GLN9GLU (rs16991652), MET54THR (rs74315447), ILE57THR (rs74315448) with long QT[33] ARG27CYS (rs74315449) with long QT[34]	Voltage-gated potassium channel.	No	5	None	rs12626687: p=0.06693889 with T axis

	PHE60LEU (rs16991654) with long QT[35]					
KCNH2	Yes 22 long QT and 1 short QT mutations reported in OMIM: ALA561VAL; ASN470ASP; IVS3, G-C, +1; ILE593ARG (rs28928904); VAL822MET; 27-BP DEL; 1-BP DEL; GLY628SER; ARG582CYS; GLY572ARG (rs9333649); ALA490THR (rs28928905); TRP1001TER; SER818LEU; ARG784TRP (rs12720441); THR65PRO; ARG752GLN; IVS10, G-A, +1; 1-BP INS, 2775G; ASN861ILE; ARG948CYS; ARG100GLY; ARG913VAL (rs77331749); ALA558PRO with long QT (http://www.ncbi.nlm.nih.gov/omim/152427?report=Variants) ASN588LYS (rs104894021) with short QT (http://www.ncbi.nlm.nih.gov/omim/152427?report=Variants)	Voltage-gated potassium channel (HERG).	Yes	9	rs2968863 with QT interval ($p=3.79x10^{-8}$)[36] rs2968864 with QT interval ($p=8x10^{-16}$) and rs4725982 with QT interval ($p=5x10^{-16}$)[37]	rs3815459: $p=9.44x10^{-9}$ with QTc interval
KCNJ2	Yes 12 mutations reported with Andersen Cardiodysrhythmic Periodic Paralysis and 1 mutation with short QT in OMIM: ASP71VAL (rs104894575); ARG218TRP (rs104894578); GLY300VAL (rs104894579); 12-BP DEL, NT513; 6-BP DEL, NT1167; ARG67TRP (rs104894580); PRO186LEU (rs104894581); VAL302MET (rs104894582); ASN216HIS (rs104894583); THR75ARG (rs104894585); CYS54PHE; THR305PRO with Andersen Cardiodysrhythmic Periodic Paralysis (http://www.ncbi.nlm.nih.gov/omim/600681?report=Variants) ASP172ASN (rs104894584) with short QT (http://www.ncbi.nlm.nih.gov/omim/600681?report=Variants)	Inwardly rectifying potassium channel expressed in heart.	Yes	3	rs17779747 with QT interval ($p=6x10^{-12}$)[36]	rs1468472: p=0.169625286 with QTc interval
KCNQ1	Yes 30 long QT, 1 short QT and 8 other mutations reported in OMIM:	Voltage-gated potassium channel	Yes	180	rs12296050 with QT interval	rs12576156: p=0.0000108 with

	SER140GLY (rs120074192) with atrial fibrillation (http://www.ncbi.nlm.nih.gov/omim/607542?report=Variants) 7-BP DEL/8-BP INS, N; 1-BP INS, 282G; TRP305SER (rs120074186); 2-BP DEL; 20-BP DEL, NT1892; THR587MET (rs120074189); IVS1 with Jervell and Lange-Nielsen syndrome 1 (http://www.ncbi.nlm.nih.gov/omim/607542?report=Variants) 3-BP DEL; ALA178PRO (rs120074177); GLY189ARG (rs104894252,104894255); ARG190GLN (rs120074178); VAL254MET (rs120074179); LEU273PHE (rs120074180); GLY306ARG (rs120074181); THR312ILE (rs120074182); ALA341GLU (rs12720459); ALA341VAL (rs12720459); GLY345GLU (rs120074183); GLY314SER (rs120074184); ARG555CYS (rs120074185); ALA300THR (rs120074187); 3-BP DEL, PHE339DEL; 9-BP DEL, NT373; IVS5, -1; CODON 344 SPLICE MUT; 1-BP INS; GLY589ASP (rs120074190); PRO117LEU (rs120074191); ARG583CYS (rs17221854); GLY269SER (rs120074193); GLY269ASP (rs120074194); VAL254MET AND VAL417; 1-BP DEL/2-BP INS, N; ARG518TER (rs17215500); ALA525THR (rs120074188); 1-BP DEL, 562T; ARG243PRO (rs120074196) with long QT (http://www.ncbi.nlm.nih.gov/omim/607542?report=Variants) VAL307LEU (rs120074195) with short QT (http://www.ncbi.nlm.nih.gov/omim/607542?report=Variants)	expressed in heart.			($p=3x10^{-17}$)[36] rs12576239 with QT interval ($p=1x10^{-15}$) and rs2074238 with QT interval ($p=3x10^{-17}$)[37]	QTc interval
LMNA	Yes 52 variants reported in OMIM, 11 with dilated cardiomyopathy and 41 with other conditions (including muscular dystrophy, charcot-marie-tooth disease, lipdystrophy and others): ARG60GLY (rs28928900); LEU85ARG (rs28933090); ASN195LYS (rs28933091); GLU203GLY (rs28933092); ARG571SER (rs80338938); 1-BP DEL, 959T; GLU161LYS (rs28933093); 1-BP INS, 28A; SER573LEU (rs60890628); ALA57PRO	Gene encodes lamins, structural proteins responsible for nuclear shape/size.	No	7	None	rs2485662: p=0.0000128 with QRS axis

	(rs28928903); LEU59ARG with dilated cardiomyopathy ARG298CYS (rs59885338); HIS222TYR (rs28928901); GLN6TER (rs61046466); ARG453TRP (rs58932704); ARG527PRO (rs57520892); LEU530PRO (rs60934003); ARG133PRO (rs60864230); GLU358LYS (rs60458016); IVS9AS, T-G, -12; GLY608GLY (rs58596362); GLY608SER (rs61064130); VAL607VAL (rs59886214); GLU145LYS (rs60310264); LEU140ARG (rs60652225); ARG482GLN (rs11575937); ARG482TRP (rs57920071); ARG482LEU (rs11575937); GLY465ASP (rs61282106); ARG582HIS (rs57830985); ARG133LEU (rs60864230); IVS8, G-C, +5; ASP230ASN (rs61214927); ARG399CYS (rs58672172); ARG527HIS (rs57520892); ARG527CYS (rs57318642); LYS542ASN (rs56673169); ALA529VAL (rs60580541); ALA529THR; ARG471CYS (rs28928902); VAL440MET; SER143PHE (rs58912633); LEU380SER; ARG249TRP; 3-BP DEL, 94AAG; ARG377HIS (rs61672878); 3-BP DEL, EXON 3; IVS9, G-C, +5; TYR259TER (rs58048078); GLN493TER (rs56699480); IVS11, G-A, +1; ARG644CYS with other conditions					
PLN	Yes ARG9CYS (rs111033559) with dilated cardiomyopathy[38] LEU39TER (rs111033560) with dilated cardiomyopathy[39] 3-BP DEL, 39AGA with dilated cardiomyopathy[40]	Phospholamban, a substrate for cAMP-dependent protein kinase in heart.	Yes	3	rs11970286 with QT interval ($p=2x10^{-24}$) and rs12210810 with QT interval ($p=2x10^{-17}$)[36] rs11153730 with QT interval ($p=2x10^{-29}$)[41] rs11756438 with QT interval ($p=5x10^{-22}$)[37] rs11153730 with	rs3752581: p=0.00143318 with QTc interval

