PHARMACOGENOMICS OF VENTRICULAR CONDUCTION IN MULTI-ETHNIC POPULATIONS

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ABSTRACT

Amanda Anne Seyerle: Pharmacogenomics of Ventricular Conduction in Multi-Ethnic Populations
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Adverse drug reactions (ADRs) pose a serious public health burden, yet the role of genetics in drug response remains incompletely characterized. Thiazide diuretics, commonly used anti-hypertensives, may cause QT interval (QT) prolongation, a major drug development barrier that increases risk for highly fatal and difficult to predict ventricular arrhythmias. We thus examined whether common SNPs modified the association between thiazide use (17% mean prevalence) and QT or its component parts (QRS interval, JT interval) by performing ancestry-specific, trans-ethnic, and cross-phenotype genome-wide analyses of European (66%), African American (15%), and Hispanic (19%) populations (N-78,199). Analyses leveraged longitudinal data, incorporated corrected standard errors to account for underestimation of interaction estimate variances, and evaluated evidence for pathway enrichment. Although no loci achieved genome-side significance ($P<5x10^{-8}$), we found suggestive evidence ($P<5x10^{-6}$) for SNPs modifying the thiazide-QT association at 22 loci, including biologically plausible ion transport loci (e.g. *NELL1*, *KCNQ3*).

Given our highly plausible, but only suggestive findings and our observational cohort setting, we next examined the influence of prevalent user bias and exposure misclassification on pharmacogenomics studies conducted in observational settings. Specifically, we simulated three study designs (longitudinal, cross-sectional, new user), two control groups

(whole cohort, active comparator), and two scenarios (extreme or modest drug effects) to enable comparison of 12 settings. For each setting, we simulated N=120,000 participants, conducted 10,000 iterations, applied an alpha=$5x10^{-8}$, and introduced varying degrees of prevalent user bias and drug exposure misclassification. When large drug effects (>10 ms change in QT) or exposure misclassification were present, drug-SNP interaction estimates were biased (bias range: 0.02–3.4 ms) across settings. Under no settings did power to detect the drug-SNP interaction estimate exceed 80% for effects less than 2 ms; detection of drug effects below 2 ms required a longitudinal design with at least 150,000 participants. Results from this dissertation suggest that despite leveraging longitudinal data in 78,199 participants, our study was likely underpowered to detect modest or clinically significant pharmacogenomics effects on QT. Future pharmacogenomics efforts will require even larger sample sizes and innovative methods to enable prevention of ADRs in the large and increasingly at-risk population exposed to medications.

To my family and friends, who put up with my ramblings during this immense undertaking and without whom I could not have accomplished this.

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LIST OF ABBREVIATIONS

AA	African American or African descent population
AC	Active comparator
ADP	Adenosine diphosphate
ADR	Adverse drug reaction
AGES	Age, Gene/Environment Susceptibility – Reykjavik Study
ARIC	Atherosclerosis Risk in Communities
AS	Asian descent population
ATP	Adenosine triphosphate
AV	Atrioventricular
BP	Blood pressure
bpm	Beats per minute
BRIGHT	British Genetics of Hypertension
Ca^{++}	Calcium ion
CACN	Calcium channel gene family
CARe	Candidate-gene Association Resource
CARDIA	The Coronary Artery Risk Development in Young Adults Study
CHARGE	Cohorts for Heart and Aging Research in Genetic Epidemiology
CHD	Coronary heart disease
CHF	Congestive heart failure
CHS	Cardiovascular Health Study
CI	Confidence interval
CKD	Chronic kidney disease
cM	Centimorgan

COGENT	Continental Origins and Genetic Epidemiology Network
CVD	Cardiovascular disease
DALY	Disability adjusted life years
DCT	Distal convoluted tubule
df	Degrees of freedom
diLQTS	Drug-induced long QT syndrome
ECG	Electrocardiogram
eMERGE	Electronic Medical Records and Genomics
ENCODE	Encyclopedia or DNA Elements
ERF	Erasmus Rucphen Family Study
ESRD	End-stage renal disease
EU	European descent population
EUROSPAN	European Special Population Research Network
FDA	Food and Drug Administration
FHS	Framingham Heart Study
GARNET	GWAS of Treatment Response in Randomized Clinical Trials
GEE	Generalized estimating equation
GenNOVA	EURAC – Institute for Genetic Medicine
GRIP	Genetic Research in Isolated Populations
GS	Gitelman syndrome
GWAS	Genome-wide association study
GxE	Gene-environment
h^2	Heritability measured in the narrow sense (i.e. only additive genetic effects)

HGP	Human Genome Project
Health ABC	Health, Aging, Body and Composition
HL	Hispanic/Latino population
HMO	Health maintenance organization
HNR	Heinz Nixdorf Recall Study
HR	Hazard ratio
ICC	Interclass correlation coefficient
JHS	Jackson Heart Study
JNC 7	"The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure"
JT	JT Interval
K^+	Potassium ion
KCN	Potassium channel gene family
kg	Kilogram
KORA	Cooperative Health Research in the Region of Agusburg
LD	Linkage disequilibrium
LIFE	Losartan Intervention for Endpoint Reduction in Hypertension
LQTS	Long QT syndrome
MAF	Minor allele frequency
MEM	Mixed effects model
MESA	Multi-Ethnic Study of Atherosclerosis
Mg^{++}	Magnesium ion
MI	Myocardial infarction
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease

MOPMAP	Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations
MRFIT	Multiple Risk Factor Intervention Trial
mg	Milligram
ms	Millisecond
MS1	Manuscript 1 of dissertation project
MS2	Manuscript 2 of dissertation project
N	Number of participants
Na^+	Sodium ion
NCC	Na^+-Cl^- cotransporter
NCX1	Na^+/Ca^{++} exchanger
NEO	The Netherlands Epidemiology of Obesity
NHANES	National Health and Nutrition Examination Survey
NU	New-user
OR	Odds ratio
PACK	Prevention of Atherosclerotic Complications with Ketanserin
PIUMA	Progetto Ipertensione Umbria Monitoraggio Amvulatoriale
PR	PR interval
Pr(ADR)	Probability of an adverse drug reaction among those on drug/probability of loss-to follow-up
PROSPER	Prospective Study of Pravastatin in the Elderly at Risk
PVC	Premature ventricular contractions
PWG	CHARGE Pharmacogenetics working group
QRS	QRS complex (also known as QRS interval)
QT	QT interval

QT_c	Heart-rate corrected QT interval
QTI	QT prolongation index
QT-IGC	QT Interval – International GWAS Consortium
QT_{mzx}	Limiting value of QT as heart rate approaches zero (656 ms)
RE	Random effects
REGARDS	REasons for Geographic and Racial Differences in Stroke
RCT	Randomized control trial
RR	RR interval
RS	Rotterdam Study
SA	Sinoatrial
SardiNIA	Progenia for the Sardinian public
SC	Sodium channel gene family
SCD	Sudden cardiac death
SD	Standard deviation
SE	Standard error
SHARe	SNP Health Association Resource
SHS	Strong Heart Study
SIDS	Sudden infant death syndrome
SLC	Solute carrier gene family
SNP	Single nucleotide polymorphism
SOL	Hispanic Community Health Study/Study of Latinos
SQTS	Short QT syndrome
TdP	Torsades de pointes

TwinsUK	Twin Registry of the United Kingdom
WC	Whole cohort
WHI	Women's Health Initiative
WHIMS	WHI Memory Study
WHO	World Health Organization
YLL	Years of life lost
YPLL	Years of potential life lost

LIST OF GENE NAMES

ACE	Angiotensin I converting enzyme
ANK2	Ankyrin 2, neuronal
ATP1B1	ATPase, Na^+/K^+ transporting, beta 1 polypeptide
CACNA1C	Calcium channel, voltage-dependent, L type, alpha 1C subunit
CACNA1D	Calcium channel, voltage-dependent, L type, alpha 1D subunit
CACNA1E	Calcium channel, voltage-dependent, R type, alpha 1E subunit
CACNA1G	Calcium channel, voltage-dependent, T type, alpha 1G subunit
CACNA1H	Calcium channel, voltage-dependent, T type, alpha 1H subunit
CACNA2D1	Calcium channel, voltage-dependent, alpha 2/delta subunit 1
CACNB2B	Calcium channel, voltage-dependent, beta 2 subunit
CAV1	Caveolin 1, caveolae protein, 22kDa
CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6
CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4
CYP11B2	Cytochrome P450, family 11, subfamily B, polypeptide 2
HCN2	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2
HCN4	Hyperpolarization activated cyclic nucleotide-gated potassium channel 4
HERG	Human ether-a-go-go related gene (a.k.a. *KCNH2*)
KCNA4	Potassium voltage-gated channel, shaker-related subfamily, member 4
KCNA5	Potassium voltage-gated channel, shaker-related subfamily, member 5
KCNA7	Potassium voltage-gated channel, shaker-related subfamily, member 7
KCNAB1	Potassium voltage-gated channel, shaker-related subfamily, beta member 1
KCNAB2	Potassium voltage-gated channel, shaker-related subfamily, beta member 2

KCNC1	Potassium voltage-gated channel, Shaw-related subfamily, member 1
KCNC4	Potassium voltage-gated channel, Shaw-related subfamily, member 4
KCND2	Potassium voltage-gated channel, Shal-related subfamily, member 2
KCND3	Potassium voltage-gated channel, Shal-related subfamily, member 3
KCNE1	Potassium voltage-gated channel, Isk-related family, member 1
KCNE2	Potassium voltage-gated channel, Isk-related family, member 2
KCNH2	Potassium voltage-gated channel, subfamily H (eag-related), member 2 (formerly *HERG*)
KCNIP2	Kv channel interacting protein 2
KCNJ2	Potassium inwardly-rectifying channel, subfamily J, member 2
KCNJ3	Potassium inwardly-rectifying channel, subfamily J, member 3
KCNJ5	Potassium inwardly-rectifying channel, subfamily J, member 5
KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11
KCNJ12	Potassium inwardly-rectifying channel, subfamily J, member 12
KCNK1	Potassium channel, subfamily K, member 1
KCNK3	Potassium channel, subfamily K, member 3
KCNK4	Potassium channel, subfamily K, member 4
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1
LIG3	Ligase III, DNA, ATP-dependent
LITAF	Lipopolysaccharide-induced TNF factor
NDRG4	NDRG family member 4
NEDD4L	Neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase
NELL1	NEL-like 1 (chicken)

NOS1AP	Nitric oxide synthase 1 (neuronal) adapter protein
PLN	Phospholamban
PRKCA	Protein kinase C, alpha
SCN1B	Sodium channel, voltage-gated type I, beta subunit
SCN2B	Sodium channel, voltage-gated, type II, beta subunit
SCN3B	Sodium channel, voltage-gated, type III, beta subunit
SCN4B	Sodium channel, voltage-gated, type IV, beta subunit
SCN5A	Sodium channel, voltage-gated, type V, alpha subunit
SCN10A	Sodium channel, voltage-gated, type X, alpha subunit
SLC12A3	Solute carrier family 12 (sodium/chloride transporter), member 3
SLC22A23	Solute carrier family 22, member 23
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1
SLCO3A1	Solute carrier organic anion transporter family, member 3A1
TRPM6	Transient receptor potential cation channel, subfamily M, member 6
TBX5	T-box 5
WNK1	WNK lysine deficient protein kinase 1
WNK4	WNK lysine deficient protein kinase 4
VKORC1	Vitamin K epoxide reductase complex, subunit 1
YEATS4	YEATS domain containing 4

CHAPTER 1: OVERVIEW

Over the past decade, the use of prescription drugs has skyrocketed, with nearly half of all Americans taking at least one prescription drug.[1] Despite the considerable increases in drug exposure, variability in drug response, a significant cause of morbidity and mortality accounting for approximately 100,000 deaths and 2.2 million serious health effects annually,[2-5] remains poorly understood.[6] One promising avenue to understanding variability in drug response is offered by pharmacogenomics,[7] which as the potential to illuminate novel pathways with the goal of informing drug development and selection,[8-10] modifying dosing regimens,[11-15] and avoiding adverse drug reactions.[16-18]

Pharmacoepidemiology is a branch of epidemiology that seeks to understand both the use of and the effects of drugs in populations. Pharmacogenomics is an extension of pharmacoepidemiology and evaluates the role of genetics in drug response. This work will perform a genome-wide association study (GWAS) that examines whether common genetic variants modify the association between thiazide diuretics and the QT interval (QT), a measure of ventricular depolarization and repolarization taken from the electrocardiogram (ECG). QT is a promising candidate for pharmacogenomic study, as it is a risk factor for ventricular tachyarrhythmia,[19] coronary heart disease,[20] congestive heart failure,[21] stroke,[22] cardiovascular mortality, and all-cause mortality.[23] Furthermore, QT is highly heritable (35-40%),[24-28] with early family studies identifying rare and highly penetrant mutations associated with long- and short-QT syndrome[29] and more recent GWAS identifying multiple

common single nucleotide polymorphisms (SNPs) associated with modest increases in QT.[30-35] Thiazide diuretics, an increasingly common antihypertensive therapy used by over a quarter of the hypertensive population in the U.S.,[36, 37] are one of many common pharmaceuticals that may cause QT prolongation.[38-40] However, the mechanisms underlying thiazide-induced QT prolongation is not well understood.[41-43] Given the rising prevalence of thiazide use, the established genetic basis of QT, the inter-individual variability in thiazide response, and the Food and Drug Administration's standard for regulating QT-prolonging medications, which requires a change of just 5 ms, a change easily obtained through both pharmaceutical and genetic exposures,[44] it is critical that pharmacogenomic interactions be identified. Pharmacogenomics remains one of the few areas where genetic research has been translated into actionable results and the pharmacogenomics of thiazides and QT prolongation is an excellent candidate for pharmacogenomics study.

