

ORIGINAL ARTICLE

Large-scale pharmacogenomic study of sulfonylureas and the QT, JT and QRS intervals: CHARGE Pharmacogenomics Working Group

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Sulfonylureas, a commonly used class of medication used to treat type 2 diabetes, have been associated with an increased risk of cardiovascular disease. Their effects on QT interval duration and related electrocardiographic phenotypes are potential mechanisms for this adverse effect. In 11 ethnically diverse cohorts that included 71 857 European, African-American and Hispanic/Latino ancestry individuals with repeated measures of medication use and electrocardiogram (ECG) measurements, we conducted a pharmacogenomic genome-wide association study of sulfonylurea use and three ECG phenotypes: QT, JT and QRS intervals. In ancestry-specific meta-analyses, eight novel pharmacogenomic loci met the threshold for genome-wide significance ($P < 5 \times 10^{-8}$), and a pharmacokinetic variant in *CYP2C9* (rs1057910) that has been associated with sulfonylurea-related treatment effects and other adverse drug reactions in previous studies was replicated. Additional research is needed to replicate the novel findings and to understand their biological basis.

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INTRODUCTION

Sulfonylureas are the oldest class of oral glucose-lowering therapy used to treat type 2 diabetes, and despite the emergence of several new classes of diabetes drugs in recent years,¹ sulfonylureas remain the most widely prescribed oral therapy after metformin.² Since the University Group Diabetes Program trial found that the first-generation sulfonylurea chlorpropamide increased the risk of cardiovascular mortality over 40 years ago,³ there have been concerns about the cardiovascular safety of sulfonylureas. Several studies since then have found that treatment with sulfonylureas is associated with an increased risk of cardiovascular events and mortality compared with other glucose-lowering drugs.^{4,5}

As one potential mechanism of cardiovascular toxicity, sulfonylureas can prolong the QT interval,^{6,7} a marker of cardiac repolarization that is associated with fatal arrhythmias and sudden cardiac death.^{8–12} Indeed, QT prolongation has been one of the most common safety issues leading to drug withdrawals from the market.^{13,14} Since 2005, the Food and Drug Administration has required clinical studies to evaluate whether a new drug prolongs the QT interval of 5 ms before regulatory approval.¹⁵

Variation in the QT interval is heritable,^{16,17} and large scale genome-wide association (GWA) studies have identified at least 35 genetic loci associated with this trait that collectively explain ~10% of interindividual variation in the QT interval.¹⁸ Pharmacogenomic studies of sulfonylurea use and the QT interval may help to unravel the biologic mechanisms underlying the cardiovascular toxicity of sulfonylureas. However, previous pharmacogenomic studies of the glucose-lowering or adverse effects of sulfonylureas have been small and focused on candidate genes,^{19–22} and most findings have not replicated.^{23,24} In the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Pharmacogenomics Working Group, a previous GWA study of sulfonylurea–QT interactions that included ~30 000 European ancestry individuals with cross-sectional measures of drug use and the QT interval did not identify any pharmacogenomic loci at genome-wide levels of significance.²⁵

To increase our power to identify novel pharmacogenomic loci for sulfonylureas, we extended this effort to include several additional diverse-ancestry cohorts with a high prevalence of sulfonylurea use. In addition, we incorporated repeated measures of drug exposure and phenotype with novel analytic methods.²⁶ Because genetic variants can have different effects on the two components of the QT interval²⁷—the JT interval, which measures primarily repolarization, and the QRS interval, which measures primarily conduction and depolarization—we also extended our analyses to include them.

MATERIALS AND METHODS

Study population and overview

Eleven cohorts participated in this meta-analysis from the CHARGE²⁸ Pharmacogenomics Working Group: Age, Gene/Environment Susceptibility – Reykjavik Study (AGES); Atherosclerosis Risk in Communities (ARIC) Study; Cardiovascular Health Study (CHS); Health, Aging, and Body Composition (Health ABC); Hispanic Community Health Study/Study of Latinos (HCHS/SOL); Jackson Heart Study (JHS); Multi-Ethnic Study of Atherosclerosis (MESA); Netherlands Epidemiology of Obesity (NEO) Study; Prospective Study of Pravastatin in the Elderly at Risk (PROSPER); Rotterdam Study cohorts 1 and 2; and the Women's Health Initiative (WHI) (Supplementary Information). Cohorts contributed results from European ancestry (EA), African-American (AA) and/or Hispanic/Latino ancestry (HA) populations. All cohorts had at least one study visit with an assessment of medication use and a resting 12-lead electrocardiogram (ECG); AGES, ARIC, CHS, the Rotterdam Study, MESA and WHI had multiple study visits with these assessments and contributed repeated measures. Each cohort followed a prespecified analysis protocol, and findings from within-cohort analyses were combined in three sets of ancestry-specific meta-analyses (EA, AA and HA) for three ECG phenotypes (QT, JT and QRS intervals), for a total of

nine primary analyses. All available cohorts were included in this single discovery effort, rather than a two-stage design with discovery and replication, to improve our power to identify significant pharmacogenomic interactions.^{29,30} This study was approved by the institutional review board of each cohort.