					QRS duration ($p=1x10^{-18}$)[17]	
PRKAG2	Yes 11 mutations reported in OMIM, 2 with Wolff-Parkinson-White syndrome, 8 with familial hypertrophic cardiomyopathy and 1 with glycogen storage disease of the heart: HIS142ARG (rs121908988); 3-BP INS, 327TTA; THR400ASN (rs28938173); ASN488ILE (rs121908989); TYR487HIS; HIS530ARG; GLU506GLN; SER548PRO with familial hypertrophic cardiomyopathy (http://www.ncbi.nlm.nih.gov/omim/602743?report=Variants) ARG302GLN (rs121908987); ARG531GLY (rs121908990) with Wolff-Parkinson-White syndrome (http://www.ncbi.nlm.nih.gov/omim/602743?report=Variants) ARG531GLN (rs121908991) with glycogen storage disease of the heart (http://www.ncbi.nlm.nih.gov/omim/602743?report=Variants)	A protein kinase with a role in metabolic stress-sensing.	No	113	None	rs1860744: p=0.001899017 with QRS duration
RYR2	Yes 10 mutations listed in OMIM, 8 with catecholaminergic polymorphic ventricular tachycardia, 2 with familial arrhythmogenic right ventricular dysplasia: SER2246LEU (rs121918597); ARG2474SER (rs121918598); ASN4104LYS (rs121918599); ARG4497CYS (rs121918600); PRO2328SER (rs121918603); VAL4653PHE (rs121918604); GLN4201ARG (rs121918605); ALA4860GLY (rs121918606) with catecholaminergic polymorphic ventricular tachycardia ASN2386ILE (rs121918601); LEU433PRO (rs121918602) with familial	Cardiac ryanodine receptor (calcium-release channel).	No	194	None	rs2152885: p=0.005047473 with QRS axis

	arrhythmogenic right ventricular dysplasia					
SCN1B	Yes CYS121TRP (rs104894718) with generalized epilepsy[42] IVS2AS, A-C, -2 with generalized epilepsy[43] 536G-A, TRP179TER with Brugada syndrome[44] GLU87GLN (rs121434627), 537G-A, TRP179TER with nonspecific cardiac conduction defect[44]	Voltage-gated sodium channel expressed in heart.	No	0	None	N/A
SCN4B	Yes LEU179PHE (rs121434386) with long QT[45]	Voltage-gated sodium channel.	No	0	None	N/A
SCN5A	Yes 41 mutations listed in OMIM, with a range of conduction-related phenotypes: VAL232ILE and LEU130 (rs41313031,45471994); ARG1232TRP AND THR16; IVS7DS, 2-BP INS; 1-BP DEL, VAL1398TER; ARG1512TRP; ALA1924THR; ARG367HIS (rs28937318); ALA735VAL; ARG1193GLN (rs41261344); TYR1795HIS; GLY1262SER; GLU1053LYS; TRP1421TER with Brugada Syndrome GLY514CYS with Nonprogressive Cardiac Conduction Defect ASP1275ASN; THR220ILE (rs45620037); 2-BP INS, NT2550; ASP1595HIS with Dilated Cardiomyopathy 1-BP DEL, 5280G with Nonprogressive Heart Block ASP1819ASN; LYS1505/PRO1506/GLN1; ARG1644HIS (rs28937316); ASN1325SER (rs28937317); ARG1623GLN; GLU1784LYS; 3-BP INS, 5537TGA;	Cardiac voltage-gated sodium channel.	Yes	40	rs11129795 with QT interval ($p=5x10^{-14}$)[36] rs12053903 with QT interval ($p=1x10^{-14}$)[37] rs11708996 with PR interval ($p=6x10^{-26}$)[46] rs3922844 with PR interval ($p=3x10^{-23}$)[47]	rs7372712: $p=6.21x10^{-10}$ with PR interval rs7374540: $p=5.87x10^{-9}$ with QRS duration

	SER941ASN; ALA997SER; ARG1826HIS; TYR1795CYS; SER1103TYR (rs7626962) with long QT IVS22DS, T-C, +2; ASP1595ASN; GLN298SER; THR512ILE AND HIS558 (rs1805124) with Progressive Familial Heart Block PRO1298LEU; GLY1408ARG; THR220ILE (rs45620037); ARG1623TER with Sick Sinus Syndrome SER1710LEU with Paroxysmal Familial Ventricular Fibrillation					
Genes associated with ECG phenotypes from genome-wide association studies (GWAS)						
ARHGAP24	No	Negative regulator of Rho GTPases.	Yes	0	rs7692808 with PR interval ($p=6x10^{-20}$)[46]	N/A
CAV1-CAV2	No	Caveolins, scaffold proteins in caveolae.	Yes	16	rs3807989 with PR interval at ($p=4x10^{-28}$)[46]	rs4730743: 0.00000443 with PR interval
CDKN1A	No	Role in cell cycle arrest following DNA damage.	Yes	18	rs9470361 with QRS duration ($p=3x10^{-27}$)[17] rs1321311 with QRS duration ($p=2.7x10^{-10}$)[48]	rs3176326: 0.000000141 with QRS duration
DKK1	No	Inhibitor of WNT signalling.	Yes	0	rs1733724 with QRS duration ($p=3x10^{-8}$)[17]	N/A

MEIS1	No	Homeobox gene implicated in leukemia.	Yes	38	rs11897119 with PR interval ($p=5x10^{-11}$)[46]	rs12619205: 0.00042975 with PR interval
MYH6	Yes 7 mutations listed in OMIM, 5 cardiomyopathy, 1 atrial septal defect and 1 hybrid gene resulting in a heavy chain variant: ILE820ASN with Atrial Septal Defect (http://www.ncbi.nlm.nih.gov/omim/160710?report=Variants) PRO830LEU; ALA1004SER; GLU1457LYS; ARG795GLN; GLN1065HIS with Familial Hypertrophic Cardiomyopathy (http://www.ncbi.nlm.nih.gov/omim/160710?report=Variants) MYH6/MYH7 HYBRID with Heavy Chain Variant of Cardiac Myosin, Cardiac (http://www.ncbi.nlm.nih.gov/omim/160710?report=Variants)	Cardiac myosin, with mutations causing cardiomyopathy.	Yes	1	rs365990 with resting heart rate ($p=4x10^{-14}$)[49]	rs365990: 0.050143516 with QRS duration
NKX2-5	Yes 17 mutations listed in OMIM, 9 atrial septal defect, 2 atrioventricular block, 4 Tetralogy of Fallot and 2 hypothyroidism: THR178MET (rs104893900); GLN170TER (rs104893901); GLN198TER (rs104893903); 7-BP DEL; 2-BP DEL, 223CG; 1-BP DEL, 262G; ARG190CYS (rs104893906); TYR256TER (rs104893907); ASP299GLY with Atrial Septal Defect With Atrioventricular Conduction Defects IVS1DS, G-T, +1 with Idiopathic Atrioventricular Block LYS183GLU with Somatic Atrioventricular Septal Defect ALA119SER; ARG161PRO with Congenital Hypothyroidism	Homolog of a mouse homebox gene assumed to be important in cardiac differentiation	Yes	1	rs251253 with PR interval ($p=9x10^{-13}$)[46]	rs703752: 0.014571864 with QRS duration

	ARG25CYS (rs28936670); GLU21GLN (rs104893904); ARG216CYS (rs104893905); ALA219VAL (rs104893902) with Tetralogy Of Fallot					
NOS1AP	No	Protein that binds the signalling molecule neuronal nitric oxide synthase.	Yes	159	rs10494366 with QT interval ($p=1x10^{-10}$)[50] rs12143842 with QT interval ($p=2x10^{-78}$) and rs4657178 with QT interval ($p=7x10^{-33}$)[36] rs12029454 with QT interval ($p=3x10^{-45}$), rs16857031 with QT interval ($p=1x10^{-34}$) and rs12143842 with QT interval ($2x10^{-78}$)[37] rs2880058 with QT interval ($2x10^{-10}$)[51]	rs12039600: $7.12x10^{-23}$ with QTc interval
SCN10A	No	Voltage-gated sodium channel not expresed in heart.	Yes	1	rs6800541 with PR interval ($p=2x10^{-74}$)[46] rs6800541 with	rs7373934: 0.071627558 with QTc interval