Pharmacogenomics studies like the one presented herein often leverage the extensive data available in large observational study settings, a setting in which pharmacoepidemiologic studies are known to be prone to multiple forms of bias (e.g. prevalent user bias, indication/contraindication, healthy-user effects, etc.).[45-51] However, it is unclear if *pharmacogenomic* studies are subject to the same biases. For example, previous work has indicated that pharmacogenomics studies may not be subjected to the same degree of bias by indication/contraindication as pharmacoepidemiologic studies.[47] However, to date, no one has evaluated how additional threats to internal validity, such as prevalent user bias, impacts pharmacogenomics studies conducted in observational settings. This dissertation will examine the effect of prevalent user bias on pharmacogenomics studies,

work which could inform the future design and interpretation of pharmacogenomics studies in large cohort studies.

CHAPTER 2: SPECIFIC AIMS

This work will be conducted through a collaboration between the Women's Health Initiative (WHI),[52] the Hispanic Community Health Study/Study of Latinos (SOL),[53] and the Cohort for Heart and Aging Research in Genomic Epidemiology (CHARGE)[54] pharmacogenomics working group (PWG) investigators, yielding a diverse population of participants of European (N=58,813), African (N=15,625), and Hispanic (N=16,657) descent. We therefore will:

Specific Aim 1: Identify genetic variants that modify the association between thiazide diuretics and QT and its component parts (QRS complex [QRS]; JT interval [JT]) in European descent, African descent, and Hispanic populations.

a. Classify thiazide diuretic exposure among all cohorts using medication inventories, which have been validated in cohort studies against physiologic measurements,[55] pharmacy databases,[56] and serum measurements.[57]
b. Conduct genome-wide, race-stratified analyses to identify significant interactions between genetic variants, thiazides, and QT and its component parts (QRS; JT), leveraging longitudinal data when possible. Study and race/ethnic-stratified results will be combined across studies using fixed-effect, trans-ethnic, and cross-phenotypic meta-analytic techniques (N_{total}=78,199).
c. Characterize identified genetic variants using *in silico* functional characterization techniques including computer databases and pathway analysis.

Specific Aim 2: Examine the influence of prevalent user bias and exposure misclassification caused by prevalent user bias on a pharmacogenomics study conducted in an observational setting.

a. Using simulations, evaluate bias, power, and type I error in the drug-SNP interaction caused by prevalent user bias and exposure misclassification
b. Compare the results of aim 2a under different study designs (e.g. whole cohort, active comparator, new-user).

CHAPTER 3: BACKGROUND AND SIGNIFICANCE

Ventricular Conduction

The role of electrical impulses in cardiac conduction was first identified in the mid-19th century by Rudolf Kollicker and Johannes Mueller, who showed that the same electrical impulses which caused a frog's legs to kick could also cause the heart to beat.[58] During the next fifty years, researchers identified and characterized all of the primary structures involved in conducting electrical impulses throughout the heart (Table 1, Figure 1).[59] These structures control the coordinated contraction and relaxation of the cardiac muscle cells, first with the rapid contraction of the atria and followed by the slower contraction of the ventricles, and together form the cardiac electrical conduction system.

Table 1. Discovery of the Structures of the Cardiac Conduction System

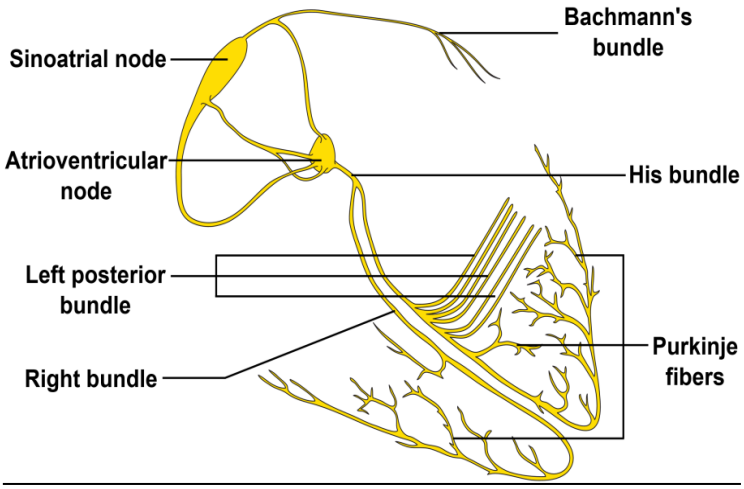
Year	Structure	Scientist
1845	Purkinje Fibers	J.E. Purkinje
1865/1893	Bundle of Kent	G. Paladino and A.F.S. Kent
1893	Bundle of His	W. His, Jr.
1906	AV Node	L. Aschoff and S. Tawara
1906/1907	Wenckebach Bundle	K.F. Wenckebach
1907	Sinus Node	A.G. Keith and M.W. Flack
1916	Bachmann Bundle	J.G. Bachmann

Adapted from Oto and Breithardt, 2001[59]

A. Electrical Conduction of the Heart

Electrical activity in the heart results from the rapid depolarization and subsequent repolarization of the cardiac cells, which creates an action potential. There are two types of cardiac cells: pacemaker cells and non-pacemaker cells, hereafter referred to as myocytes. Pacemaker cells are capable of generating regular, spontaneous action potentials and are

Figure 1. Electrical Conduction System of the Heart



Adapted from http://en.wikipedia.org/wiki/Atrioventricular_node

primarily found in the sinoatrial (SA) node and the atrioventricular (AV) node. These cells are responsible for generating the initial depolarizing current of the heartbeat. Myocytes make up the majority of cardiac cells but cannot generate their own action potential.

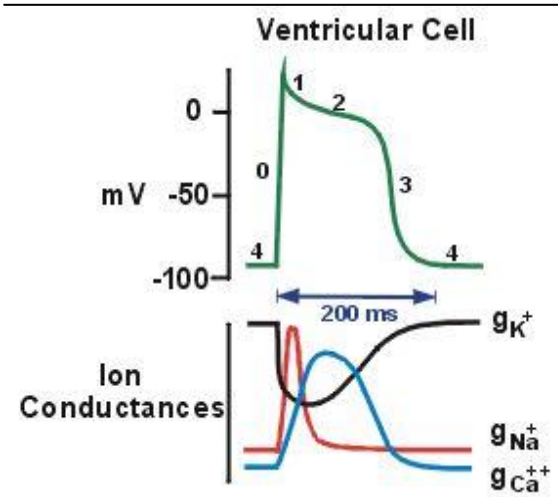
Action potentials in the heart are primarily initiated in the SA node, which is the heart's primary pacemaker site and provide an intrinsically automated rate of depolarizations that drives the overall electrical activity of the heart.[60] From the SA node, the depolarization current spreads through the myocytes of the atria. However, the AV valves, which separate the atria and the ventricles, are composed on non-conductive connective tissue which prevents the action potentials generated by the SA node from entering the ventricles directly.[58, 60] Instead, the action potential enters through the AV node, a specialized region of pacemaker cells in the wall between the atria and ventricles. The AV node conducts electrical impulses at 1/10th the rate of the atrial cells and thus delays the conduction between the atria and ventricles, ensuring enough time for blood to exit the atria and fill the ventricles. However, once the action potential leaves the AV node, it spreads rapidly through the His-Purkinje system (Figure 1) in a process known as rapid depolarization, ensuring the spread of

depolarization throughout the ventricles simultaneously. From here, the action potential spreads to the remaining myocytes of the ventricles through cell-to-cell conduction, causing the ventricles to contract.

Rapid ventricular depolarization (Phase 0) is followed by a much slower period of repolarization, which consists of four phases (Figure 2). Phase 1 consists of a short, initial burst of repolarization which is then followed by a plateau phase (Phase 2), where there is minimal repolarization activity. Finally, cells undergo rapid repolarization (Phase 3) and return to their resting state (Phase 4).

Progression through each of the five action potential phases is controlled by the movement of sodium, calcium, and potassium ions into and out of the cardiac cells. Both pacemaker cells and non-pacemaker cells have multiple ion channels embedded in their membranes which control the movement of ions into and out of the cells. In their resting

Figure 2. Action Potential of the Ventricular Cell and Associated Ion Conductances



Adapted from Klabunde 2012[56]
Phase 0: Rapid depolarization; Phase 1: Initial repolarization; Phase 2: Plateau phase; Phase 3: Repolarization; Phase 4: Resting potential; gK^+: Potassium conductance; gNa^+: Sodium conductance; gCa^{++}: Calcium conductance

state, cardiac cells have a negative electrical potential relative to the outside of the cell.[58, 60] The net negative electrical potential is produced through a combination of ion concentrations. K^+ ions are present in higher concentrations inside the cell relative to outside while both Ca^{++} and Na^+ ions are present in higher concentrations outside the cell relative to inside.[60] Depolarization (Phase 0) occurs with the movement of Na^+ into the cell. Phase 1 of repolarization is caused by the movement of K^+ ions out of the cell and is then slowed (Phase 2) by the continued, slow movement of Ca^{++} into the cell. Phase 3 is brought about by the end of inward Ca^{++} movement and the continued outward movement of K^+. Resting potential (Phase 4) is maintained through the movement of K^+ ions back into the cell. The ion gradients needed to control the electrical impulses of the heart are controlled by a series of ion channels.

A.1. Sodium Channels

Table 2. Cardiac Ion Channels

Channels	Gating	Characteristics
Sodium		
Fast Na^+	Voltage	Phase 0 of myocytes
Slow Na^+	Voltage/Receptor	Contributes to Phase 4 pacemaker current in SA and AV nodal cells
Calcium		
L-type	Voltage	Slow inward, long-lasting current; Phase 2 of myocytes and phases 4 and 0 of SA and AV nodal cells
T-type	Voltage	Transient current; contributes to Phase 4 pacemaker current in SA and AV nodal cells
Potassium		
Inward rectifier	Voltage	Maintains negative potential in Phase 4; Closes with Depolarization
Transient outward	Voltage	Contributes to Phase 1 in myocytes
Delayed rectifier	Voltage	Phase 3 repolarization
ATP-sensitive	Receptor	Inhibited by ATP; opens when ATP decreases during cellular hypoxia
Acetylcholine activated	Receptor	Activated by acetylcholine and adenosine; Gi-protein coupled; Slows SA nodal firing
Calcium activated	Receptor	Activated by high cytosolic calcium; Accelerates repolarization

Adapted from Klabunde 2012[60]

Sodium channels are the most common ion channels found in cardiac cells, with over 100,000 sodium channels expressed in each cardiac cell and over 1 million expressed in cells of the Purkinje fibers.[61] Two types of sodium channels are critical to regulating the electrical activity of the heart: fast acting and slow acting (Table 2). Fast acting sodium channels are responsible for the rapid depolarization of the myocyte. The activation gates are opened when the depolarization current spreads from cell to cell, which increases the conductance of Na^+ across the cell membrane (Figure 2). This allows Na^+ to move into the cell but the channels close rapidly, limiting the length of time in which sodium can enter the cell.[60] Slow acting sodium channels play a minor role in myocytes but are involved in the spontaneous depolarization of cardiac pacemaker cells where the slow inward movement of Na^+ is partly responsible for the spontaneous depolarizing current, or pacemaker current, which differentiates pacemaker cells from myocytes.[60]

Sodium channels are expressed in virtually all eukaryotic organisms; Ren *et al.* identified a primitive counterpart to the eukaryotic sodium channel which is expressed in prokaryotes,[61, 62] and the genes encoding sodium channel genes are highly conserved across organisms.[61] The primary gene involved in the cardiac isoform of the sodium channels is *SCN5A*.[61, 63] However, many additional genes are involved in the encoding of human sodium channels in the heart, including many from the sodium channel (SC) family of genes such as *SCN10A*, *SCN4B*, *SCN1B*, *SCN2B*, *SCN3B*, and *SNC4B*.[64-66] Mutations in the genes encoding the primary cardiac isoforms have been implicated in rare familial cardiac conduction disorders (See Section QT Interval Genetics).