Inclusion and exclusion criteria

Participants with genome-wide genotype data and with ECG measurements and medication assessments at the same study visits were eligible. The following exclusion criteria were applied: poor ECG quality; atrial fibrillation; second- or third-degree atrioventricular heart block; QRS interval > 120 ms; a paced rhythm; history of heart failure; pacemaker implantation; pregnancy; and ancestry other than European, African American or Hispanic/Latino. For studies with repeated measures, exclusion criteria were applied for each visit-specific observation.

Drug exposure assessment

Sulfonylurea drugs are listed in Supplementary Table 1. Sulfonylurea use was assessed through medication inventories conducted at study visits, or using information from a pharmacy database for the Rotterdam Study (Supplementary Table 2). Some cohorts assessed medication use on the day of the study visit, whereas others assessed medication use within an interval of time before the study visit, typically 2 weeks. For cohorts with repeated measures, the number of participants exposed to sulfonylureas (N_{exposed}) was the sum of the estimated number of independent observations at which each participant was exposed, calculated from the following equation:

$$N_{\text{exposed}} = \sum_i \frac{n_i}{1 + (n_i - 1)\hat{\rho}} \frac{\#\{E_{it} = 1\}}{n_i}$$

where the summand is the product of the estimated number of independent observations and the proportion of observations at which a participant was exposed,³¹ with n_i being the number of observations for participant i , $\hat{\rho}$ an estimate of the pairwise visit-to-visit correlation in outcome within participants from a generalized estimating equation-exchangeable model that does not contain genetic data and $\#\{E_{it} = 1\}$ the number of observations for which participant i was exposed.²⁶

Phenotype measurement

QT and QRS intervals were recorded from resting, supine or semi-recumbent standard 12-lead ECGs (Supplementary Table 2). Across all cohorts, comparable procedures were used for preparing participants, placing electrodes, recording, transmitting, processing and controlling the quality of ECGs. Cohorts used Marquette MAC 5000, MAC 1200 or MAC PC (GE Healthcare, Milwaukee, WI, USA), Burdick Eclipse 850i (Cardiac Science, Manchester, UK) or ACTA (EASOTE, Florence, Italy) machines. Recordings were processed using Marquette 12SL, MEANS or University of Glasgow software (Glasgow, Scotland, UK). The JT interval was calculated by the formula: JT = QT – QRS.

Genotyping and imputation

All cohorts performed genome-wide genotyping with either Affymetrix (Santa Clara, CA, USA) or Illumina (San Diego, CA, USA) arrays, and used similar quality control thresholds for excluding samples and single-nucleotide polymorphisms (SNPs) (Supplementary Table 3). Sex mismatches, duplicate samples and first-degree relatives (except in HCHS/SOL and JHS) were excluded. DNA samples and SNPs with call rates of < 90–98%, depending on the cohort, were excluded. Within each cohort, SNPs with minor allele frequencies (MAFs) of < 1% or that failed Hardy–Weinberg equilibrium were excluded.

Genotypes were imputed using ancestry-specific HapMap2,^{32–34} HapMap3, 1000 Genomes Phase 1 or 1000 Genomes Phase 3 reference panels (Supplementary Table 3).^{35,36} Genotypes imputed from build 37 of the human genome were lifted over to build 36 (refs 37) to enable comparisons between imputation platforms, and all results were restricted to SNPs present in HapMap2.

Statistical analysis

GWA analyses were performed by each cohort separately, and ancestry-specific results for each ECG phenotype were combined with meta-analysis. Within each cohort, for ~2.5 million genotyped or imputed

autosomal SNPs, sulfonylurea–SNP interactions were estimated with an additive genetic model using mixed effects models, generalized estimating equation or linear regression with robust standard errors. The analytic model varied based on the study design and the availability of longitudinal data (Supplementary Table 4). All analyses were adjusted for age, sex, study site or region, principal components of genetic ancestry, visit-specific RR interval (inversely related to heart rate) and visit-specific use of QT-prolonging medications. The QT-prolonging effect of medications was categorized as definite, possible or conditional, according to the University of Arizona Center for Education and Research on Therapeutics (UAZ CERT) system of classification, and adjusted for as binary variables for each category (presence of any versus none).³⁸ HCHS/SOL incorporated estimates of relatedness into all analyses. Cohort-specific results were corrected for genomic inflation.

Previous simulations demonstrated that models using robust standard errors underestimate the variance of coefficient estimates for SNPs with low MAFs.²⁶ To account for this, corrected standard errors were calculated using a *t* distribution as the reference distribution. Cohort and SNP-specific degrees of freedom (d.f.) for the *t* distribution were estimated primarily using the method of Satterthwaite.³⁹ For cohorts unable to implement the method of Satterthwaite,³⁹ an approximate d.f. was calculated as two times the cohort- and SNP-specific product of the SNP imputation quality (0–1), MAF (0.00–0.50) and N_{exposed} . Standard errors were then corrected by assuming a normal reference distribution that yielded the *t* distribution-based *P*-values from the coefficient estimates. Furthermore, because simulations demonstrated that corrected standard errors were unstable when minor allele counts among the exposed were low, an approximate d.f. filter of 10 was applied to cohort-specific results across all SNPs.