					PR interval ($p=9.7x10^{-17}$)[52]	
SOX5	No	Related to SRY.	Yes	5	rs11047543 with PR interval ($p=3x10^{-13}$)[46]	rs4147498: 0.006349122 with QRS duration
TBX5-TBX3			Yes	21		rs1946293: 0.000556618 with PR interval
WNT11			Yes	0		N/A

Supplementary Table 2: Trait and Covariate Correlations

		PR Interval	QRS Axis	QRS Duration	QTc Interval
BWHHS	**PR Interval**	***1.000***			
GRAPHIC					
WHI II					
BWHHS	**QRS Axis**	-0.121	***1.000***		
GRAPHIC		-0.184			
WHII		-0.148			
BWHHS	**QRS Duration**	0.061	-0.140	***1.000***	
GRAPHIC		0.041	-0.123		
WHII		0.066	-0.201		
BWHHS	**QTc Interval (QT corrected for heart rate)**	0.017	-0.116	0.428	***1.000***
GRAPHIC		-0.003	-0.141	0.072	
WHII		0.016	-0.123	0.30	
BWHHS	**Sex**	NA	NA	NA	NA
GRAPHIC		-0.135	0.103	-0.419	0.267
WHI II		-0.12	0.119	-0.23	0.17
BWHHS	**Age**	0.091	-0.134	0.101	0.097
GRAPHIC		0.282	-0.392	-0.002	0.263
WHI II		0.15	-0.184	0.078	0.15
BWHHS	**BMI**	0.112	-0.180	0.096	0.095
GRAPHIC		0.156	-0.411	0.077	0.166
WHI II		0.088	-0.279	0.044	0.09
BWHHS	**Systolic BP (corrected for BP medication)**	0.047	-0.107	0.117	0.111
GRAPHIC		0.110	-0.280	0.191	0.024
WHI II		0.095	-0.232	0.12	0.15

Supplementary table 3: Minor allele frequencies and missingness details for SNPs reported in table 2.

			Whitehall II (N=5059)			BWHHS (N=3443)			GRAPHIC (N=2024)		
SNP	**Major allele**	**Minor (coded) allele**	**MAF**	**N with genotype and phenotype**	**N with genotype**	**MAF**	**N with genotype and phenotype**	**N with genotype**	**MAF**	**N with genotype and phenotype**	**N with genotype**
PR Interval											
rs7372712	C	T	0.1922	4865	5011	0.1965	3002	3405	0.1787	1887	2024
rs3807989	G	A	0.4051	4880	5026	0.4090	3006	3408	0.3843	1887	2024
QRS Duration											
rs7374540	A	C	0.3977	4873	5019	0.3855	3116	3397	0.3955	1875	2024
rs3176326	G	A	0.1974	4871	5016	0.1955	3125	3409	0.2075	1875	2024
rs10988728	A	G	0.01194	4880	5026	0.01555	3125	3408	0.01416	1875	2024
QTc Interval											
rs12039600	G	A	0.1154	4879	5025	0.1153	3125	3408	0.1075	1881	2023
rs12271931	A	G	0.1377	4876	5022	0.1334	3123	3406	0.1401	1881	2024
rs3815459	G	A	0.2079	4876	5022	0.2081	3118	3400	0.2056	1881	2024
rs2267368	A	A	0.01899	4882	5028	0.01687	3125	3408	0.01709	1881	2024
QRS Axis											
rs2132570	A	A	0.2212	4880	5026	0.2235	3074	3403	0.2206	1893	2022
rs1934968	A	A	0.1128	4880	5026	0.1054	3123	3406	0.1212	1893	2023

Supplementary table 4: SNPs reaching array-wide significance (threshold $p < 1 \times 10^{-5}$) for all four phenotypes.

SNP	CHR	BP	coded allele	non-coded allele	Gene	I^2	Fixed Effects Beta (SE)	Fixed Effects P-Value	Random Effects Beta	Random Effects P-Value
QRS Axis										
rs2132570	7	45928988	A	C	*IGFBP3*	0	2.41 (0.51)	2.89E-06	2.41 (0.51)	2.89E-06
rs10255707	7	45921217	T	C	*IGFBP3*	0	2.42 (0.52)	3.23E-06	2.42 (0.52)	3.23E-06
rs903889	7	45931520	C	A	*IGFBP3*	0	2.37 (0.51)	4.21E-06	2.37 (0.51)	4.21E-06
rs1934968	10	96731807	A	G	*CYP2C9*	0	-3.17 (0.68)	3.43E-06	-3.17 (0.68)	3.43E-06
QRS Duration										
rs7374540	3	38609146	C	A	*SCN5A*	0	0.00104 (0.00018)	5.87E-09	0.00104 (0.00018)	5.87E-09
rs9861242	3	38584338	A	G	*SCN5A*	1.57	0.00112 (0.00020)	1.20E-08	0.00112 (0.00020)	1.59E-08
rs1805126	3	38567410	C	T	*SCN5A*	0	0.00099 (0.00018)	6.93E-08	0.00099 (0.00018)	6.93E-08
rs11710077	3	38632903	T	A	*SCN5A*	0	-0.00113 (0.00022)	1.80E-07	-0.00113 (0.00022)	1.80E-07
rs9833086	3	38585475	G	A	*SCN5A*	0	0.00099 (0.00019)	2.05E-07	0.00099 (0.00019)	2.05E-07
rs12053903	3	38568397	C	T	*SCN5A*	0	0.00088 (0.00019)	1.97E-06	0.00088 (0.00019)	1.97E-06
rs10154914	3	38607634	A	T	*SCN5A*	79.59	-0.00101 (0.00022)	4.35E-06	-0.00101 (0.00050)	4.37E-02
rs6797133	3	38631037	A	G	*SCN5A*	0	-0.00080 (0.00018)	8.73E-06	-0.00080 (0.00018)	8.73E-06
rs3176326	6	36755267	A	G	*CDKN1A*	0	0.00115 (0.00022)	1.41E-07	0.00115 (0.00022)	1.41E-07
rs3176320	6	36754766	G	A	*CDKN1A*	43.04	0.00096 (0.00018)	1.94E-07	0.00100 (0.00025)	5.77E-05
rs733590	6	36753181	C	T	*CDKN1A*	67.8	0.00091 (0.00018)	4.19E-07	0.00102 (0.00033)	1.93E-03
rs3176323	6	36754827	C	T	*CDKN1A*	10.67	0.00093 (0.00019)	6.47E-07	0.00094 (0.00020)	2.46E-06
rs2395655	6	36753674	G	A	*CDKN1A*	76.13	0.00089 (0.00018)	7.89E-07	0.00102 (0.00038)	6.99E-03
rs762624	6	36753566	C	A	*CDKN1A*	61.67	0.00095 (0.00020)	1.70E-06	0.00104 (0.00033)	1.61E-03
rs10988728	9	1.01E+08	G	A	*TGFBR1*	0	0.00352 (0.00079)	7.90E-06	0.00352 (0.00079)	7.90E-06
PR Interval										
rs7372712	3	38661196	T	C	*SCN5A*	0	0.00305 (0.00045)	8.08E-12	0.00302 (0.00049)	6.21E-10
rs12053903	3	38568397	C	T	*SCN5A*	0	0.00216 (0.00037)	4.59E-09	0.00216 (0.00037)	4.59E-09
rs7374540	3	38609146	C	A	*SCN5A*	0	0.00186 (0.00035)	9.50E-08	0.00186 (0.00035)	9.50E-08
rs1805126	3	38567410	C	T	*SCN5A*	0	0.00174 (0.00037)	2.07E-06	0.00174 (0.00037)	2.07E-06
rs7624535	3	38640206	G	T	*SCN5A*	28.77	-0.00194 (0.00042)	4.33E-06	-0.00194 (0.00042)	4.33E-06
rs6768664	3	38659470	C	A	*SCN5A*	66.65	-0.00156 (0.00034)	4.75E-06	-0.00156 (0.00034)	4.75E-06