A.2. Calcium Channels

Similarly to sodium channels, there are two types of calcium channels influencing cardiac conduction: L-type and T-type (Table 2).[60] However, the average myocyte has approximately 1/5th as many calcium channels as sodium channels.[61] Despite the smaller number, calcium channels play a critical role in cardiac electrophysiology. After depolarization, L-type calcium channels continue to allow Ca^{++} to flow into the myocyte. Unlike the fast acting sodium channels which cause depolarization, L-type calcium channels remain open for a longer period of time and are the primary cause of the plateau phase (Phase 2 in Figure 2).[60] T-type calcium channels are, similarly to slow acting sodium channels, primarily involved in the spontaneous depolarization of pacemaker cells and play little role in the action potential of general myocytes.

Calcium channel genes are highly conserved across vertebrates.[64] There are at least ten calcium channel genes in the human genome but only half are expressed in cardiac cells. Calcium channel genes belong to the CACN gene family and include *CACNA1C*, *CACNA1D*, *CACNA1E*, *CACNA1G*, and *CACNA1H*.[61] The first three CACN genes encode isoforms of the L-type channel while the latter two encode isoforms of the T-type channel. Of the three L-type calcium channel genes, *CACNA1C* produces the primary isoform found in cardiac cells.[61]

A.3. Potassium Channels

Unlike sodium and calcium channels which both have two main subtypes, potassium channels have six main subtypes (Table 2) and transient outward channels and delayed rectifier channels can be further broken down into subclasses based on their speed of action (Table 3). Transient outward K^+ channels are responsible for initial repolarization (Phase 1

in Figure 2) while delayed rectifier K^+ channels are responsible for the increase in K^+ conductance that causes Phase 3 repolarization.[60] Inward rectifiers are involved in the last phases of repolarization and in setting the resting potential (Phase 4).[61]

Given the wide range of potassium channel subtypes, it is therefore unsurprising to find a wide variety of genes encode potassium channel subunits. These genes are highly conserved across eukaryotes and comprise the KCN gene family.[66, 67] The KCN gene family is composed of over 90 genes but only a subset are expressed in the heart.[65] In addition to the genes which encode alpha subunits of the numerous cardiac potassium channels (Table 3), multiple accessory subunits are also expressed in cardiac cells: *KCNIP2*, *KCNAB1*, *KCNAB2*, *KCNE2*, and *KCNE1*.[61] Mutations in genes in the KCN family have been linked to inherited forms of Long QT Syndrome (LQTS), a Mendelian disorder with an increased duration of ventricular repolarization, and with the overall duration of ventricular repolarization (See Section QT Interval Genetics).

Table 3. Alpha Subunits of Cardiac Potassium Channels

Current	Description	Gene(s)	Action Potential Phase	Activation Mechanism
$I_{to,f}$	Transient Outward Current (Fast)	*KCND2, KCND3*	Phase 1	Voltage (depolarization)
$I_{to,s}$	Transient Outward Current (Slow)	*KCNA4, KCNA7, KCNC4*	Phase 1	Voltage (depolarization)
I_{Kur}	Ultra-Rapid Delayed Rectifier	*KCNA5, KCNC1*	Phase 2	Voltage (depolarization)
I_{Kr}	Rapid Delayed Rectifier	*KCNH2*	Phase 3	Voltage (depolarization)
I_{Ks}	Slow Delayed Rectifier	*KCNQ1*	Phase 3	Voltage (depolarization)
I_{K1}	Inward Rectifier (Strong)	*KCNJ2, KCNJ12*	Phase 3, Phase 4	Voltage (depolarization)
I_{KATP}	ADP Activated	*KCNJ11*	Phase 1, Phase 2	↑ADP/ATP Ratio (ATP depletion)
I_{KACh}	M2 Receptor Gated K^+ Channel	*KCNJ3, KCNJ5*	Phase 4	Acetylcholine
I_{Kp}	Background K^+ Channels	*KCNK1/6, KCNK3, KCNK4*	All Phases	Metabolic parameters, Membrane stretch
I_h	Pacemaker Channel	*HCN2, HCN4*	Phase 4	Voltage (hyperpolarization)

Adapted from Zipes 2004[61]

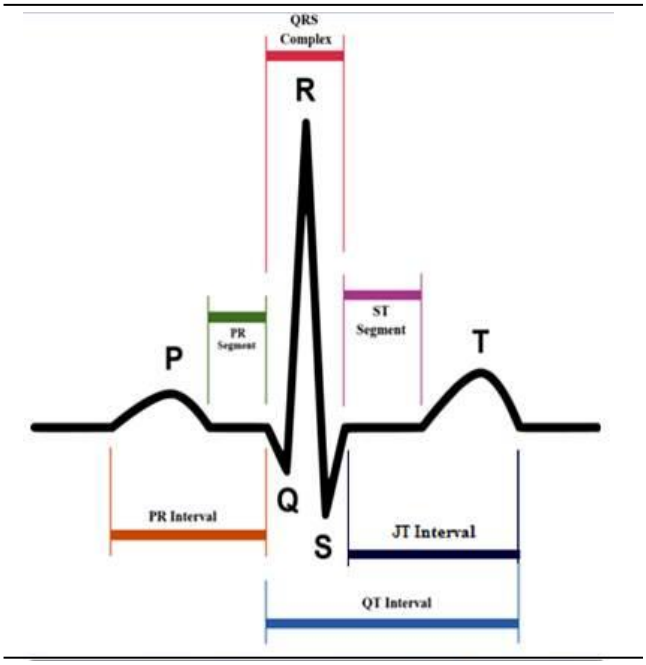
B. Ventricular Conduction on the Electrocardiogram

In 1887, a French scientist by the name of Gabriel Lippmann first demonstrated that the electrical impulses of the heart could be recorded from the body's surface.[59] Fifteen years later, Dutch physiologist Willem Einthoven published the first modern tracings from a surface electrocardiogram (ECG).[59] He identified five distinct points on the ECG rhythm, which he labeled P, Q, R, S, and T, nomenclature which is still used over a century later to describe points on the ECG (Figure 3). The P wave is produced as a depolarization wave is sent from the SA node and spreads through the atria. The break between the P wave and the Q point corresponds to the slowing of the depolarization wave as it enters the AV node. As depolarization is rapidly spread through the ventricles, the QRS complex (QRS) is produced (Figure 4).[68] This is then followed by another break, which represents the plateau phase of repolarization. The final wave on the ECG, the T wave, represents the rapid phase of repolarization (Figure 4).[58, 68] Together, these points produce a number of commonly studied intervals (Figure 3). The PR interval (PR) represents the period of atrial depolarization and AV nodal conduction, including the propagation of the impulse through the bundle of His, the bundle branches, and the Purkinje fibers.[69] The QT interval (QT) is a measure of the ventricular action potential and can be broken down into the QRS complex (QRS, ventricular depolarization) and the JT interval (JT, ventricular repolarization).[60, 69]

B.1. QT Interval

The QT interval, the subject of this dissertation, is a measure of both ventricular depolarization and repolarization. It is measured from the onset of the QRS complex to the end of the T wave. In a standard 12-lead ECG, with 3 standard limb leads, 3 augmented limb leads, and 6 precordial chest leads, QT can vary between leads, a phenomenon called QT

Figure 3. ECG Rhythm with Labeled Intervals

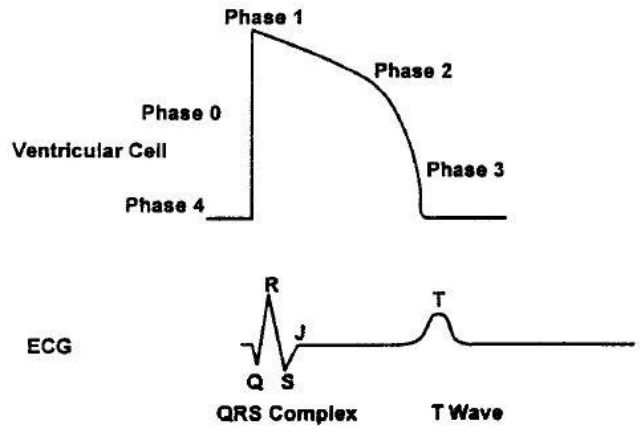


Adapted from http://en.wikipedia.org/wiki/QT interval

dispersion. To standardize measurement, QT is measured from the lead that has the largest T wave with the most distinct termination.[69] The latter feature is particularly important, as the T wave can sometimes be difficult to define and can be influenced with by the presence of a U wave.[68, 70] The U wave is a small wave sometimes seen on the ECG following the T wave; its origins are unknown but it is believed to represent repolarization of the Purkinje fibers or the prolonged repolarization of cells in the mid-myocardium.[71]

Despite the potential introduction of measurement error through lead placement or external environmental factors, repeatability studies have found that QT measurements are reliable.[72-74] Savelieva *et al*. found that, over the course of 10 consecutive ECGs, QT interval measurement demonstrated a modest 1-2% coefficient of variation, or the ratio of the standard deviation to the mean, among the general population, among a population of myocardial infarction (MI) patients, and among patients with hypertrophic cardiomyopathy.[73] Similarly, Vaidean *et al.* found that the interclass correlation coefficient (ICC), which is the

Figure 4. Action Potential of the Ventricle Cell and Corresponding Surface ECG Components



Adapted from Bednar 2001[68]
Phase 0: Rapid depolarization; Phase 1: Initial repolarization; Phase 2: Plateau phase; Phase 3: Repolarization; Phase 4: Resting potential

ratio of between-person variance to the total variance in the study, for QT was 0.86 (95% Confidence Interval [CI]: 0.81 – 0.92),[74] suggesting low within-person variance in QT. Furthermore, Vaidean and colleagues demonstrated that, as the total sample size increases, the precision of the mean QT measurement for a group of study participants increases significantly, allowing studies with large sample sizes to reliably study QT and QT correlates.[74]

Heart Rate Correction Formulas for QT Interval

Table 4. Heart Rate Correction Formulae for QT Interval

Formula	Mathematical Form
Bazett[75]	$QT_c = \frac{QT}{\sqrt{RR}}$
Fridericia[76]	$QT_c = \frac{QT}{\sqrt[3]{RR}}$
Hodges[77]	$QT_c = QT + 1.75(Heart\ Rate - 60)$
Framingham[78]	$QT_c = QT + 0.154(1 - RR)$
Normogram[79]	$QT_c = QT + Correction\ Factor$

Adapted from Aytemir 1999[80]
QT: Uncorrected QT interval; QT_c: Corrected QT interval; RR: RR interval

Normal QT intervals range from 200 to 400 ms.[60] However, despite the overall reliability of QT measurements, inter-individual variation remains high, largely reflecting the

influence of heart rate. QT is expected to be prolonged at slower heart rates and shortened at faster heart rates.[38, 75, 81, 82] This range can be extreme. Data from the Framingham Heart Study (FHS) have shown that in men, QT can range from 450 ms at 40 beats per minute (bpm) to 300 ms at 120 bpm, and in women, QT can range from 465 ms at 40 bpm to 310 ms at 120 bpm.[69, 78] Because of the large influence of heart rate, studies of QT commonly account for heart rate in their analysis, either through simple adjustment or through the use of one of the numerous correction formulae available in the literature. After adjustment, corrected QT (QT_c) is expected to be no greater than 440 ms and QT_c greater than 500 ms is considered critically prolonged.[38, 60] One of the most commonly used correction formula is Bazett's formula.[38, 75] However, Bazett's correction can be inaccurate at elevated heart rates.[38]

Because of the potential for inaccuracy when using Bazett's formula, numerous alternatives have been suggested. Fridericia, a contemporary of Bazett's, suggested using the cubed root of the RR interval (RR), an inverse measure of heart rate, rather than the squared root.[76] In 1936, Shipley and Hallaran modified Bazett's formula to $QT_c = k\sqrt{RR}$ where k is 0.397 in men and 0.415 in women.[69, 83] Despite these alternatives, many researchers have remained skeptical of the accuracy of the existing formulas and multiple additional formulas have been proposed (Table 4).[80] In 1992, the FHS offered a new method to correct for heart rate based on a large population based cohort.[78] The normogram formula attempted to develop a heart rate correction formula that had a correction factor that varied by heart rate, making it more accurate at the extreme heart rates and allowing it to vary by population. Rautaharju *et al.* have also proposed the QT prolongation index (QTI), which is calculated as a proportion of the limiting value of QT when heart rate approaches zero (QT_{max}):

$$QTI = \frac{QT \times (Heart\ Rate + 100)}{QT_{max}}$$

where QT_{max} = 656 ms; because this is a proportion, the mean value is 100 and the upper 2% of prolonged QT have a value greater than 110, making it difficult to compare to other studies which used one of the standard correction formulae.[84, 85] However even with the wide variety of correction formulae available, there is still no consensus on the preferred approach, but the suggestion has been made that it is may be necessary for each individual study to investigate which correction model best fits their data.[80, 86, 87]