Primary analyses

For each ECG phenotype and for each ancestral population, SNP-by-treatment interaction coefficients and corrected standard errors were combined with inverse-variance weighted meta-analysis using METAL.⁴⁰ SNPs had to meet quality control criteria and pass the d.f. filter in at least two studies to be included. The threshold for statistical significance was $P < 5 \times 10^{-8}$, and this has been used in other GWA studies of correlated phenotypes.^{41,42} For each locus with multiple SNPs meeting the threshold for statistical significance, a lead SNP with the lowest *P*-value was identified. Significant loci and loci at suggestive levels of statistical significance ($P < 10^{-6}$) were annotated using information from several genomics and bioinformatics databases. RefSeq genes within 500 kb of lead SNPs were identified from the UCSC (University of California, Santa Cruz) Genome Browser.⁴³ The NHGRI-EBI GWAS Catalog was queried for other traits associated with lead SNPs in GWA studies.⁴⁴ HaploReg (Broad Institute, Cambridge, MA, USA) was queried to identify missense coding variants in linkage disequilibrium (LD) ($R^2 < 0.8$) with lead SNPs.⁴⁵ *Cis*-expression quantitative trait loci (*cis*-eQTLs) in LD with lead SNPs were identified from several gene expression databases, including ScanDB and the Broad Institute GTEx Portal, that include samples from multiple cell lines and tissue sites, including whole blood, leukocytes, subcutaneous adipose, skeletal muscle, lung, skin, fibroblasts, arterial wall and left ventricular and atrial heart tissue.⁴⁶

Secondary analyses

All ancestry-specific summary results were combined in a transethnic inverse-variance weighted meta-analysis using METAL. Because effects

Table 1. Characteristics of study populations

Cohort	N	N_{exposed} (%)	Age, years (s.d.)	Female, N (%)	QT interval, ms (s.d.)	JT interval, ms (s.d.)	QRS interval, ms (s.d.)
<i>European Ancestry</i>							
AGES	2587	64 (2.5)	75 (4.7)	925 (64)	406 (34)	316 (33)	90 (10)
ARIC	8597	379 (4.4)	54 (5.7)	4453 (53)	399 (29)	308 (29)	91 (10)
CHS	3055	280 (9.2)	72 (5.3)	1880 (63)	414 (32)	321 (30)	88 (10)
Health ABC	1441	81 (5.6)	74 (2.8)	714 (49)	414 (32)	324 (32)	90 (11)
MESA	2256	71 (3.1)	62 (10.1)	1156 (52)	412 (29)	320 (29)	93 (9)
NEO	5366	94 (1.8)	56 (5.9)	2521 (47)	406 (29)	313 (29)	93 (10)
PROSPER	4555	243 (5.3)	75 (3.3)	2445 (47)	414 (36)	320 (35)	94 (11)
Rotterdam 1	4805	216 (4.5)	69 (8.6)	2891 (60)	397 (29)	300 (28)	97 (11)
Rotterdam 2	1889	84 (4.4)	65 (7.6)	1070 (57)	403 (28)	305 (28)	98 (11)
WHI GARNET	3943	304 (7.7)	66 (6.8)	3642 (100)	400 (32)	314 (31)	86 (9)
WHI MOPMAP	1324	36 (2.7)	63 (6.6)	1224 (100)	402 (30)	316 (30)	86 (8)
WHIMS	5184	243 (4.7)	69 (6.0)	4811 (100)	401 (30)	315 (30)	86 (9)
Total	45 002	2 095 (4.7)					
<i>African American</i>							
ARIC	2191	213 (9.7)	53 (5.8)	1322 (62)	400 (33)	310 (32)	90 (10)
CHS	707	141 (20.0)	73 (5.6)	447 (65)	409 (35)	317 (36)	88 (11)
Health ABC	1020	111 (10.9)	73 (2.9)	588 (58)	411 (35)	322 (34)	88 (11)
JHS	2122	117 (5.5)	50 (11.8)	1244 (61)	410 (30)	319 (30)	92 (1)
MESA	1464	135 (9.2)	62 (10.0)	796 (54)	410 (32)	319 (31)	91 (10)
WHI SHARe	4227	450 (10.6)	61 (6.8)	3860 (100)	401 (34)	316 (33)	85 (9)
Total	11 731	1 167 (9.9)					
<i>Hispanic/Latino</i>							
HCHS/SOL	12 024	518 (4.3)	46 (13.8)	7155 (60)	416 (28)	325 (29)	91 (10)
MESA	1316	134 (10.2)	61 (10.3)	681 (52)	409 (30)	318 (30)	91 (10)
WHI SHARe	1784	142 (7.9)	60 (6.4)	1627 (100)	402 (30)	316 (30)	86 (9)
Total	15 124	794 (5.2)					
Total, all ancestries	71 857	4056 (5.6)					

Abbreviations: AGES, Age, Gene/Environment Susceptibility–Reykjavik Study; ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Health Study; Health ABC, Health, Aging, and Body Composition Study; HCHS/SOL, Hispanic Community Health Study/Study of Latinos; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; NEO, Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; Rotterdam 1, first cohort of the Rotterdam Study; Rotterdam 2, second cohort of the Rotterdam study; WHI GARNET, Women’s Health Initiative Genome-wide Association Research Network into Effects of Treatment; WHI MOPMAP, Women’s Health Initiative Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; WHI SHARe, Women’s Health Initiative SNP Health Association Resource; WHIMS, Women’s Health Initiative Memory Study. $N_{\text{exposed}} = \sum_i \frac{n_i}{1+(n_i-1)\rho} \frac{\#\{E_i=1\}}{n_i}$.