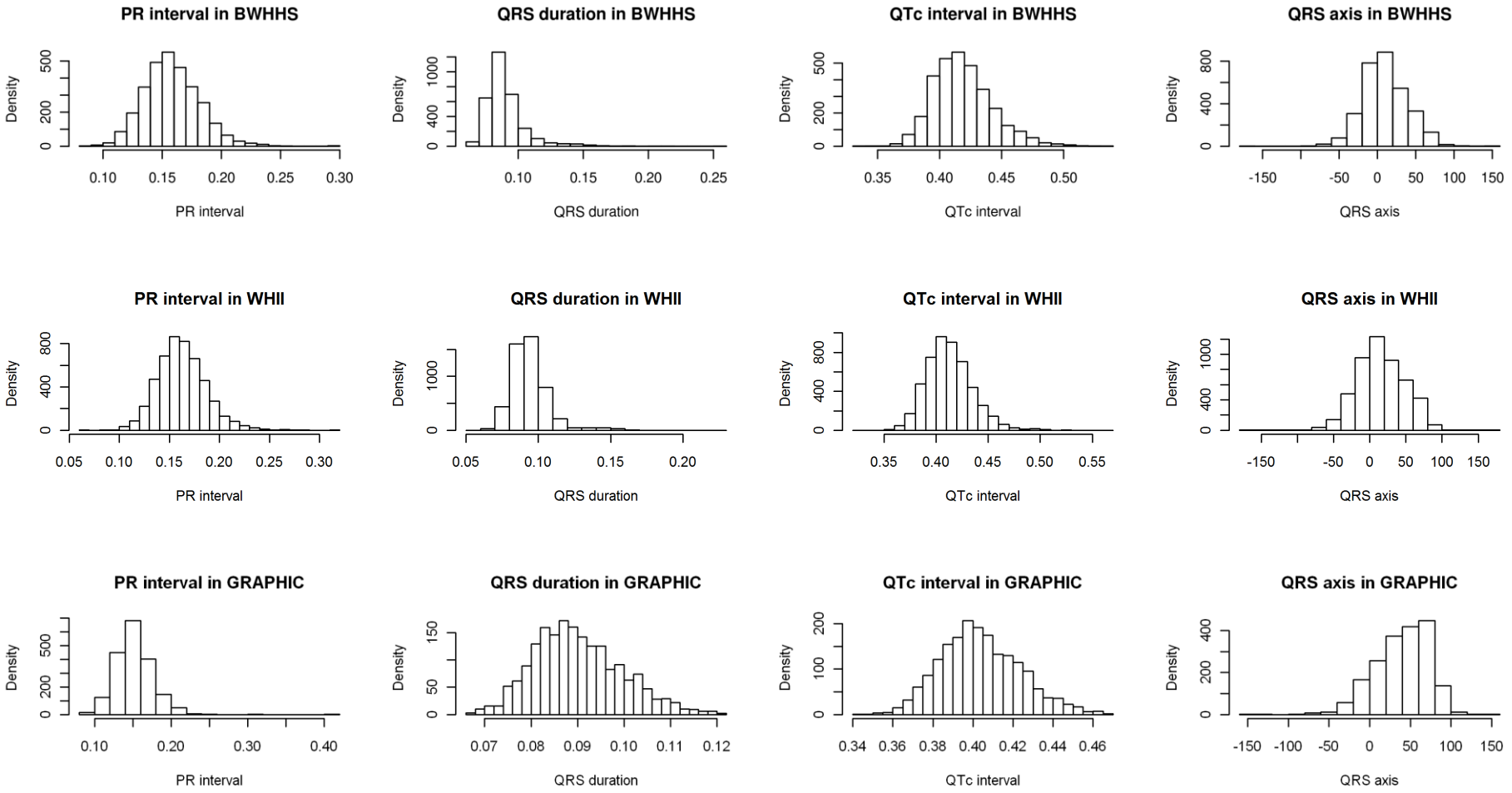
rs7373102	3	38655632	T	C	*SCN5A*	64.75	-0.00155 (0.00034)	5.32E-06	-0.00155 (0.00034)	5.32E-06
rs3807989	7	1.16E+08	A	G	*CAV1*	67.89	0.00199 (0.00036)	1.99E-08	0.00199 (0.00036)	1.99E-08
rs3807986	7	1.16E+08	G	A	*CAV1*	64.59	0.00193 (0.00041)	2.55E-06	0.00186 (0.00049)	1.69E-04
rs4730743	7	1.16E+08	A	T	*CAV2*	4.1	-0.00160 (0.00035)	4.43E-06	-0.00160 (0.00035)	4.43E-06
rs1049334	7	1.16E+08	A	G	*CAV1*	38.28	0.00295 (0.00066)	8.10E-06	0.00295 (0.00066)	8.10E-06
QTc Interval										
rs12039600	1	1.60E+08	A	G	*NOS1AP*	0	0.00476 (0.00048)	7.12E-23	0.00476 (0.00048)	7.12E-23
rs7534004	1	1.60E+08	A	G	*NOS1AP*	15.18	0.00422 (0.00044)	7.89E-22	0.00422 (0.00044)	7.89E-22
rs10918859	1	1.60E+08	A	G	*NOS1AP*	7.57	0.00372 (0.00040)	3.48E-20	0.00372 (0.00040)	3.48E-20
rs10800352	1	1.60E+08	G	A	*NOS1AP*	0	0.00348 (0.00038)	6.12E-20	0.00348 (0.00038)	6.12E-20
rs10918594	1	1.60E+08	G	C	*NOS1AP*	39.98	0.00283 (0.00033)	4.49E-18	0.00283 (0.00033)	4.49E-18
rs7522678	1	1.60E+08	A	C	*NOS1AP*	0	0.00337 (0.00039)	5.44E-18	0.00337 (0.00039)	5.44E-18
rs4657139	1	1.60E+08	A	T	*NOS1AP*	57.31	0.00272 (0.00032)	3.80E-17	0.00272 (0.00032)	3.80E-17
rs6664702	1	1.60E+08	C	T	*NOS1AP*	0	0.00299 (0.00036)	1.28E-16	0.00299 (0.00036)	1.28E-16
rs6427664	1	1.60E+08	A	G	*NOS1AP*	0	0.00302 (0.00037)	1.52E-16	0.00297 (0.00047)	2.97E-10
rs10399680	1	1.60E+08	C	T	*NOS1AP*	0	0.00294 (0.00036)	2.20E-16	0.00285 (0.00050)	1.57E-08
rs12733821	1	1.60E+08	G	C	*NOS1AP*	74.69	0.00260 (0.00032)	4.92E-16	0.00260 (0.00032)	4.92E-16
rs10494366	1	1.60E+08	G	T	*NOS1AP*	72.21	0.00253 (0.00032)	1.70E-15	0.00253 (0.00032)	1.70E-15
rs11579850	1	1.60E+08	G	T	*NOS1AP*	75.81	0.00249 (0.00032)	3.66E-15	0.00249 (0.00032)	3.66E-15
rs1415257	1	1.60E+08	G	A	*NOS1AP*	72.34	0.00249 (0.00032)	4.10E-15	0.00249 (0.00032)	4.10E-15
rs16847548	1	1.60E+08	C	T	*NOS1AP*	0	0.00291 (0.00037)	7.33E-15	0.00303 (0.00057)	1.34E-07
rs1415262	1	1.60E+08	C	G	*NOS1AP*	48.33	0.00246 (0.00032)	1.07E-14	0.00246 (0.00032)	1.07E-14
rs5000342	1	1.60E+08	G	A	*NOS1AP*	0	0.00254 (0.00033)	1.24E-14	0.00254 (0.00033)	1.24E-14
rs10800409	1	1.61E+08	T	C	*NOS1AP*	0	0.00344 (0.00045)	1.35E-14	0.00331 (0.00063)	1.23E-07
rs945708	1	1.60E+08	A	G	*NOS1AP*	59.52	0.00242 (0.00032)	1.50E-14	0.00242 (0.00032)	1.50E-14
rs1932933	1	1.60E+08	A	G	*NOS1AP*	61.87	0.00241 (0.00031)	1.69E-14	0.00241 (0.00031)	1.69E-14
rs6660701	1	1.60E+08	C	G	*NOS1AP*	53.06	0.00242 (0.00032)	2.28E-14	0.00242 (0.00032)	2.28E-14
rs10919035	1	1.61E+08	T	C	*NOS1AP*	8.96	0.00338 (0.00045)	5.17E-14	0.00324 (0.00070)	3.32E-06
rs4657166	1	1.60E+08	G	C	*NOS1AP*	68.68	0.00251 (0.00034)	1.26E-13	0.00251 (0.00034)	1.26E-13