B.2. QRS Complex

The QRS complex, sometimes referred to the QRS interval, is a measure of ventricular depolarization (Figure 3, Figure 4). It also measures an early component of ventricular repolarization (Phase 1).[88] Its duration is controlled by the His-Purkinje system, composed of the His bundle, the left and right bundle branches, and the Purkinje fibers (Figure 1). The His-Purkinje system ensures the spread of the depolarization impulse from the AV node through both ventricles simultaneously. It is also during the QRS that atrial repolarization occurs but, due to its short duration and small amplitude, this process is masked by ventricular repolarization on the surface ECG.[60] QRS is measured on the lead with the widest QRS complex with the sharpest onset and termination, usually one of the six precordial chest leads.[69] Because QRS includes an early phase of repolarization, the transition from the QRS complex to the ST segment can be gradual making it hard to define the J point (Figure 4). Further complicating the definition of the QRS complex is the Q wave, which is often absent on ECGs.[58]

When the Q wave is present, its duration is used in the diagnosis of MI. A widened Q wave on limb leads I, II, aV_L, or aV_F is indicative of an MI. However, use of limb lead III or

aV_R can lead to a false diagnosis, as the Q wave is typically wider on these leads.[69] Widening of the whole QRS complex can also be indicative of malfunctions of the cardiac conduction system, e.g. bundle branch blocks. The QRS is typically wider in young populations,[89] in males,[90, 91] and in Whites.[92] Widening of the interval is also seen in hyperkalemic populations,[93-95] in obese populations,[96] in populations using certain anti-arrhythmic medications[93, 97, 98] and in populations on hemodialysis.[99] A normal QRS duration is between 60 and 100 ms, with about half of the general population falling near 80 ms,[60, 69] although a QRS duration of as high as 110 ms is not considered abnormal.[69] However, a QRS greater than 120 ms is a very specific marker of ventricular dysfunction.[100, 101]

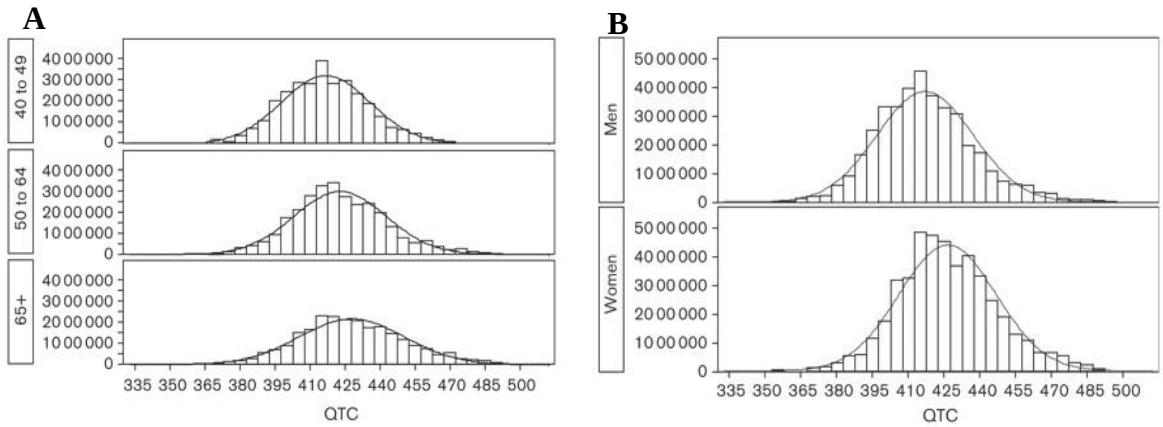
B.3. JT Interval

The JT interval is a measure of ventricular repolarization and is composed of the ST segment and the T wave. The ST segment represents the plateau phase of repolarization while the T wave represents phase 3 repolarization.[58] JT is generally calculated as $JT = QT - QRS$ rather than measured directly from the surface ECG. JT is highly correlated with QT but, unlike QT and QRS, JT has not been as commonly studied.[28] However, it has been suggested that, JT is better than QT for monitoring increased risks due to prolongation of ventricular repolarization, as JT represents the repolarization phase of QT and it is this phase which is predicted to be most clinically relevant.[102] Tsai *et al.* have demonstrated that JT is a better marker of changes in repolarization duration when monitoring patients on antiarrhythmic drug therapy, a common cause of prolonged QT (See Section Drug-Induced QT Prolongation).[103] Additionally, JT is a better marker than QT when studying conditions with a wide QRS.[104] For example, prolonged QT is considered a risk factor for coronary

heart disease (CHD) (See Section *Coronary Heart Disease*); however, Crow *et al.* found that JT was actually a better predictor of CHD mortality than QT in cases where a wide QRS was present.[105] These findings indicate that studies of JT are informative in addition to studies of QT and QRS.

QT Interval Prolongation

Figure 5. QTc Distribution in the U.S. Population by Age and Sex



Adapted from Benoit 2005[106]
Data from the Third National Health and Nutrition Evaluation Survey, 1988 – 1994
QT_c corrected for heart rate using the Fridericia formula

QT ranges from 200-400 ms in the general population; after adjustment for heart rate, the distribution of QT shifts upward so that intervals up to 440 ms are considered normal. QT is normally distributed and is shifted upwards in females and in older populations (Figure 5).[75, 106, 107] However, malfunction of the ion channels associated with the cardiac conduction system and disruptions in the action potential of the heart, both achieved through multiple mechanisms, can lead to a shortening or lengthening of the QT interval beyond the normal range. While short QT syndrome can be pathogenic, it is exceedingly rare and is primarily congenital.[108, 109] QT prolongation, however, is more prevalent in the population and can be caused by many common innate and acquired risk factors.

C. Risk Factors

QT prolongation can occur through multiple mechanisms and numerous risk factors for prolonged QT have been identified. Broadly, risk factors for prolonged QT can be classified into three categories: clinical conditions, congenital conditions, and electrolyte imbalances.

C.1. Acquired Clinical Conditions

Table 5. Acquired Clinical Causes of QT Prolongation

Myocardial Infarction
Valvular Disease*
Cardiomyopathy*
Bradycardia
Subclinical Cardiovascular Disease
Liver Function Impairment
Diabetes Mellitus
Hypothyroidism
Obesity
Anorexia
Use of QT-Prolonging Medications

*Can be either acquired or congenital

Numerous clinical conditions have been identified as risk factors for QT prolongation (Table 5). Multiple diseases of the heart can interfere with normal cardiac conduction, including myocardial infarction,[69, 110, 111] structural heart disease (e.g. valvular disease, cardiomyopathy),[65, 108] and bradycardia (slow heart rate),[112, 113] all of which can lead to QT prolongation. Furthermore, some research has suggested that QT prolongation could serve as a marker of subclinical CVD[114, 115]

Non-cardiac diseases also confer a risk of QT prolongation. QT prolongation is present in populations with cirrhosis of the liver,[116-118] with diabetes,[119] and with hypothyroidism.[120, 121] Liver disease has been shown to confer a 3-4 fold increase in the risk of QT prolongation associated with liver disease.[122] Based on NHANES data, diabetes confers a 1.6-fold increase in risk (95% CI: 1.1-2.3) of prolonged QT,[123] and hypothyroidism could increase the risk of QT prolongation by over 2-fold.[106] Additionally, studies have

found between 20-30% of obese individuals have a prolonged QT,[124-126] which suggests that, given the high prevalence of obesity in the U.S. (~35% of adults), obesity may be one of the most common causes of prolonged QT.[69, 127] On the opposite end of the weight spectrum, QT prolongation is also more common in cases of anorexia nervosa than in the general population.[128-130] Finally, many prescription medications can cause QT prolongation (See Section Drug-Induced QT Prolongation).[39]

C.2. Congenital Conditions

Several congenital conditions have been associated with a prolonged QT, including the congenital versions of several structural heart diseases including valvular disease and cardiomyopathy, which manifest similarly to their acquired counterparts.[110, 111] QT prolongation also has a strong genetic component.[24, 25, 33, 131] Congenital LQTS was first described in 1957 by Anton Jervell and Fred Lange-Nielsen.[132] There are two predominant forms, Jervell and Lang-Nielsen syndrome and Romano-Ward syndrome, named after the researchers who first described the two subtypes.[59] These conditions are caused by mutations in the genes encoding the Na^+, K^+, and Ca^{++} ion channel expressed in the heart.[69] For greater detail on the genetics of the QT interval, see Section QT Interval Genetics.

C.3. Electrolyte Imbalances

Electrolyte imbalances are a common cause of QT prolongation, second only to drug-induced LQTS (diLQTS).[133] In fact, it has been suggested that electrolyte imbalances may be responsible for the underlying mechanism of the associations seen with several clinical conditions discussed above, such as anorexia and diabetes.[134, 135] The three most common electrolyte imbalances associated with QT prolongation are hypokalemia, hypocalcemia, and hypomagnesemia, three disorders which represent decreased levels of potassium, calcium,

and magnesium, respectively, in the blood.[111] Linkages between electrolytes and QT were first documented in case reports in the late twentieth century.[136-138] Subsequently, Zeltser and colleagues found that hypokalemia was present in 28% of a population of 249 patients who developed a highly fatal ventricular arrhythmia associated with prolonged QT, torsades de pointes (TdP).[139] The prevalence of hypokalemia in Zeltser's study was significantly higher than that seen in the general U.S. populations according to the NHANES study (3%).[140] Larger, population-based studies have shown that the risk of developing prolonged QT increases between 2 and 4-fold in the presence of hypokalemia, although it is unclear if this association is the same in both men and women.[106, 122] Additional evidence of the role of electrolyte imbalances in the role of QT prolongation was provided by Hoshino and colleagues, who showed that treatment with magnesium sulfate was successful in the treatment of TdP associated with prolonged QT in the presence of hypomagnesemia.[141] A study by Benoit *et al.* in the NHANES III population with over 4,000 men and 4,000 women, suggested that hypocalcemia conferred an increased risk of QT prolongation (OR=6.12 [95% CI: 1.03-36.53]).[106] However, given the imprecision of the results, further work is needed to confirm this association. One possible avenue to investigate is the effect of electrolyte imbalances on mean QT, rather on QT prolongation. However, few studies to date have examined the association any QT risk factors other than genetics with mean QT. The role of electrolyte imbalances in QT prolongation is unsurprising; these electrolyte imbalances can impair the function of the ion channels which are responsible for the electrical conduction of the heart, especially the I_{kr} current, which plays a critical role in ventricular repolarization (Table 3).[134, 142]

D. Drug-Induced QT Prolongation

The most common cause of acquired LQTS is the use of prescription drugs.[133] In 1964, Seizer and Wray first identified drug-induced Long QT Syndrome in patients using the antiarrhythmic quinidine.[143, 144] Drug-induced QT prolongation was the most common cause for medications to withdrawn from the market after approval by the U.S. Food and Drug Administration (FDA) over the past decade.[39, 145] FDA guidelines begin regulating medications after an increase in QT duration of just 5 ms, a modest change in the overall length of the QT interval relative to the mean.[44] As the use of prescription drugs continues to rise[1] and the number of QT prolonging medications identified continues to grow,[146, 147] the importance of understanding the mechanisms of diLQTS will remain critical.

D.1. QT-Prolonging Medications

The University of Arizona's Center for Education and Research on Therapeutics (UAZ-CERT) maintains a database of all medications reported to prolong the QT interval.[146] This database currently includes over 170 medications, most of which are still available in the U.S. market. Of these 170 medications, 107 are known to prolong QT, 36 prolong QT under specific conditions, and a further 28 should be avoided by those with congenital LQTS.[146] Of medications which prolong QT beyond FDA guidelines, there is a broad range of prolongation. A recent study by Iribarren *et al*. found that aripirazole, an antipsychotic, prolonged QT by 7.6 ms while amiodarone, an antiarrhythmic , prolonged QT by 25.2 ms.[148]

Cardiac Medications

Numerous medications used to treat CVD can result in QT prolongation (Table 6). Unsurprisingly, many additional anti-arrhythmic medications have also been found to prolong QT, as these medications interfere directly with the ion channels of the heart.[69]

Table 6. List of Cardiac Medications by Category That Prolong the QT Interval

Anti-anginal	Antiarrhythmic	Antihypertensive	Diuretics	Vasodilators
Bepridil	Amiodarone	Isradipine	Furosemide	Anagrelide
Ivabradine	Disopyramide	Moexipril	Hydrochlorothiazide	Vardenafil
Ranolazine	Dofetilide	Nicardipine	Indapamide	
	Dronedarone			
	Flecainide			
	Ibutilide			
	Procainamide			
	Quinidine			
	Sotalol			

List obtained from UAZ-CERT crediblemeds.org on November 17, 2014[146]

Class 1A antiarrhythmics (disopyramide, procainamide, quinidine) are also known to prolong the QRS interval and JT intervals when examined separately, while class III antiarrhythmics (amiodarone, dofetilide, ibutilide, sotalol) prolong only the JT interval of QT; conversely, class 1C antiarrhythmics (flecainide, trycyclic anti-depressants) are known to prolong QRS but not JT.[69] In addition to antiarrhythmics, anti-anginals, antihypertensives, diuretics, and vasodilators are also known to prolong QT. Of particular interest, hydrochlorothiazide is included in the UAZ-CERT database as a conditional QT prolonging agent but is still a commonly used anti-hypertensive.