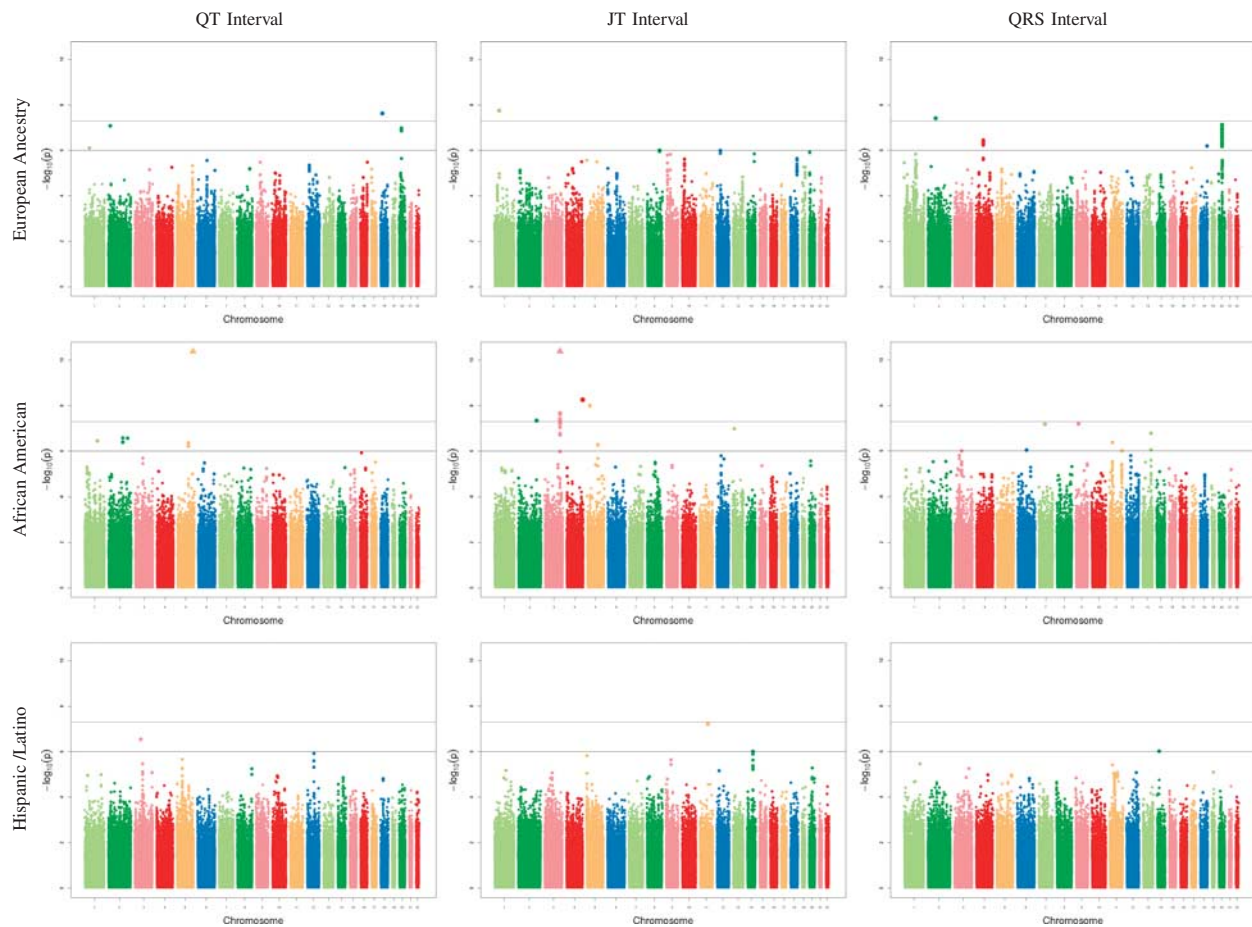


Figure 1. Manhattan plots from each ancestry-specific meta-analysis (row) for sulfonylurea–single-nucleotide polymorphism (SNP) interaction associations with each electrocardiogram (ECG) phenotype (column). The dashed line is the genome-wide threshold for significance ($P < 5 \times 10^{-8}$). The solid line is the threshold for suggestive associations ($P < 10^{-6}$). SNPs with P -values of $< 10^{-10}$, outside of the range of the y axis, are denoted by triangles.

may be heterogeneous across different racial/ethnic populations,^{47,48} we conducted additional transethnic analyses using the Bayesian MANTRA method, with a genome-wide significance threshold of $\log_{10}(\text{Bayes factor}) > 6$.⁴⁹

Previous candidate gene pharmacogenetic studies have identified several pharmacokinetic and pharmacodynamic loci for sulfonylurea-associated glucose-lowering effects and hypoglycemia.^{19–23,50–53} In addition, large-scale GWA studies have identified 35 replicated genetic loci for QT interval main effects.¹⁸ For these candidate SNPs, the P -value threshold for statistical significance was 0.05 divided by the total number of tests conducted across all ECG phenotypes and populations: $0.05/158 = 3.2 \times 10^{-4}$.