rs4233385	1	1.60E+08	C	T	*NOS1AP*	30.08	0.00240 (0.00033)	1.63E-13	0.00240 (0.00033)	1.63E-13
rs880296	1	1.60E+08	C	G	*NOS1AP*	45.88	0.00279 (0.00038)	1.63E-13	0.00279 (0.00038)	1.63E-13
rs10918762	1	1.60E+08	G	A	*NOS1AP*	9.34	0.00280 (0.00038)	1.96E-13	0.00280 (0.00038)	1.96E-13
rs4657154	1	1.60E+08	A	G	*NOS1AP*	66.61	0.00238 (0.00035)	5.03E-12	0.00238 (0.00035)	5.03E-12
rs10800279	1	1.60E+08	C	T	*NOS1AP*	4.57	0.00229 (0.00034)	1.30E-11	0.00238 (0.00044)	7.03E-08
rs7515045	1	1.60E+08	A	C	*NOS1AP*	34.99	0.00230 (0.00034)	2.00E-11	0.00230 (0.00034)	2.00E-11
rs10494365	1	1.60E+08	G	C	*NOS1AP*	0	0.00313 (0.00047)	2.25E-11	0.00357 (0.00104)	5.99E-04
rs10918602	1	1.60E+08	C	T	*NOS1AP*	2.56	0.00226 (0.00034)	2.97E-11	0.00232 (0.00041)	1.16E-08
rs6702936	1	1.60E+08	G	A	*NOS1AP*	0	0.00223 (0.00034)	4.49E-11	0.00225 (0.00036)	3.06E-10
rs6659759	1	1.60E+08	C	T	*NOS1AP*	12.83	0.00221 (0.00034)	5.71E-11	0.00225 (0.00038)	3.26E-09
rs4531275	1	1.60E+08	T	C	*NOS1AP*	0	0.00202 (0.00034)	2.08E-09	0.00202 (0.00034)	2.08E-09
rs1123217	1	1.60E+08	C	G	*NOS1AP*	0	0.00461 (0.00078)	3.16E-09	0.00380 (0.00228)	9.55E-02
rs12128479	1	1.61E+08	G	A	*NOS1AP*	45.53	0.00316 (0.00053)	3.33E-09	0.00316 (0.00053)	3.33E-09
rs10800366	1	1.60E+08	T	C	*NOS1AP*	4	0.00195 (0.00033)	4.75E-09	0.00195 (0.00033)	4.75E-09
rs12026452	1	1.60E+08	A	G	*NOS1AP*	55.72	0.00260 (0.00045)	7.18E-09	0.00254 (0.00051)	6.12E-07
rs10800397	1	1.61E+08	T	C	*NOS1AP*	0	0.00213 (0.00037)	1.14E-08	0.00213 (0.00037)	1.14E-08
rs16857031	1	1.60E+08	G	C	*NOS1AP*	66.94	0.00248 (0.00044)	1.34E-08	0.00246 (0.00046)	8.78E-08
rs10458392	1	1.60E+08	G	T	*NOS1AP*	3.71	0.00211 (0.00037)	1.47E-08	0.00211 (0.00037)	1.47E-08
rs3923368	1	1.60E+08	C	A	*NOS1AP*	5.56	0.00179 (0.00032)	3.10E-08	0.00179 (0.00032)	3.10E-08
rs10800404	1	1.61E+08	T	G	*NOS1AP*	0	0.00207 (0.00037)	3.26E-08	0.00207 (0.00037)	3.26E-08
rs16860185	1	1.61E+08	A	G	*NOS1AP*	64.97	0.00279 (0.00052)	6.37E-08	0.00279 (0.00052)	6.37E-08
rs7513132	1	1.60E+08	A	G	*NOS1AP*	49.67	0.00173 (0.00032)	7.03E-08	0.00173 (0.00032)	7.03E-08
rs10918936	1	1.60E+08	A	G	*NOS1AP*	0	0.00170 (0.00032)	7.57E-08	0.00170 (0.00032)	7.57E-08
rs7540690	1	1.60E+08	A	G	*NOS1AP*	30.88	0.00201 (0.00038)	1.11E-07	0.00210 (0.00048)	1.08E-05
rs4657161	1	1.60E+08	G	A	*NOS1AP*	80.95	0.00165 (0.00032)	1.81E-07	0.00165 (0.00032)	1.81E-07
rs12135795	1	1.60E+08	A	G	*NOS1AP*	79.64	0.00164 (0.00032)	1.98E-07	0.00164 (0.00032)	1.98E-07
rs3927640	1	1.60E+08	T	C	*NOS1AP*	77.61	0.00162 (0.00032)	2.71E-07	0.00162 (0.00032)	2.71E-07
rs10753765	1	1.60E+08	G	A	*NOS1AP*	80.06	0.00160 (0.00031)	3.86E-07	0.00160 (0.00031)	3.86E-07
rs4557949	1	1.60E+08	A	T	*NOS1AP*	0	0.00156 (0.00031)	4.44E-07	0.00156 (0.00031)	4.44E-07

rs12734991	1	1.60E+08	C	T	*NOS1AP*	0	0.00157 (0.00031)	4.65E-07	0.00157 (0.00031)	4.65E-07
rs12022557	1	1.60E+08	A	G	*NOS1AP*	19.92	0.00184 (0.00037)	5.65E-07	0.00184 (0.00037)	6.64E-07
rs10918615	1	1.60E+08	A	G	*NOS1AP*	31.62	0.00182 (0.00037)	7.21E-07	0.00182 (0.00037)	7.21E-07
rs4145621	1	1.60E+08	C	T	*NOS1AP*	0	0.00149 (0.00031)	1.44E-06	0.00149 (0.00031)	1.44E-06
rs12742393	1	1.60E+08	A	C	*NOS1AP*	0	0.00150 (0.00032)	1.95E-06	0.00150 (0.00032)	1.95E-06
rs2661818	1	1.61E+08	G	C	*NOS1AP*	37.18	0.00146 (0.00031)	2.35E-06	0.00146 (0.00031)	2.35E-06
rs3815459	7	1.50E+08	A	G	*KCNH2*	0	0.00219 (0.00038)	9.44E-09	0.00219 (0.00038)	9.44E-09
rs6972137	7	1.50E+08	G	T	*KCNH2*	0	0.00233 (0.00041)	1.22E-08	0.00233 (0.00041)	1.22E-08
rs6947240	7	1.50E+08	A	G	*KCNH2*	0	-0.00179 (0.00035)	4.54E-07	-0.00181 (0.00038)	2.43E-06
rs1805123	7	1.50E+08	C	A	*KCNH2*	0	-0.00174 (0.00035)	8.68E-07	-0.00174 (0.00035)	8.68E-07
rs3807375	7	1.50E+08	A	G	*KCNH2*	5.3	0.00143 (0.00032)	9.49E-06	0.00143 (0.00032)	9.49E-06
rs12271931	11	2435095	G	A	*KCNQ1*	36.93	-0.00292 (0.00044)	3.17E-11	-0.00301 (0.00074)	4.36E-05
rs2267368	22	36895155	A	G	*PLA2G6*	72.98	-0.00499 (0.00112)	7.80E-06	-0.00499 (0.00112)	7.80E-06