Additionally, Iribarren and colleagues found that indapamide, a thiazide-like diuretic, prolonged QT by an average of 9.4 ms and by more than 20 ms in 43% of participants.[148] For greater detail on hydrochlorothiazide and other thiazide and thiazide-like diuretics, the subject of this proposal, see Section Thiazide Diuretics.

Non-Cardiac Medications

In addition to cardiac medications, there are many medications that are not designed for CVD treatment which also have a risk of QT prolongation. In fact, more than 120 of the drugs listed on the UAZ-CERT QT prolonging drug list are not primarily designed to CVD (Table 7).[146] These medications cover a broad range of therapeutic classes, including

Table 7. List of Non-Cardiac Medications by Category That Prolong the QT Interval

Antibiotic	**Anti-cancer**	**Anticonvulsant**	**Antidepressant**	**Antifungal**	**Antihistamine**
Azithromycin	Tamoxifen	Felbamate	Trazodone	Fluconazole	Astemizole
Ciprofloxacin	Lapatinib	Fosphenytoin	Venlafaxine	Itraconazole	Terfenadine
Clarithromycin	Arsenic trioxide		Citalopram	Ketoconazole	Diphenhydra-mine
Gatifloxacin		**Antimalarial**	Fluoxetine	Posaconazole	
Grepafloxacin	Nilotinib	Dihydroartemisinin+	Paroxetine	Voriconazole	**Anti-nausea**
Gemifloxacin	Vorinostat	Piperaquine	Sertraline		Domperidone
Grepafloxacin	Dabrafenib	Chloroquine	Escitalopram	**Antipsychotic**	Dolasetron
Levofloxacin	Eribulin	Halofantrine	Amoxapine	Pipamperone	Granisetron
Moxifloxacin	Sunitinib	Quinine sulfate	Mirtazapine	Mesoridazine	
Norfloxacin	Vandetanib		Amitriptyline	Thioridazine	**Anti-viral**
Ofloxacin		**Kinase Inhibitor**	Clomipramine	Haloperidol	Amantadine
Roxithromycin	**Muscle Relaxant**	Crizotinib	Desipramine	Pimozide	Telaprevir
Sparfloxacin		Vemurafenib	Doxepin	Droperidol	Atazanavir
Telavancin	Tolterodine	Pazopanib	Imipramine	Promethazine	Foscarnet
Trimethoprim-	Tizanidine	Sorafenib	Nortriptyline	Chlorpromazine	Nelfinavir
Sulfamethoxazole	Solifenacin	Bosutinib	Protriptyline	Sertindole	Rilpivirine
Erythromycin		Dasatinib	Trimipramine	Amisulpride	Ritonavir
Pentamidine	**Opiate**			Aripiprazole	Saquinavir
Metronidazole	Levomethadyl	**Miscellaneous**		Clozapine	
Bedaquiline	Methadone	Famotidine	Sevoflurane	Iloperidone	**Sedative**
		Tacrolimus	Ondansetron	Paliperidone	Dexmedeto-mide
		Cocaine	Probucol	Quetiapine	
		Tetrabenazine	Lithium	Risperidone	Chloral hydrate
		Oxytocin	Mirabegron	Sulpiride	
		Mifepristone	Galantamine	Ziprasidone	
		Bortezomib	Apomorphine	Olanzapine	
		Pantoprazole	Toremifene		
		Pasireotide	Cisapride		
		Fingolimod			

List obtained from UAZ-CERT crediblemeds.org on November 17, 2014[146]

antibiotics and antivirals, cancer treatments, antidepressants and antipsychotics, sedatives, and pain medications, in addition to numerous others. However, identifying non-cardiac medications is particularly difficult because the risk of QT prolongation is rarely identified in clinical trials but is rather identified after the medications have been approved, marketed to the public, and commonly used, sometimes after many years.[149] For example, in a large, population based study based in the Netherlands, van Noord *et al.* studied antipsychotics and anti-depressants, two classes of medication which are commonly found to prolong QT. In the study, the antipsychotic thioridazine was found to prolong QT 28.3 ms (95% C.I.: 5.9-50.8) compared with nonusers.[150] A further six medications which significantly prolonged QT were found to increase QT by more than the minimum FDA guidelines (5 ms): lithium (10.1 ms), olanzapine (22.9 ms), amitriptyline (5.1 ms), maprotiline (9.6 ms), imipralnine (12.8 ms), and nortriptyline (23.3 ms).[150] Furthermore, when Iribarren *et al*. examined 90 medications that had been reported to prolong QT in a population-based cohort (N=59,531), they found 78 (87%) significantly prolonged QT and of these 78 medications, 63 were non-cardiac medications.[148]

D.2. Prevalence of QT-Prolonging Medication Use

Despite the rising awareness in both clinical and research settings, the use of QT prolonging drugs continues to be a concern. In a study of 2 million members of health maintenance organizations (HMOs) over a two and a half year period, over 180,000 members filled a prescription for a high-risk QT prolonging medication.[151] Among patients who were admitted to a hospital in Switzerland over a 3 month period who had prolonged QT at admission, defined as $QT_c \geq 450$ ms in men and 460 ms in women, half were subsequently administered a known QT prolonging medication.[122] Similarly, in a study of admissions to a

cardiac care unit, a third of patients who had prolonged QT at admission were later administered a QT prolonging medication and 42% of those who had a $QT_c \geq 500$ ms (extreme prolongation) were administered a QT prolonging agent.[152] These findings indicate that diLQTS remains a prominent concern and more work is needed to prevent diLQTS, either through the development of new medications that do not prolong QT or through the identification of those most at risk for QT prolongation in order to better prescribe QT prolonging medications and avoid potential adverse reactions.

D.3. Clinical Considerations

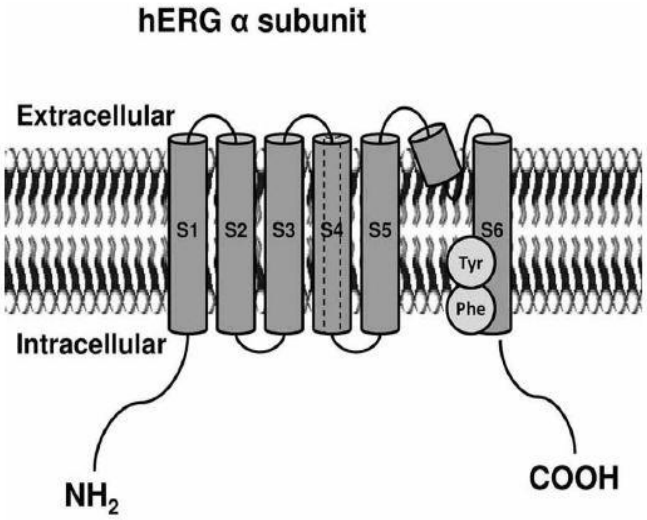
The continued use of QT prolonging medications despite the risk of severe negative outcomes has been widely acknowledged by the medical community.[151, 152] Physicians must weigh the risks and benefits of the use of such medications. In cases where effective alternative treatments are available, the risks of using a QT prolonging agent would outweigh the benefits.[39] Also, in cases where multiple risk factors for prolonged QT are present, adding a QT prolonging medication presents particular concern.[38] However, in cases where an effective alternative treatment is not available, such as with the use of arsenic trioxide to treat a relapse of promyelocytic leukemia, then these medications can and should be used.[39] But in these cases, it is critical to monitor patients. While some clinicians do not consider regular ECGs, both before and after subscribing a QT prolonging agent, cost effective, as for many of these drugs, a thousand patients would need to be assessed to identify a single person at risk, experts in QT pharmacology recommend that patients be screened by ECG prior to administering a QT prolonging medication.[38] Continued, long term monitoring is also important, as Zeltser *et al*. found that, among published cases of torsades de pointes (TdP), an arrhythmia which results diLQTS, only 18% of patients developed TdP within 72

hours of the onset of drug therapy, while 42% developed TdP between 3 and 30 days after the onset of therapy and 40% developed TdP more than a month after the onset of therapy.[139] It is critical that both researchers and clinicians continue to work to identify those at risk of QT prolongation and the mechanisms of this risk to better prescribe, monitor, and prevent diLQTS.

D.4. Mechanisms

Drug-induced LQTS is caused when prescription medications interfere with the normal action of the ion channels of the cardiac conduction system. The ion channel most commonly disrupted is the rapid delayed rectifier K^+ channel, or the I_{Kr}. This channel, encoded by the *KCNH2* gene, also known as the human ether-a-go-go related gene (*HERG*). The HERG channel is composed of six transmembrane subunits and it is on the sixth subunit that the two most important drug binding sites are located: Tyr652 and Phe656 (Figure 6).[143]

Figure 6. Schematic Representation of the HERG (KCNH2) Channel



Adapted from Ponte 2010[144]
The fourth membrane's planning unit (S4) contains positively charged residues and functions as the voltage sensor.
The residues between S5 and S6 form the ion selective pore.
Tyr652 (Tyr) and Phe656 (Phe), marked in the diagram, are the two most important drug binding sites

When drugs bind to the tyrosine located at the 652nd amino acid or phenylalanine at the 656th amino acid, they can reorient these amino acids, subsequently trapping the drug in the central cavity of the channel and preventing the conduction of K^+ ions.[143, 153] The blockage of the I_{Kr} current primarily affects the Purkinje fibers and the mid-myocardium (M cells).[143] The M cells are particularly responsive to drug exposure.[154] In addition to blockage of the HERG channel, M cells can prolong QT through pharmacologic interference of the slow delayed rectifier potassium channel, the sodium channels, and the sodium-calcium exchangers, which while less common than the disruption of the I_{Kr}, make the M cells a primary source in prolongation of Phase 2 and 3 of the action potential of the heart.[154]

E. Categorical Versus Continuous Measures of QT Prolongation

QT prolongation does not have a single, standard threshold. When evaluating QT prolongation suing a threshold, a common cut-point is 450 ms in men and 460 ms in women,[20, 155-158] a threshold which, according to the NHANES population, only 2% of men and 3% of women exceed.[159] In clinical settings, the risk of adverse outcomes is believed to increase substantially at 500 ms.[39, 111] Despite these commonly-used cutpoints, there is no clear threshold at which risks due to QT prolongation increase, and many studies of QT use alternate cut-points or study QT as a continuous outcome. Another alternative is to study QT as a continuous variable and report results for a standard deviation of the population distribution.[160, 161] However, reporting results for a standard deviation prevents generalization across populations and cannot be used in meta-analysis efforts, such as large genetics consortia. Furthermore, it is conceivable that risk factors for QT prolongation may prolong QT a small amount and it is only through a combination of risk factors that higher levels of prolongation are achieved. This is particularly true of the genetic component of QT duration (See Section QT Interval Genetics). Thus, we have chosen to evaluate QT as a

continuous outcome in order to identify risk factors which have smaller although still important effects on QT.

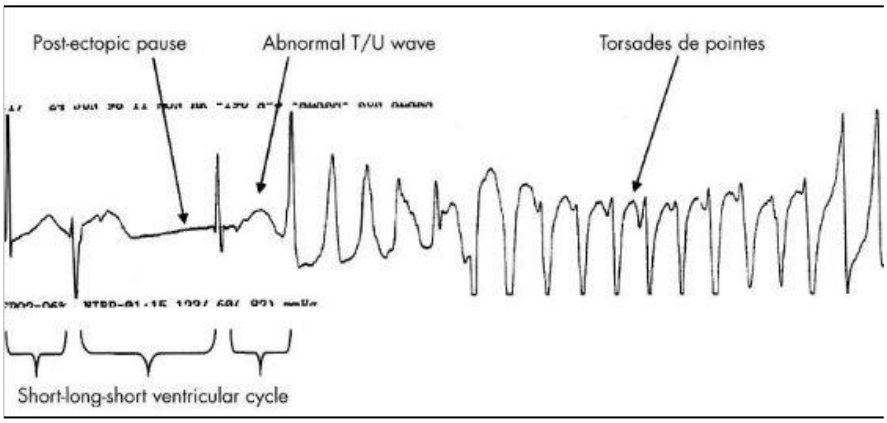
F. Potential Clinical Outcomes of Prolonged QT

QT prolongation has been extensively studied. QT prolongation was first described in association with sudden death in 1957 by Anton Jervell and Fred Lange-Nielsen, for whom the subtype of familial long QT syndrome (LQTS) which they described is named.[132] Torsades de pointes (TdP), the ventricular tachyarrhythmia commonly associated with prolonged QT, was also first described in the mid-20th century (1966) by the French scientist Dessertenne.[162, 163] Since then, prolonged QT has been identified as a risk factor for not just TdP but also numerous other clinical conditions, including coronary heart disease (CHD),[20] congestive heart failure (CHF),[164] stroke,[165] and both cardiovascular and all-cause mortality.[23]