For the QT interval, we also assessed for enrichment of candidate SNP-by-treatment interactions with a high probability of being functional for cardiac conduction and repolarization phenotypes. SNPs that fell within 50 kb of transcripts that are preferentially expressed in the left ventricle were identified using the GTEx database (839 transcripts). SNPs in these gene regions were filtered to those falling within DNase I hypersensitivity, H3K4me3 or CTCF chromatin immunoprecipitation sequencing peaks assayed in human cardiomyocytes from the NIH (National Institutes of Health) Roadmap Epigenomics Consortium (<http://www.roadmapepigenomics.org>). In addition, SNPs that were eQTLs in left ventricle tissue ($P < 1 \times 10^{-10}$) were selected.^{54,55} All variants were pruned using ancestry-matched LD patterns from the 1000 Genomes project at a level of $R^2 > 0.5$,⁵⁶ resulting in 9004, 8424 and 5437 candidate SNPs for EA, AA and HA analyses, respectively. The P -value threshold for statistical significance for these candidate SNP analyses was 0.05 divided by the total number of SNPs selected ($P < 5.6 \times 10^{-6}$ for EA, $P < 5.9 \times 10^{-6}$ for AA and $P < 5.6 \times 10^{-6}$ for HA). The selection of candidate SNPs was validated by

evaluating enrichment for low P -value variants using main-effect SNP associations from the QT Interval-International GWAS Consortium.¹⁸

RESULTS

The characteristics of the 11 cohorts and 21 ancestry-specific analysis populations are listed in Table 1. There were 45 002 EA participants ($N_{\text{exposed}} 2095$ (4.7%)), 11 731 AA participants ($N_{\text{exposed}} 1167$ (9.9%)) and 15 124 HA participants ($N_{\text{exposed}} 794$ (5.2%)), for a total of 71 857 ($N_{\text{exposed}} 4056$ (5.6%)). Mean durations of ECG intervals ranged from 397 to 414 ms for QT, 300 to 325 ms for JT and 85 to 98 ms for QRS. The correlation between traits was evaluated among EA and AA participants of CHS: QRS and JT were highly correlated ($R^2 > 0.5$), whereas QRS was not correlated with either QRS or JT ($R^2 < 0.1$).

Primary analysis results

Sulfonylurea–SNP interaction results from cohort-specific GWA analyses were well calibrated: genomic inflation factors for ancestry-specific meta-analyzed results ranged from 0.97 to 1.04 (Supplementary Table 5). A total of 31 sulfonylurea–SNP interaction associations met the genome-wide threshold for significance, comprising 8 unique loci (Figure 1 and Table 2). Each locus was significant for only one of the three ECG phenotypes (2 QT, 5 JT and 1 QRS) and in only one racial/ethnic population (3 EA and 5 AA). Absolute values for effect sizes ranged from 4 to 16 ms. All

Table 2. Summary of significant sulfonylurea–SNP interaction associations with QT, JT and QRS intervals from ancestry-specific GWAS meta-analyses ($P < 5 \times 10^{-8}$)

Lead SNP	Chr:position (hg19)	Nearest gene	Race	Studies	Min/alt alleles	MAF	Effect	s.e.	P	Function	Other GWAS	Coding	eQTL ($P < 5 \times 10^{-8}$)
<i>QT interval</i>													
rs9966832	18:23405188	SS18	EA	3	G/A	0.03	−10.4	1.9	2.3E−08	Intergenic	Periodontitis ⁶⁵		
rs830233	5:165403746		AA	4	A/G	0.05	−16.3	2.3	2.5E−12	Intergenic			
<i>JT interval</i>													
rs1890262	1:62114402	TM2D1, NFIA	EA	2	A/G	0.03	14.9	2.6	1.8E−08	Intergenic			
rs12468579	2:191832264		GLS, STAT1	AA	6	G/A	0.49	4.1	0.8	4.5E−08	Intergenic		GLS ^{59–62} MFSD6 ⁵⁹
rs1478173	3:162276405		AA	2	C/A	0.03	−15.0	2.1	1.0E−12	Intergenic			
rs17281245	4:182635289	TENM3	AA	5	C/T	0.06	8.8	1.5	5.4E−09	Intergenic			
rs7713675	5:28750307	LSP1P3	AA	4	C/T	0.05	−12.2	2.1	9.8E−09	Intergenic			
<i>QRS interval</i>													
rs7595140	2:71551621	ZNF638, PAIP2B	EA	4	G/C	0.03	−5.7	1.0	3.8E−08	Intergenic			

Abbreviations: AA, African American; Chr, chromosome; EA, European ancestry; eQTL, expression quantitative trait locus; GWAS, genome-wide association study; HA, Hispanic/Latino ancestry; MAF, minor allele frequency; SNP, single-nucleotide polymorphism. Studies indicate number of cohorts contributing to ancestry-specific analysis. Other GWAS indicates phenotypes associated with lead SNP ($P < 5 \times 10^{-8}$) in other GWASs. Coding indicates lead SNP in linkage disequilibrium ($r^2 > 0.8$) with a protein-coding variant. The eQTLs indicate transcripts associated with SNPs in linkage disequilibrium ($r^2 > 0.8$) with lead SNP.

loci were intergenic and none had substantial LD with coding variants. Supplementary Table 6 lists the SNP–phenotype associations for the eight significant loci in each ancestry-specific meta-analysis; none reached even nominal levels of significance in the other populations ($P < 0.05$).