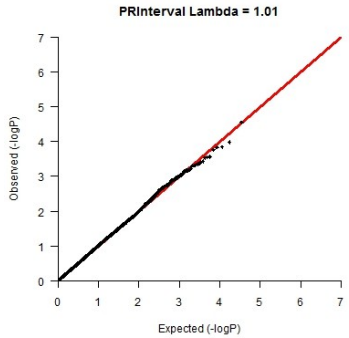
Supplementary Figures

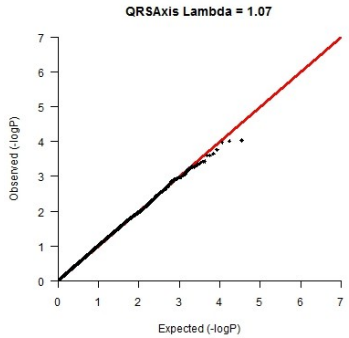
Supplementary Figure 1: Phenotype distributions in the three cohorts

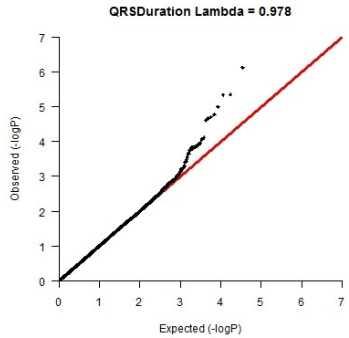


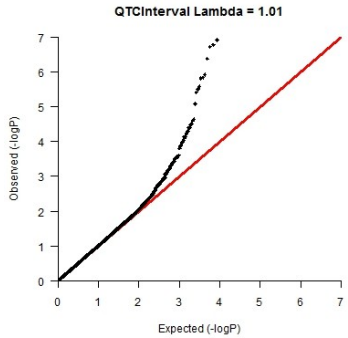
Supplementary Figure 2: QQ plots for each trait

BWHHS:









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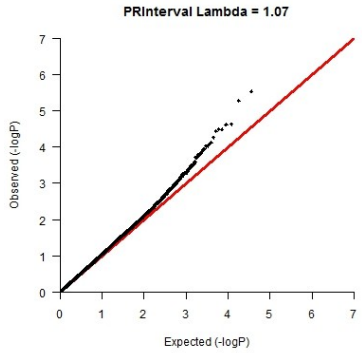
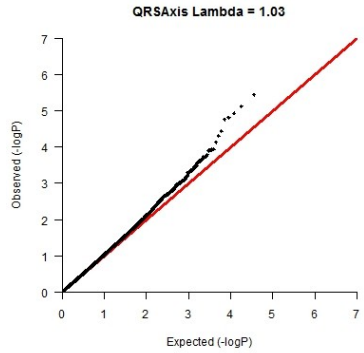
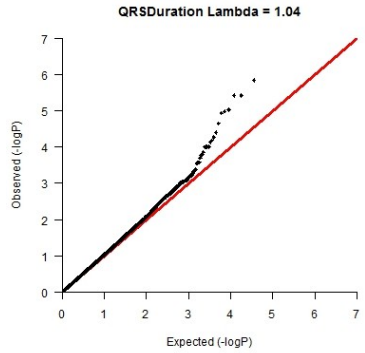
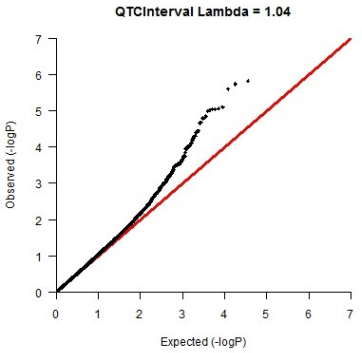
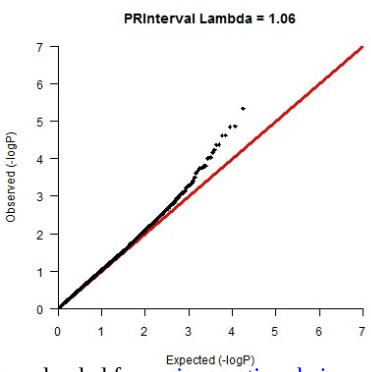