F.1. Arrhythmias

Cardiac arrhythmias, or abnormal heart rhythms, are the most common cause of sudden cardiac death (SCD), which is defined as unexpected death which occurs within one hour of the onset of symptoms if the death is witnessed or within 24 hours of last being seen alive if the death was unwitnessed.[166] SCD accounts for between 200,000 and 400,000 deaths annually in the U.S. and for more than 60% of all cardiovascular deaths, the leading cause of the death in the U.S.[166-168] TdP is the distinctive ventricular tachycardia, a rapid heart rhythm, associated with both congenital and acquired LQTS.[68, 163, 169] TdP is thusly named because it is characterized by a twisting of the QRS peaks through the axis of the ECG (Figure 7). Intermittent TdP often results in syncope (loss of consciousness) before reverting back to a normal rhythm while sustained TdP often devolves into ventricular

Figure 7. ECG Rhythm Strip in a Patient with Torsades de Pointes



Adapted from Yap and Camm 2003[153]

fibrillation and cardiac arrest, often leading to SCD.[19, 163] SCD peaks both in elderly age and in infancy, the latter peak associated with sudden infant death syndrome (SIDS).[166, 167]

Very little is known about the underlying epidemiology of TdP. Drug-induced TdP is the most closely monitored form of TdP and is reported as an adverse drug reaction (ADR) to the World Health Organization's (WHO) Drug Monitoring Centre. For drug-induced TdP, there were 750 total cases reported from 1990 to 1999,[149] a number likely to be an extreme underrepresentation, given the high level of underreporting found for ADRs (as high as 95%).[170] Further complicating the measurement of TdP prevalence are cases of syncope and SCD. In both cases, patients usually present without ECG, making it unclear if TdP was the underlying cause.[171]

It is also worth noting that both a widened QRS and a severely prolonged QT_c were independent predictors of another arrhythmia, atrial fibrillation.[172] In a study of 42,751 participants, 1,050 of whom developed atrial fibrillation during the study period, QRS > 110ms was associated with a hazard ratio (HR) of 1.9 (95% CI: 1.7-2.2) and QT_c > 450 ms was associated with HR = 1.7 (95% CI: 1.5-2.0) of developing atrial fibrillation.[172] Atrial fibrillation is the most common arrhythmia in the U.S., affecting an estimated 2.2 million

people.[173] It is highly associated with both stroke and mortality, accounting for approximately 75,000 strokes per year[174] and a nearly 2-fold increase in the risk of death.[175] The association between QT prolongation and both TdP and atrial fibrillation, one a highly fatal arrhythmia, the other a highly prevalent arrhythmia, underscores the importance of studying QT prolongation and its risk factors.

F.2. Coronary Heart Disease

Coronary heart disease (CHD) is the clinical manifestation of the blockage of the arteries supplying blood to the myocardium, most often through atherosclerosis of the coronary arteries. As of 2010, CHD affected an estimated 15.4 million Americans over the age of 20 and makes up more than half of all CVD events in men and women under age 75.[36] QT_c prolongation is an established risk factor for CHD and CHD mortality.[20, 158, 176-180] Multiple studies have found that QT_c prolongation, corrected using Bazett's formula, is associated with CHD in both black and white men and women (Table 8). Broadly, a prolonged QT has been found to have between a 1.5 and a 2-fold increase in the risk of developing incident CHD or CHD mortality. Using data from the Atherosclerosis Risk in Communities (ARIC) study, Dekker and colleagues found that prolonged QT_c imparted a greater risk of CHD in blacks than in whites when comparing the top 10% of the QT_c distribution to the rest of the population (HR = 2.07 [95% CI: 1.24-3.46] and 1.39 [95%CI: 1.00-1.92], respectively).[20] Maebuchi *et al.* also conducted a study of prolonged QT and CHD in Japanese adults and reported that prolonged QT_c, corrected using Bazett's formula, was associated with incident CHD in Japanese men but not in women (HR = 4.50 [95% CI: 2.18-9.27] and 0.99 [95% CI: 0.37-2.65], respectively) when comparing prolonged QT_c,

Table 8. Review of Four Studies of QT Prolongation and CHD Risk in Black and White Men and Women

Author	Year	Study	N	% Male	% Black	Outcome	Prolonged QT (ms)	Reference QT (ms)	HR (95% CI)
Dekker[176]	1994	Zutphen	851	100	0	CHD Mortality	>420	<385	4.4 (1.2-16.4)
Dekker[27]	2004	ARIC	14,548	43.4	27.0	Incident CHD	Male: >440 Female: >454	<403	2.34* (1.72-3.19) 1.55** (1.08-2.23)
							Male: >450 Female: >465	Male: ≤450 Female: ≤465	2.14 * (1.71-2.69) 1.51** (1.15-1.89)
Robbins[113]	2003	CHS	4,988	40.1	14.7	CHD Mortality	>450	≤410	1.6† (1.0-2.5) 2.0†† (1.1-3.7)
Schillaci[179]	2006	PIUMA	2,110	55	0	Incident CHD	Male: >440 Female: >450	Male: ≤440 Female: ≤450	1.95 (1.12-3.42)

*Adjusted for age, gender, and race

**Adjusted for age, gender, race, and CVD risk factors (heart rate, hypertension, systolic blood pressure, ECG abnormalities, body mass index, waist-hip ratio, cigarette smoking status, cigarette years, total cholesterol, HDL cholesterol, triglycerides, cardiac medications, diabetes, intima-media thickness

†Hazard ratio among participants without CHD at baseline

††Hazard ratio among participants with CHD at baseline

ARIC: Atherosclerosis Risk in Communities; CHD: Coronary heart disease; CHS: Cardiovascular Health Study; CI: Confidence Interval; HR: Hazard ratio; N: Number of study participants; PIUMA: Progetto Ipertensione Umbria Monitoraggio Ambulatoriale

defined as $QT_c \geq 440$ ms to the referent category of $QT_c < 400$ ms, although results were imprecise.[178] This association has also been generalized to populations with other CHD risk factors (type II diabetes mellitus, hypertension, and chronic kidney disease [CKD]).[177, 179, 180] It has been hypothesized that the underlying mechanisms of this association may be irregular regulation of cardiac ion channels, leading to cardiac instability, a mechanism also believed to be the cause of QT-related arrhythmias.[179]

F.3. Chronic Heart Failure

Congestive heart failure (CHF) is characterized by the impaired pumping function of the left ventricle, which results in the heart's inability to meet the body's cardiometabolic demands. CHF is estimated to affect more than 5 million Americans over the age of 20 and is expected to increase in prevalence by 25% between 2013 and 2030.[36, 181] Both prolonged QT and QRS have been associated with CHF.[21, 164, 177, 182] Dhingra *et al.* found that, in an elderly population of 1,759 white men and women, extreme values of QRS (QRS ≥ 120 ms) conferred a significant increase in heart failure risk over normal QRS (QRS < 100 ms), with a HR of 1.74 (95% CI: 1.28-2.35). Furthermore, intermediate levels of QRS prolongation (QRS 100-119 ms) had a HR for risk of heart failure of 1.43 (95% CI: 1.05-1.96) when compared to normal QRS.[182] Similarly, studies have found that prolonged QT confers approximately a 2-fold risk of incident CHF. In a study of 32,283 multiethnic participants in the Women's Health Initiative (WHI), prolonged QT ($QT_c \geq 437$ ms corrected using the linear-scale model) conferred an HR of 1.80 (95% CI: 1.40-2.31) of incident heart failure compared to the rest of the population; this represented an additional 26 cases of incident CHF for every 10,000 women attributable to prolonged QT.[21] A study of 13,555 participants from the ARIC study (57% female, 26% black) found that prolonged QT ($QT_c > 436$ ms in

men and 442 ms in women, corrected using the linear-scale model) resulted in a HR of 1.99 (95% CI: 1.53-2.58) in men.[164] It has also been shown that the risk of CHF associated with prolonged QT is higher in populations with decreased kidney function compared to populations with normal kidney function (HR=4.95 [95% CI: 1.99-12.34], HR=1.66 [95% CI: 1.08-2.58], respectively).[177] It is speculated that both QT and QRS are markers of other underlying causes of CHF, such as structural heart defects or electrolyte abnormalities, rather than direct causes of CHF.

F.4. Stroke

Strokes are a cardiovascular event caused by the acute interruption of blood flow to one or more sections of the brain; there are two main types of stroke: ischemic (most common form) caused by the formation of a blood clot, and hemorrhagic caused by the buildup of blood in the brain or skull. An estimated 6.8 million Americans over age 20 have suffered at least one stroke, with nearly 800,000 new and recurrent strokes occurring annually and over 125,000 annual deaths due to stroke.[36] Several studies have found prolonged QT to be a predictor of incident stroke (both ischemic and hemorrhagic). Early work by Goldstein suggested an association between QT and stroke, finding that 45% of acute stroke patients had a prolonged QT compared with only 12% of the control group, although the direction of the association was not clear.[183] Cardoso and colleagues expanded this work and examined a population of 471 participants with type II diabetes mellitus and found prolonged QT ($QT_c \geq 470$ ms, corrected using Bazett's formula), increased the risk of incident stroke 2.78-fold (95% CI: 1.33-5.81) and increased the risk of incident or recurrent stroke 2.63-fold (95% CI: 1.21-5.28).[22] Soliman and colleagues further expanded this, examining a population of 27,411 participants in the REasons for Geographic and Racial

Differences in Stroke (REGARDS) study. They found a prolonged QT (QTc ≥ 460 ms in women and 450 ms in men, corrected using the Framingham formula) was associated with a smaller increase in the risk of incident stroke (HR = 1.12 [95% CI: 1.03-1.21]) and that using a continuous measure of QT also produced associations as good as using the cut-points, suggesting that the use of specific thresholds may mask some associations.[165] Sensitivity analysis found the same association for ischemic stroke as for both stroke types combined.[165] Similarly to the association between CHF and QT, researchers hypothesize that QT is actually marking subclinical atherosclerosis, which is responsible for the association between QT and stroke.

F.5. Mortality

CVD is the number one cause of death worldwide.[181] The relationship between ECG traits and mortality has been extensively studied and both QT and QRS prolongation have been associated with CVD and all-cause mortality (Table 9). This relationship generally falls around a 1.5-3-fold increase in the risk of death with a prolonged QT or QRS interval, although modest variation by heart rate correction method has been observed.[184, 185] It is also unclear if there is a difference in associated risk in men versus women.[184, 186] However, the relationship between QT, QRS, and mortality has been broadly studied and, while most studies have been conducted in white populations, some work has been done in blacks,[20, 158, 177, 185] Hispanics,[185] and American Indians,[157] suggesting that the association generalizes across race/ethnicities. Finally, a recent meta-analysis of the extensive literature on the association between QT and mortality and found that prolonged QT was associated with a 1.35-fold increase in risk (95% CI: 1.24-1.46) of all-cause mortality and a 1.51-fold increase in risk (95% CI: 1.29-1.78) of CVD mortality. However, it is worth noting that, as is

evidenced in Table 9, there is no single definition for prolonged QT or for the reference category used by these studies and, as mentioned above in regards to stroke, the relationship is likely more continuous than indicated with the use of thresholds.

QT Interval Genetics

As described above, many genes are involved in the electrical conduction system of the heart. These include the many genes in the gene families encoding the multitude of different ion channels involved in cardiac conduction (SCN, CACN, and KCN gene families) as well as numerous other genes or gene regions (loci) which have been implicated in cardiac conduction. In many cases, the genes in addition to ion channels have been associated with measures of cardiac conduction but their function remains unknown.

A. Heritability

The QT interval is heavily influenced by an individual's genetic code. Broadly, a trait's heritability takes into account both Mendelian inheritance patterns (i.e. dominant, recessive, etc.) and the more complex modes of inheritance, most commonly represented as additive effects. While it is difficult to measure a broad sense of heritability, population-specific heritability (h^2), estimated using only genetic effects which are additive, can be measured, which is interpreted as the proportion of the inter-individual variability of a particular phenotype, or trait, which is determined by genetics. For QT, estimates indicate that between a quarter and a half of the phenotypic variation is explained by genetics, which represents a moderately strong genetic influence.[24, 26, 28, 131, 187-190] Given the known forms of congenital LQTS which are caused by dominant, loss-of-function mutations and are therefore not accounted for in the above h^2 estimates, it can therefore be expected that the actual broad heritability of QT is larger than 25-50%.