The *TM2D1–NFIA* locus (rs1890262) on chromosome 1 was ~200 kb away from a locus associated with QRS interval main effects; *NFIA* encodes a transcription factor of unknown significance for cardiac tissue development.⁵⁸ A locus on chromosome 2 (rs12468579) was 2 kb away from *GLS* and was also identified as a *cis*-eQTL for *GLS* and *MFSD6* transcripts in blood, lung and prostate;^{59–62} *GLS* encodes glutaminase that catalyzes the production of glutamine, the most abundant excitatory neurotransmitter in the central nervous system.⁶³ The chromosome 3 locus (rs1478173) was ~115 kb away from a locus for coronary artery disease.⁶⁴ The only locus associated with another trait (periodontitis) in a previous GWA study was rs9966832 near *SS18* on chromosome 18.⁶⁵

Among the 37 suggestive associations (P -value $< 10^{-6}$ but $> 5 \times 10^{-8}$; Supplementary Table 7), 15 (41%) were intronic, 1 was a missense variant, 3 were in LD ($r^2 > 0.8$) with missense variants and 5 were *cis*-eQTLs in multiple tissues. Several of the subthreshold loci were located in or near genes that might be relevant to cardiac conduction, repolarization or arrhythmogenesis. For example, rs6035275 is an intronic SNP in *SLC24A3*, a potassium-dependent sodium/calcium ion exchanger that plays a role in calcium homeostasis,⁶⁶ and rs624896 is located 24 kb away from *KCNN2*, a voltage-independent calcium-activated potassium channel that helps to regulate neuronal electrical conduction.⁶⁷

Secondary analysis results

Transethnic fixed effects meta-analyses and MANTRA analyses did not identify any additional loci (results not shown). Among the candidate SNPs, only one was significantly associated with an ECG phenotype when multiple comparisons were accounted for (Table 3). This SNP, rs1057910 (Ile359Leu), is a loss-of-function variant that defines the *3 haplotype of *CYP2C9*, a highly polymorphic cytochrome P450 (CYP) enzyme that metabolizes 15–20% of all known drugs that undergo phase I oxidative metabolism.⁶⁸ For the sulfonylurea–SNP interaction, the minor allele of rs1057910 was associated with a 7.6 ms (s.e. 2.1 ms) decrease in the QT interval ($P = 2.3 \times 10^{-4}$) in HA cohorts (MAF 0.05), but not in EA cohorts (MAF 0.07). This SNP did not meet filtering criteria for meta-analysis in the AA cohorts. The more

common functional variant (rs1799853) that defines the *2 haplotype of *CYP2C9* (MAF 0.13 in EA and 0.09 in HA) was also evaluated, but it was not significantly associated with any of the ECG phenotypes.

Selecting additional candidate SNPs based on bioinformatic analysis of annotation from cardiac gene expression and regulatory marks active in cardiomyocytes did not identify additional loci. Although these variants were enriched for signals among main-effect QT analyses (Supplementary Figure 1), none met our statistical significance threshold for sulfonylurea–SNP interactions with the QT, JT or QRS intervals (Supplementary Figure 2).

DISCUSSION

In this study, we identified eight novel loci for sulfonylurea–genetic interactions with the QT, JT and QRS intervals. For seven of these pharmacogenomic associations, the effect size was > 5 ms, the threshold for regulatory concern established by the FDA (Food and Drug Administration). Compared with our previous effort, which included 869 sulfonylurea users among ~30 000 EA participants and failed to identify any genome-wide significant loci, this effort included over 4000 sulfonylurea users among over 70 000 participants from diverse ancestries. Broadening the racial/ethnic composition of the study population and extending our investigation to related ECG phenotypes improved our ability to identify pharmacogenomic loci; most were identified in AA populations and for the JT interval.

Some of the novel pharmacogenomic loci discovered in our study were near (but not in LD with) loci for related traits, such as the *NFIA* locus for QRS interval main effects⁵⁸ and a locus on chromosome 3 for coronary artery disease.⁶⁴ None of the eight loci were near genes that have a clear role in cardiac conduction or repolarization, and even with the use of several bioinformatics resources, the biologic mechanism that would explain these drug–gene interactions are unknown. Among the loci that did not meet the genome-wide threshold for statistical significance but had a P -value of $< 10^{-6}$, several were located in or near potassium ion channels or ion exchanger genes involved in electrical conduction. Without rigorous statistical evidence to support these subthreshold associations, however, their validity is uncertain and replication is needed.