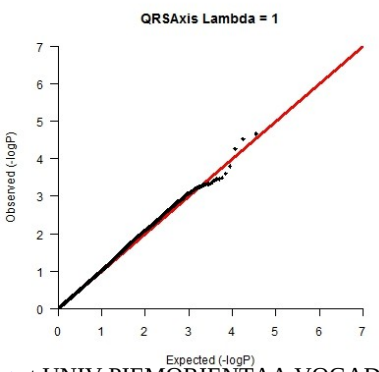


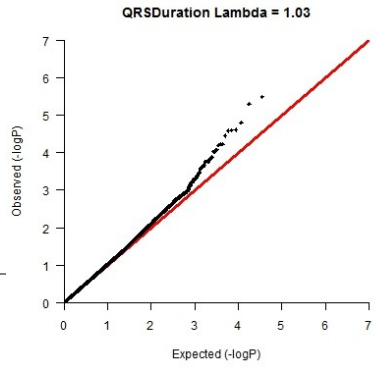


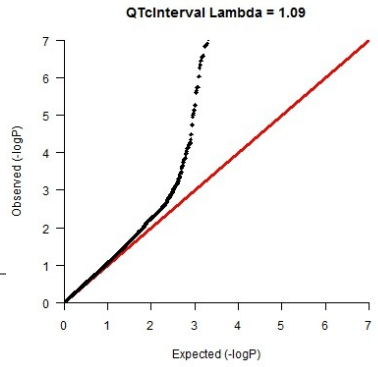


WHII

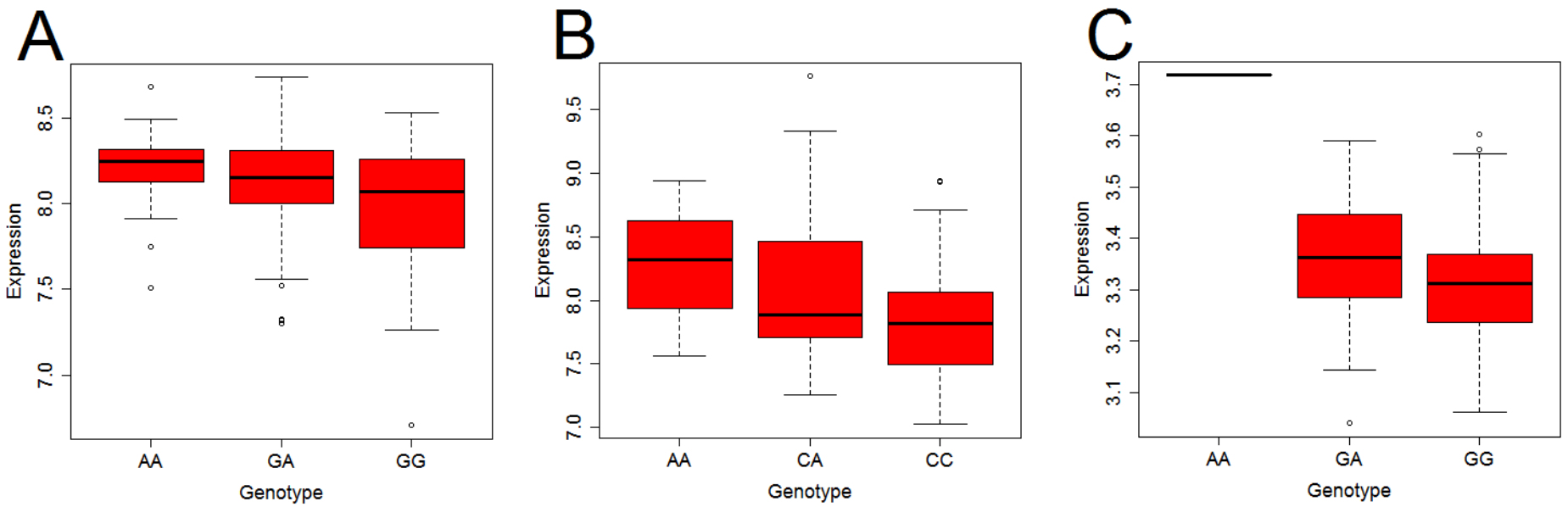




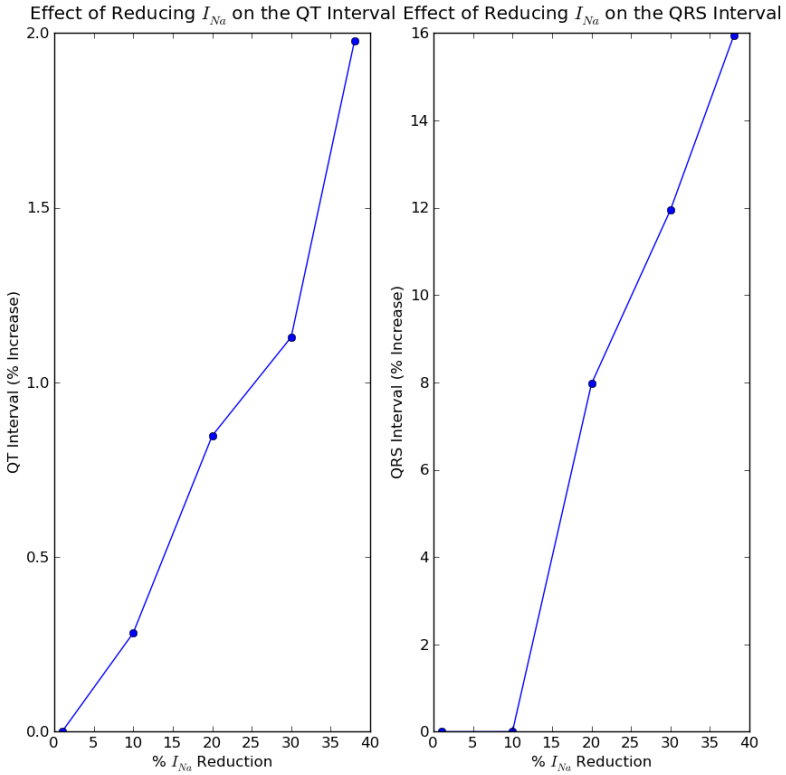




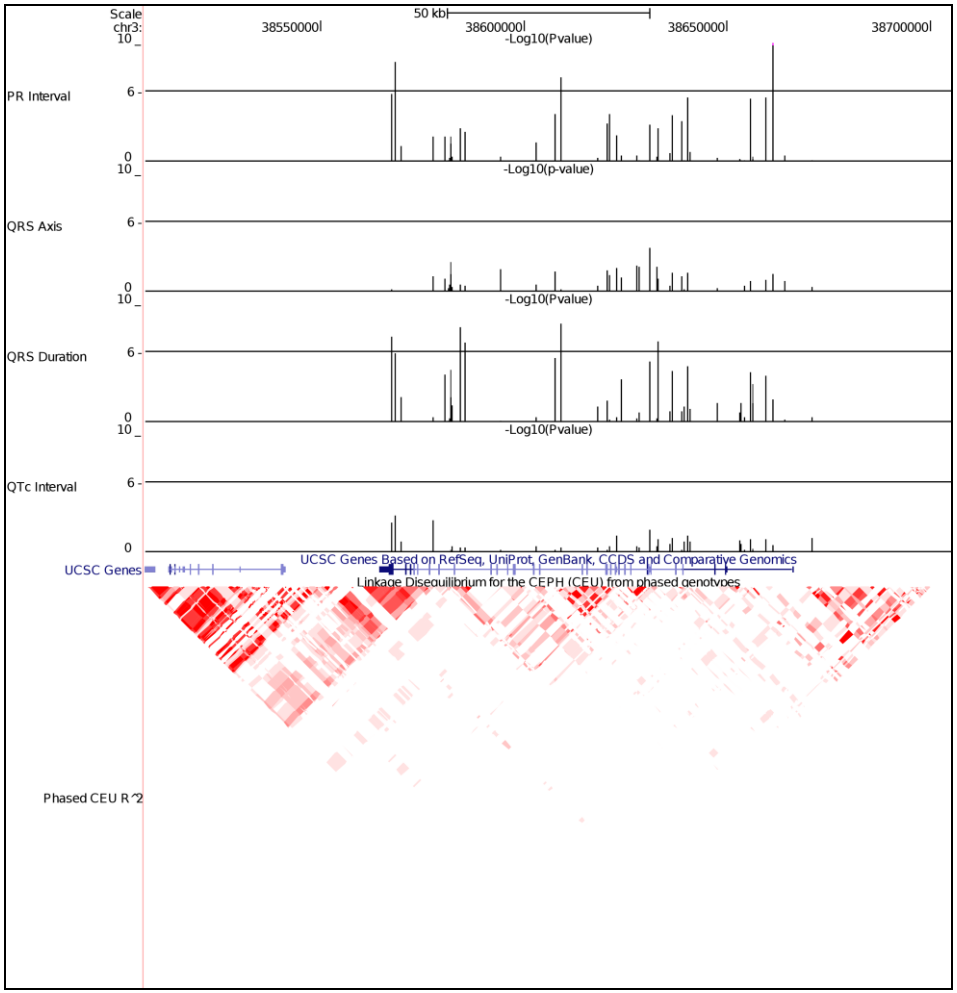
Supplementary Figure 3: eQTL plots illustrating differential expression of (A) *CAV1* by rs3807989 genotype, (B) *IGFBP3* by rs2132570 genotype, and (C) *CYP2C9* by rs1934968 genotype.