Table 9. Review of 11 Studies of QT Prolongation and All-Cause or CVD Mortality Risk

Author	Year	N	Race	Prolonged (ms)	Reference (ms)	Outcome (Mortality)	HR (95% CI)
Algra[191]	1991	6,693	White	≥440	<440	All-Cause	2.1 (1.4-3.1)
de Bruyne[184]	1999	5,241	White	>446[a] >437[b]	<418[a] <406[b]	All-Cause	1.8 (1.3-2.4)
						CVD	1.7 (1.0-2.7)
Dekker[27]	2004	14,548	27% Black 73% White	>454[a] >440[b]	<417[a] <403[b]	All-Cause	2.28 (1.73-3.00)
						CVD	3.91 (2.40-6.37)
Elming[192]	1998	3,455	White	≥440	310-380	All-Cause	1.89 (1.04-3.37)
						CVD	3.31 (1.04-9.91)
Hage[155]	2010	280	White	≥460[a] ≥450[b]	<460[a] <450[b]	All-Cause	1.008 (1.001-1.014)
Nilsson[193]	2006	433	White	≥430	<430	All-Cause	2.4 (1.5-3.7)
Noseworthy[156]	2012	6,895	White	>470[a] >450[b]	≤470[a] ≤450[b]	All-Cause	1.21 (0.88-1.66)
						CVD	1.78 (0.90-3.50)
Okin[157]	2000	1,839	American Indian	>460	≤460	All-Cause	2.6 (1.8-3.7)
						CVD	2.3 (1.2-4.6)
Schillaci[179]	2006	2,110	White	≥450[a] ≥440[b]	<450[a] <440[b]	CVD	2.05 (1.03-4.37)
Sohaib[161]	2008	3,596	White	1 SD (26 ms)[c]		All-Cause	1.13 (1.05-1.22)
						CVD	1.17 (1.05-1.31)
Zhang[185]	2011	7,828	9% Black 4% Hispanic 87% White	≥439	401-421	All-Cause	2.03 (1.46-2.81)
						CVD	2.55 (1.59-4.09)

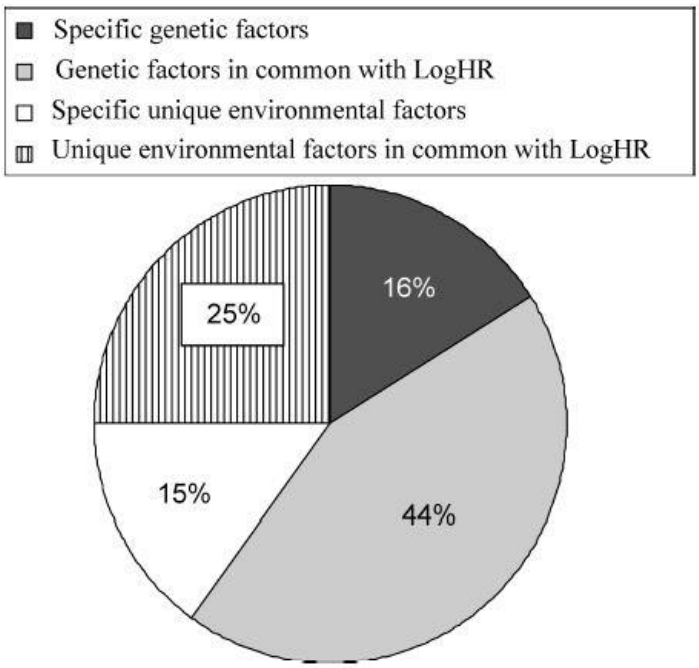
CI: Confidence interval; CVD: Cardiovascular disease; HR: Hazard ratio; ms: millisecond; N: Number of participants; QT: QT interval; SD: Standard deviation

a. In female populations

b. In male populations

c. Linear regression used with ECG variable as continuous variable; HR reported for a unit increase

Figure 8. Sources of Variance in QT Interval



Adapted from Dalageorgou 2008[190]
LogHR refers to the logarithmic transformation of resting heart rate

Several studies have attempted to better understand the heritability of QT. Dalageorgou *et al.*, who found the heritability of QT to be upwards of 50%; 16% of the estimated heritability was explained by genetic factors unique to QT, while the remainder was explained by genetic factors which were also associated with resting heart rate, a relationship which was also mimicked in the environmental determinants of QT (Figure 8).[187] Yang *et al.* determined the amount of the variance which was explained by common single nucleotide polymorphisms (SNPs) and found a lower heritability estimate for QT (h^2 = 21%) using only common variants.[189] They also evaluated heritability by chromosomes and found that the heritability estimates for QT explained by each chromosome were proportional to both the length of the individual chromosome and the length of the genes on the chromosome.[189] Furthermore, Yang and colleagues found that a substantial portion of the 21% heritability they found was explained by intergenic variants (7.5%), with the remainder

explained by genic variants (13.5%).[189] Researchers have also sought to understand the heritability of not just QT but also its component parts. Two studies of twins found heritability estimates between 40-50% for QRS.[26, 190] A more recent study by Newton-Cheh *et al.* found the heritability of JT to be 25%.[28] Combined, these findings suggest that QT and its component parts are strong candidates for genetic study and that researchers are likely to find genetic variants influencing QT in both the coding and noncoding regions of the human genome.

B. Early Studies

Early work in genetics focused on two research strategies. Monogenic diseases, which often followed a Mendelian mode of inheritance (i.e. dominant, recessive), were studied using segregation and linkage analysis.[194] In relation to QT, these included congenital long and short QT syndromes. The second strategy used family and twin studies that were not ascertained for Mendelian diseases to identify genes associated with complex diseases and traits, again using segregation and linkage analysis methods. These early studies were successful in identifying several highly associated regions but struggled to replicate findings across studies and failed to explain much of the heritability observed in the above studies.

B.1. Congenital Long and Short QT Syndrome

As stated above, congenital long QT syndrome was first described by Jervell and Lange-Nielsen in 1957.[132] However, it wasn't until 1999 that the first case of congenital short QT syndrome (SQTS) was described in humans.[195] With both LQTS and SQTS, researchers have since identified numerous genetic mutations believed to cause the two disorders, almost all in gene encoding cardiac ion channels (Table 10). Overall, hundreds of

distinct rare mutations have been linked to congenital LQTS within the six ion channel genes associated with the disorder.[29]

In addition to rare mutations in ion channels, a single mutation in *ANK2*, which encodes ankyrin B, a scaffolding protein, has been linked to LQTS, highlighting the role of non-ion channel genes in QT duration.[196] Ankyrin B influences the functional expression of both ion channels and transporter proteins.[29]

Table 10. Genes Associated With Congenital Forms of Long and Short QT Syndrome

Disease Subtype	Chromosome	Gene	Protein	Protein Function	Inheritance Pattern
LQTS					
LQT1	11p15	KCNQ1	KvLQT1 (I_{Ks})	I_{Ks} channel (α subunit)	AD
LQT2	7q35	KCNH2	HERG (I_{Kr})	I_{Kr} channel (α subunit)	AD
LQT3	3p21	SCN5A	Na Channel	I_{Na} channel	AD
LQT4	4q25	ANK2	Ankyrin B	Ankyrin	AD
LQT5	21q22	KCNE1	MinK (I_{Ks})	I_{Ks} channel (β subunit)	AD
LQT6	21q22	KCNE2	MiRP1 (I_{Kr})	I_{Kr} channel (β subunit)	AD
LQT7	17	KCNJ2	IK1	I_{K1} channel (α subunit)	AD
LQT-JLN1	11p15	KCNQ1	KvLQT1 (I_{Ks})	I_{Ks} channel (α subunit)	AR
LQT-JLN2	21q22	KCNE1	MinK (I_{Ks})	I_{Ks} channel (β subunit)	AR
SQTS					
SQTS	7	KCNH2	HERG (I_{Kr})	I_{Kr} channel (α subunit)	AD
SQTS	11	KCNQ1	KvLQT1 (I_{Ks})	I_{Ks} channel (α subunit)	AD

Adapted from Shah 2005[36] and Zipes 2004[61]
AD, Autosomal dominant; AR, Autosomal recessive

SQTS is similarly associated with numerous ion channel genes. The first to be identified was *KCNH2* (also known as *HERG*), where Brugada *et al*. identified two missense mutations, both of which change the 588th amino acid in the HERG protein from an asparagine (neutrally charged) to a lysine (positively charged), causing a substantial increase in I_{Kr}.[197] Additionally, five more genes have been associated with SQTS, beyond those described in Table 10, when patients with both SQTS and Brugada syndrome phenotypes are studied.[29] Brugada syndrome is another disorder that is characterized by ECG abnormalities, in this case elevation of the ST segment. The five genes include one potassium ion channel gene (*KCNJ2*) and three calcium channel genes (*CACNA1C*, *CACNB2B*, *CACNA2D1*).[29, 198-

[200] In total, there have been ten genes associated with congenital forms of LQTS and SQTS and they further enhance the evidence for ion channels, particularly potassium channels, involvement in prolonging QT.

B.2. Family/Twin Studies

While research in families with LQTS or SQTS worked in identifying the genes associated with the rare congenital forms of the disorders, they did not establish if the same genes, or others, influenced QT variability on a population level. To determine if the same genes were involved, early research on QT used linkage analysis in twin and family studies taken from populations without congenital LQTS. These linkage studies often focused on regions of the genome harboring genes already associated with the congenital form of LQTS. One of the first studies to successfully link a LQTS gene to QT duration in a population not ascertained for LQTS or SQTS was conducted by Busjahn and colleagues in 1999. Busjahn *et al.* examined 166 pairs of twins and found strong evidence for linkage between QT and the genetic regions containing *KCNQ1* and *ANK2*.[190] Newton-Cheh *et al.* expanded on this work in 2005, using FHS families to conduct a genome-wide linkage scan in 10 centimorgan (cM) intervals. A cM measures a genetic distance in which 0.01 crossover events are expected to occur each generation. This family-based linkage study was a precursor to the later genome wide association studies discussed in the next section and allowed Newton-Cheh and colleagues to identify linkages between QT and three genetic regions including the region surrounding *SCN5A* on chromosome 3, a region on chromosome 9 at 104 cM, and a region on chromosome 15 at 102 cM.[28]

B.3. Candidate Gene Studies

While linkage analyses of QT were successful in identifying many of the same regions that harbored genes associated with congenital LQTS, they only identified large regions of the genome. Candidate gene studies, on the other hand, relied on genotyped SNPs and could evaluate specific genetic variants. However, candidate gene studies required *a priori* hypotheses and were thus limited to examining a handful of SNPs from a limited number of loci underlying previously identified linkage peaks or loci associated with Mendelian forms of LQTS/SQTS.[201] For example, Pfeufer *et al*. examined 174 SNPs from four candidate genes (*KCNQ1*, *KCNH2*, *KCNE1*, and *KCNE2*) and were able to identify one SNP in *KCNQ1* and *KCNE1* and two independent loci in *KCNH2*.[202] However, the identified SNPs explained only 1% of the variance in QT. Because of this, candidate gene studies, like linkage analyses, have not been successful in expanding our knowledge of the genetic underpinnings of ventricular conduction. Instead, the field has moved into a GWAS era.

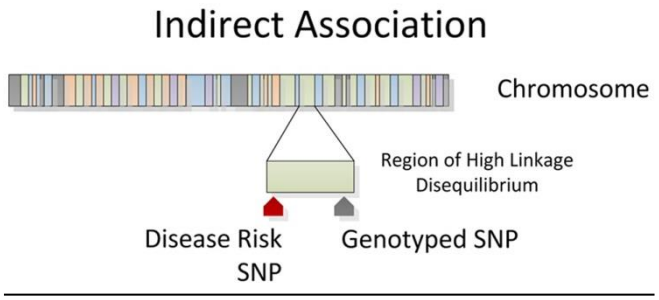
C. Genome-Wide Association Studies

The completion of the Human Genome Project (HGP), which sought to catalog human genetic variation by identifying all human genes and sequencing the three billion bases in the human genome, allowed researchers to conduct large-scale human genome studies such as GWAS.[203, 204] Specifically, GWAS enabled researchers to test associations between complex traits of interest and thousands-to-millions of SNPs throughout the human genome, greatly increasing the coverage from linkage analyses and candidate gene studies.[8] One reason for the success of GWAS is that they leverage an important property of SNPs in the human genome: linkage disequilibrium (LD). LD describes the degree to which one allele, or variant, of a SNP is correlated with another SNP on the same chromosome within a

population.[205] Two SNPs in high LD with each other tend to be inherited together and when the allele of one SNP is known, it can be used to infer the allele of the second SNP. Because of LD, researchers do not need to genotype every SNP in the human genome to make inferences about large segments of the genome. Instead, GWAS rely on indirect associations (Figure 9). Using indirect association, researchers expect that in most cases, the specific SNP identified in a GWAS is not actually the causal SNP but is rather a marker of the causal SNP, with which it is in high LD. Because GWAS can be conducted in large, unrelated populations and do not require a candidate region to be identified *a priori*, they have been highly successful in unraveling the genomic etiology of many complex diseases and identifying thousands of novel associations.

ECG traits have been a popular phenotype for GWAS inquiry. To date, there have been eleven GWAS performed for QT, two of which also evaluated QRS separately from QT, and another three which evaluated QRS but not for QT (Table 11). The earliest of these was conducted by Arking and colleagues on approximately 4,000 European descent individuals and identified three genetic loci (regions) which were associated with QT, including *NOS1AP*, which has since become the top finding in many subsequent GWAS (Table 11, Appendix 1).[30] However, Arking studied only the extremes of the QT

Figure 9. Indirect Associations in GWAS Using Linkage Disequilibrium



Adapted from Bush 2012[201]

distribution. Later GWAS which evaluated the whole QT distribution had even greater success in identifying and replicating novel associations which are now considered valid QT loci (Table 11). Most GWAS of QT and QRS have been conducted in European descent populations; however, there have been three GWAS in Asian/Pacific Islander populations[31, 206, 207] and one in African descent populations.[208] The results of these GWAS have confirmed the role of ion channel genes in ventricular conduction across global populations, as well as identified numerous novel associations.