We also assessed candidate SNPs involved in the pharmacokinetics and pharmacodynamics of sulfonylureas and SNPs associated with the QT interval in main-effect GWA analyses. Among

Table 3. Results for pharmacokinetic, pharmacodynamic and QT main-effect candidate SNPs

SNP	Chr	Gene	P-values								
			QT			JT			QRS		
			EA	AA	HA	EA	AA	HA	EA	AA	HA
<i>Pharmacokinetic</i>											
rs1057910 ¹⁹	10	CYP2C9	0.42		2.3E-4	0.06		0.55	0.38		4.1E-3
rs1799853 ¹⁹	10	CYP2C9	0.99		0.33	0.81		0.25	0.75		0.62
<i>Pharmacodynamic</i>											
rs10494355 ⁵⁰	1	NOS1AP	0.27	0.51	0.89	0.87	0.88	0.62	0.37	0.07	0.74
rs7903146 ^{51,52}	10	TCF7L2	0.30	0.94	0.70	0.70	0.44	0.24	0.51	0.89	0.79
rs12255372 ^{51,52}	10	TCF7L2	0.39	0.12	0.71	0.77	0.22	0.50	0.51	0.04	0.86
rs5215 ^{23,53}	11	KCNJ11	0.93	0.83	0.57	0.16	0.01	0.84	0.33	0.40	0.76
rs757110 ²¹	11	ABCC8	1.00	0.68	0.47	0.08	2.5E-3	0.60	0.24	0.15	0.66
<i>QT main effect¹⁸</i>											
rs2298632	1	TCEA3	0.29	0.88	0.20	0.78	0.89	0.78	0.58	0.87	0.75
rs846111	1	RNF207	1.00	0.88	0.79	0.82	0.34	0.84	0.64	0.67	0.91
rs10919070	1	ATP1B1	0.91		0.40	0.25		0.90	0.48		0.35
rs12143842	1	NOS1AP	0.44	0.88	0.75	0.67	0.29	0.52	0.90	0.49	0.97
rs295140	2	SPATS2L	0.12	0.54	0.88	0.12	0.42	0.29	0.67	0.83	0.67
rs938291	2	SP3	0.79	0.41	0.07	0.41	0.10	0.83	0.75	0.58	0.65
rs7561149	2	TTN-CCDC141	0.85	0.72	0.96	0.84	0.41	0.44	0.43	0.69	0.49
rs12997023	2	SLC8A1	0.29	0.51	0.61	0.23	0.50	0.15	0.77	0.44	0.22
rs6793245	3	SCN5A-SCN10A	0.95	0.48	0.55	0.17	0.57	0.85	0.80	0.65	0.94
rs17784882	3	C3ORF75	0.16	0.26	0.31	0.55	0.91	0.32	0.12	0.40	0.57
rs3857067	4	SMARCD1	0.82	0.18	0.46	0.76	0.32	0.81	0.33	0.78	0.41
rs2363719	4	SLC4A4	0.23	0.72	0.05	0.89	0.95	0.51	0.27	0.84	0.28
rs10040989	5	GFRA3	0.93	0.70	0.12	0.14	0.12	0.39	0.35	0.82	0.09
rs7765828	6	GMPR	0.63	0.44	0.23	0.37	0.19	0.05	0.99	0.03	0.40
rs11153730	6	SLC35F1-PLN	0.84	0.67	0.27	0.24	0.52	0.70	0.45	0.16	0.37
rs9920	7	CAV1	0.36		0.01	0.52		0.64	0.08		0.85
rs2072413	7	KCNH2	0.30	0.88	0.75	0.27	0.38	0.77	0.82	0.70	0.95
rs1961102	8	AZIN1	0.33	0.22	0.18	0.30	1.00	0.96	0.44	0.51	0.19
rs11779860	8	LAPTM4B	0.74	0.74	0.08	0.14	0.46	0.65	0.23	0.82	0.16
rs16936870	8	NCOA2	0.08	0.11	0.96	0.24	0.82	0.16	0.02	0.19	0.54
rs174583	10	FEN1-FADS2	0.87	0.26	0.98	0.98	0.57	0.16	0.98	0.35	0.48
rs2485376	10	GBF1	0.86	0.50	0.51	0.03	0.41	0.07	0.13	0.73	0.79
rs7122937	11	KCNQ1	0.25	0.31	0.11	0.20	0.15	0.38	0.12	0.54	0.29
rs3026445	12	ATP2A2	0.94	0.29	0.42	0.23	0.81	0.89	0.33	0.28	0.50
rs728926	13	KLF12	0.30	0.29	0.50	0.46	0.70	0.20	0.75	0.21	0.16
rs2273905	14	ANKRD9	0.38	0.31	0.16	0.71	0.66	0.50	0.21	0.13	0.09
rs3105593	15	USP50-TPRM7	0.71	0.89	0.44	0.73	0.91	0.41	0.80	0.35	0.29
rs735951	16	LITAF	0.34	0.08	0.52	0.28	0.43	0.23	0.59	0.10	0.92
rs1052536	17	LIG3	0.58	0.70	0.77	0.65	0.67	0.39	0.65	0.40	0.70
rs246185	16	MKL2	0.11	0.99	0.31	0.81	0.71	0.54	0.32	0.73	0.28
rs246196	16	CNOT1	0.38	0.96	0.35	0.74	0.97	0.91	0.19	0.60	0.39
rs1296720	16	CREBBP	0.73	0.32	0.33	0.29		0.29	0.36		0.14
rs1396515	17	KCNJ2	0.76	0.98	0.78	0.41	0.19	0.64	0.72	0.69	0.64
rs9892651	17	PRKCA	0.49	0.54	0.29	0.44	0.38	0.98	0.24	0.94	0.37
rs1805128	21	KCNE1	0.69			0.48			0.36		