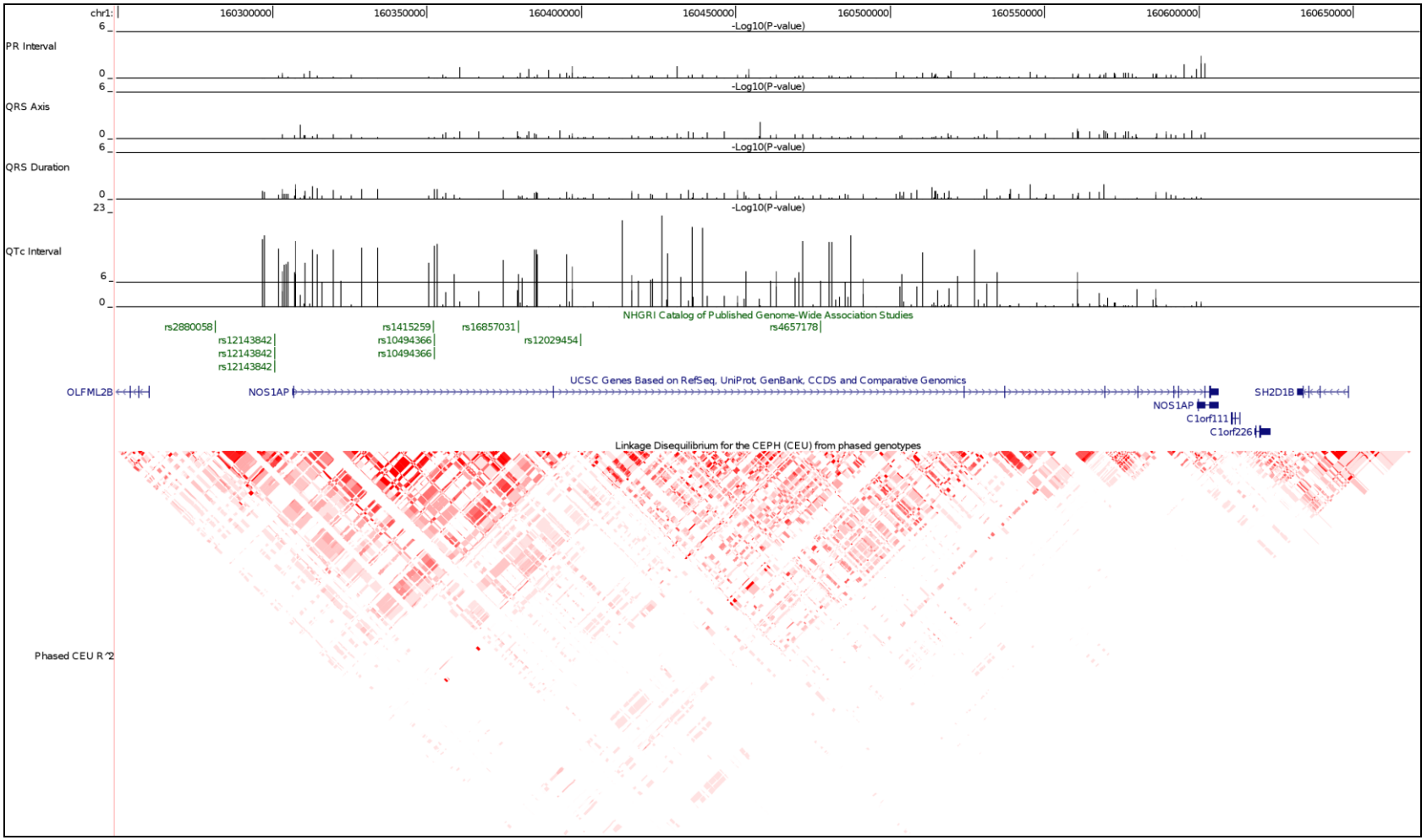
Supplementary Figure 4: Illustrative example of modelling the effects on QT interval and QRS duration for a given change of I_{Na}. In both cases as the % I_{Na} reduction increases (x-axis) the predicted QT interval increases (y-axis).



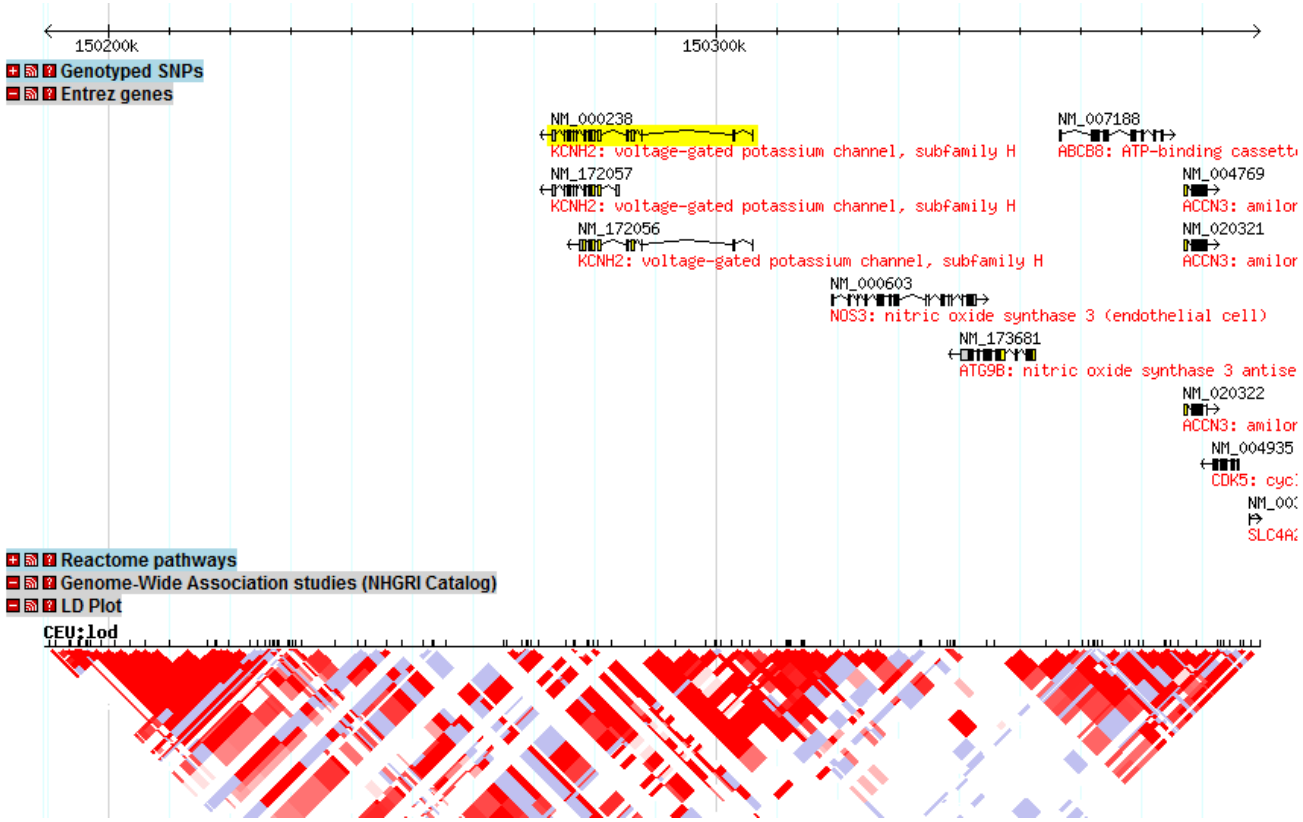
Supplementary Figure 5: SNP associations with all four traits across the *SCN5A* region illustrated in the UCSC genome browser (http://genome.ucsc.edu/). Genomic location is represented on the horizontal axis, pairwise linkage disequilibrium illustrated by red in the triangular heat map (bottom) and SNP associations by the height of bars in the four charts (top)



Supplementary Figure 6: SNP associations with all four traits across the *NOS1AP* region illustrated in the UCSC genome browser (http://genome.ucsc.edu/). Genomic location is represented on the horizontal axis, pairwise linkage disequilibrium illustrated by red in the triangular heat map (bottom) and SNP associations by the height of bars in the four charts (top)



Supplementary Figure 7: Linkage disequilibrium block around *KCNH2* and *NOS3* as presented in the HapMap browser (www.hapmap.org) with genomic location on the horizontal axis and pairwise linkage disequilibrium illustrated by red in the triangular heat map (bottom).



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