Abbreviations: AA, African American; Chr, chromosome; EA, European ancestry; HA, Hispanic/Latino ancestry; SNP, single-nucleotide polymorphism. With Bonferroni correction for 158 tests, the threshold for statistical significance was 3.1×10^{-4} . Significant associations are in bold.

these SNPs, only a well-known functional variant in *CYP2C9* was identified as a pharmacogenomic locus in our study, and among HA participants only. Variant rs1057910 (*CYP2C9**3) reduces the catalytic activity of *CYP2C9*, the main CYP isoenzyme involved in the metabolism of sulfonylureas,^{68,69} and this variant has been associated with severe skin reactions from phenytoin use⁷⁰ and warfarin-related hemorrhage.^{71,72} Allele frequencies for rs1057910 were similar among HA and EA participants in our study that has also been reported elsewhere.^{68,73} To our knowledge, only one previous study has identified *CYP2C9* as a pharmacogenomic locus in a HA population;⁷⁴ among 122 male Puerto Rican patients on warfarin therapy, functional variants in *CYP2C9* and *VKORC1* were

associated with lower warfarin dose requirements and a higher risk of warfarin adverse effects.⁷⁵ Other studies, conducted primarily in EA populations, have evaluated the impact of *CYP2C9* functional variants on sulfonylurea-related treatment response and adverse effects. In one study, the presence of either the *CYP2C9**2 or the *CYP2C9**3 haplotype was associated with an increased reduction in hemoglobin A1c and an increased probability of achieving adequate glycemic control,¹⁹ and in another study these variants were associated with an increased risk of hypoglycemia among elderly individuals.⁷⁶

In our study, the variant rs1057910 was associated with a shorter QT interval among HA participants. This was a surprising

finding, because reduced function variants in *CYP2C9* decrease the clearance of sulfonylureas⁶⁹ that would be expected to prolong the QT interval. A short QT interval, which can be hereditary or acquired, has been associated with cardiac arrhythmias and an increased risk of death.^{77–79} Various drugs can also shorten the QT interval, and whether drug-induced shortening of the QT interval causes cardiac arrhythmias is an area of debate.⁸⁰ Although many pharmacogenomic findings for diabetes drugs^{23,24} and for other types of drug therapies^{81,82} have failed to replicate in the past, there is now a growing body of evidence that rs1057910 may be a genuine pharmacogenomic locus for sulfonylureas. Whether this variant contributes to the increased cardiovascular risk associated with sulfonylureas in a subset of the population is uncertain.

The strengths of our study include repeated high-quality phenotype measurements recorded from ECGs conducted at study visits, a large sample size and the inclusion of diverse ancestry populations. There were also several limitations. With the exception of the two cohorts from the Rotterdam Study, medication use was assessed with the inventory method,⁸³ and some participants classified as sulfonylurea users may have failed to take the medication on the day of the study visit. However, changes in diabetes medications typically occur over a period of months or years rather than weeks, and this type of misclassification would bias associations toward the null, decreasing power to identify pharmacogenomic associations. By the same rationale, this type of misclassification is expected to decrease rather than increase the chance of false positive findings.

Because all available analysis populations from the CHARGE consortium were included in a single-stage discovery analysis, which is a more powerful approach than a two-stage approach that includes separate discovery and validation samples,^{29,30} there was no opportunity to assess the validity of our findings through replication in independent study populations. The increasing availability of electronic health data and the decreasing cost of genotyping has led to the emergence of a new model for genomic discovery research: biobanks that link genetic data on tens or even hundreds of thousands of individuals with prescription records and other electronic health data to create large data repositories. Some biobank studies, such as the UK Biobank,⁸⁴ have conducted ECGs as a part of study visits, whereas others⁸⁵ may have access to ECGs obtained through clinical care. Although the large sample sizes in these biobank studies may be attractive for pharmacogenomics research, results from ECGs and other clinical tests that are conducted during the course of clinical care may be related to the indication for conducting the test that can result in confounding and false positive associations.

In conclusion, we have identified several novel loci for sulfonylurea-related changes in various ECG phenotypes in a large multisite pharmacogenomics study conducted within the CHARGE consortium. Although these findings may explain some of the cardiovascular risk associated with sulfonylureas for some individuals, replication in independent study populations is necessary and further work is needed to determine the genetic and biologic mechanisms of these drug–gene interactions.

CONFLICT OF INTEREST

BMP serves on the DSMB of a clinical trial of a device funded by the manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. The other authors declare no conflict of interest.

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