C.1. Ion Channel Genes

Seven different ion channel genes have been associated with QT or QRS in at least one GWAS (Table 11). The majority of the ion channel genes associated with QT are potassium channel genes (*KCNE1, KCNH2, KCNJ2, KCNQ1*). All four of the potassium channel genes identified through GWAS were previously associated with congenital LQTS and SQTS (Table 10), but the identified variants are more common in the population than the rare variants associated with LQTS and SQTS. Each of these four genes was identified by at least two different studies with approximately 10,000 participants or more. Within the four potassium channel genes, fifteen different SNPs have been associated with QT in the five largest studies with ~10,000 participants or more, making it unclear what the causal SNP or SNPs are (Appendix 1). In addition to the potassium channel genes, two sodium channel genes have also been identified, *SCN5A* and *SCN10A*, two genes which are next to each other on the chromosome three but are not in strong LD. However, while *SCN5A* has been associated with both QT and QRS,[35, 209, 210] *SCN10A* has only been associated with QRS,[210, 211] suggesting that it may not be critical to the repolarization process but may be involved in depolarization. While both sodium and potassium channels have been heavily implicated in

the duration of ventricular conduction, the evidence for calcium channels is far weaker. Only one calcium channel gene (*CACNA1D*) has been associated with QRS and none have been associated with QT in a GWAS.[210] Furthermore, the single calcium channel gene identified is not the same as the calcium channel genes potentially associated with congenital LQTS.[198, 210]

C.2. Novel Associations

In addition to the ion channel genes, GWAS have identified a multitude of novel associations with QT. In the five largest studies alone, more than 70 SNPs have been associated at more than 30 loci across the genome (Appendix 1). The most consistently associated locus in GWAS of QT is *NOS1AP*. Within this locus, rs12143842 is the most commonly identified SNP. Six of the eleven GWAS of QT have found an association between rs12143842 and QT, with effect sizes near 3 ms.[31, 33-35, 208, 209] This association has also undergone functional characterization. Kapoor *et al.* examined rs12143842 as well as all SNPs in high LD with it and found that rs7539120 is the most likely functional variant underlying this association, as the T allele of rs7539120 increases expression of *NOS1AP* and that increased *NOS1AP* expression does alter cardiac electrophysiology, potentially through the propagation of the electrical current rather than directly through the depolarization and repolarization currents.[214]

In addition to *NOS1AP*, other notable loci associated with QT are listed in Table 11. Many of these loci have been associated not just with QT but with other measures of cardiac conduction. For example, in addition to its association with QT and QRS, *TBX5* has also been associated with the PR interval, as have *CAV1*, *SLC8A1*, *SCN5A*, and *SCN10A*.[214-217] Furthermore, *PLN* has also been associated with left ventricular structure and is known to

Table 11. Summary Results of QT and QRS Genome-Wide Association Studies

Author, Year	ECG Trait(s)	Race	N	Populations	Notable Results: Ion Channel Genes	Notable Results: Novel Associations
Arking, 2006[37]	QT	EU	3,996	FHS, KORA		*NOS1AP*
Newton-Cheh, 2007[212]	QT	EU	1,345	FHS		*NOS1AP*
Marroni, 2009[32]	QT	EU	2,325	EUROSPAN		*NOS1AP*
Newton-Cheh, 2009[33]	QT	EU	13,685	CHS, FHS, RS	*KCNH2* *KCNQ1* *SCN5A*	*LIG3* *LITAF* *NDRG4* *NOS1AP* *PLN*
Nolte, 2009[34]	QT	EU	3,558	BRIGHT, DCCT/EDIC, TwinsUK		*NOS1AP* *PLN*
Pfeufer, 2009[35]	QT	EU	15,842	ARIC, KORA, SardiNIA, GenNOVA, HNR	*KCNH2* *KCNJ2* *KCNQ1* *SCN5A*	*ATP1B1* *LITAF* *NDRG4* *NOS1AP* *PLN*
Smith, 2009[207]	QRS	AS	1,604	Kosrae		
Chambers, 2010[31]	QT QRS	AS	6,543	London Life Sciences on the Indian Subcontinent	*KCNH2* *KCNJ2* *SCN5A* *SCN10A*	*ATP1B1* *LITAF* *NOS1AP* *PLN*
Holm, 2010[213]	QT QRS	EU	9,860	Icelandic Cohort	*KCNE1* *KCNH2* *KCNQ1*	*ATP1B1* *LITAF* *NDRG4* *TBX5*
Sotoodehnia, 2010[210]	QRS	EU	40,407	CHARGE	*CACNA1D* *SCN10A* *SCN5A*	*PLN* *TBX5*
Kim, 2012[206]	QT	AS	6,805	KARE	*KCNH2* *KCNQ1* *SCN5A*	*NDRG4* *NOS1AP* *PLN* *SLC8A1*
Smith, 2012[208]	QT	AA	12,097	COGENT, CARe, WHI		*ATP1B1* *NOS1AP* *SLC8A1*
Ritchie, 2013[211]	QRS	EU	5,272	eMERGE	*SCN5A* *SCN10A*	
Arking, 2014[209]	QT	EU	76,061	QT-IGC	*KCNE1* *KCNH2* *KCNJ2* *KCNQ1* *SCN5A*	*ATP1B1* *CAV1* *LIG3* *LITAF* *NDRG4* *NOS1AP* *PLN* *SLC8A1*

AA, African descent population; ARIC, Atherosclerosis Risk in Communities; AS, Asian descent population; BRIGHT, British Genetics of Hypertension; CARe, Candidate-gene Association Resource; CHARGE, Cohorts for Heart and Aging Research in Genetic Epidemiology; CHS, Cardiovascular Health Study; COGENT, Continental Origins and Genetic Epidemiology Network; DCCT/EDIC, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications; eMERGE, Electronic Medical Records and Genomics; EU, European descent population; EUROSPAN, European Special

Population Research Network; FHS, Framingham Heart Study; GenNOVA, EURAC-Institute for Genetic Medicine; HNR, Heinz Nixdorf Recall Study; KARE, Korea Association Resource; KORA, Cooperative Health Research in the Region of Agusburg; N, Number of study participants; QT-IGC, QT Interval-International GWAS Consortium; RS, Rotterdam Study; SardiNIA, Progenia for the Sardinian public; TwinsUK, Twin Registry of the United Kingdom; WHI, Women's Health Initiative

affect rates of cardiac contraction in mice.[34, 218] Together, these results suggest that ventricular conduction is influenced not just by the ion channels directly involved in ventricular depolarization and repolarization, but also by many other factors broadly involved in overall cardiac electrophysiology. This emphasizes the potential for GWAS to identify novel biology underlying complex traits.

C.3. Replication in Multi-Ethnic Populations

As previously mentioned, most GWAS to date have been conducted in population of European descent. However, there has been some effort to generalize the results from European descent populations (EU) to multi-ethnic populations. In particular, results from the *NOS1AP* locus have been generalized to African descent (AA), Asian descent (AS), American Indian, and Hispanic/Latino populations (HL).[219-221] Furthermore, SNPs from *NDRG4*, *KCNE1*, *SCN5A*, *SCN10A*, and *KCNH2* have been generalized to AA populations for QT and QRS.[220, 222] Additionally, the following loci have been generalized to American Indian populations: *ATP1B1*, *SCN5A*, *PLN*, *KCNH2*, *KCNQ1*, *LITAF*, and *NDRG4*.[220] Only four additional loci have been generalized to HLs: *ATP1B1*, *KCNH2*, *LITAF*, and *NDRG4*.[220] However, global genetic architecture varies by race/ethnic group, with EU populations having the largest regions of LD and AA populations having the smallest regions of LD.[223] Given the underlying differences in LD patterns between different ancestral populations, it is not surprising that in many cases, the index, or most highly associated, SNP in EU populations is not associated with QT in multi-ethnic populations or there is a better marker of the signal in these populations.[224] For example, a fine mapping study of QT found that of

the six loci which generalized to the AA, four had different index signals in the AA populations than the one which had been previously identified in EU populations and for two of these four, the index signal identified in EU populations was not significantly associated with QT.[225] Furthermore, the *SLC8A1* locus was not identified in EU populations but has been identified and replicated in AS populations for not only QT but also other ECG metrics.[206, 215, 226] This illustrates why it is imperative to expand genetic studies beyond EU populations and include a diverse range of populations.

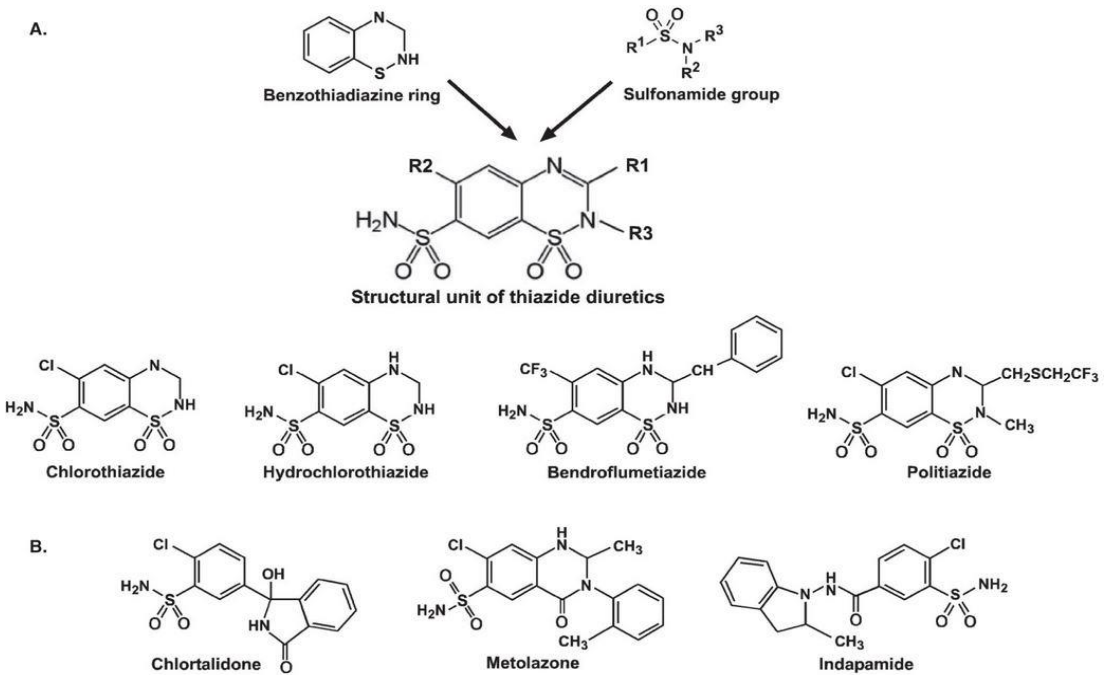
D. Gene-Environment Studies

Several lines of evidence suggest that environmental influences, including potassium levels, moderate the genetic associations with QT. Two family studies of congenital LQTS identified two different mutations in the *KCNQ1* gene, which is mutated in LQT1 patients, both of which are only identified in patients presenting with hypokalemia at the time of their ECG.[227, 228] Furthermore, a recent study in AAs identified a mutation in *SCN5A* for which hypokalemia can moderate its association.[229] Further evidence for the potential role of gene-environment (GxE) interactions in QT is provided by the concept of "missing heritability." While GWAS have identified a multitude of genetic variants associated with QT, they only explain 10% of the variance in QT.[209] One common hypothesis for this "missing heritability" is GxE interactions.[230] However, GxE studies require a much greater sample size to achieve sufficient power to detect associations and for this reason, there have been few well-powered GxE studies of QT. To successfully identify GxE interactions, studies must often combine into large consortia, as is proposed in this work, to achieve the sample sizes required to detect GxE associations. Such further work in GxE studies could help illuminate the underlying biology of the missing heritability of QT.

Thiazide Diuretics

Thiazide diuretics are a promising candidate for GxE inquiry in QT, as this class of medications has been associated with QT prolongation (Section Thiazide-Induced QT Prolongation). Briefly, thiazide and thiazide-like diuretics are members of a class of pharmaceuticals which increase renal excretion of sodium and water. Thiazides were first released in 1957 as an antihypertensive and have since become a critical drug in hypertension treatment.[40, 231] Thiazide and thiazide-like diuretics are distinguished by their molecular structure (Figure 10). Thiazides are derived from the bezothiadiazine core while thiazide-like diuretics are derived from sulfonamide.[40] Thiazides and thiazide-like medications are commonly considered together, as they have a similar mechanism of action. They will be referred to jointly as thiazides for the remainder of this proposal. Below, I will examine the

Figure 10. Molecular Structure of Thiazide and Thiazide-Like Diuretics



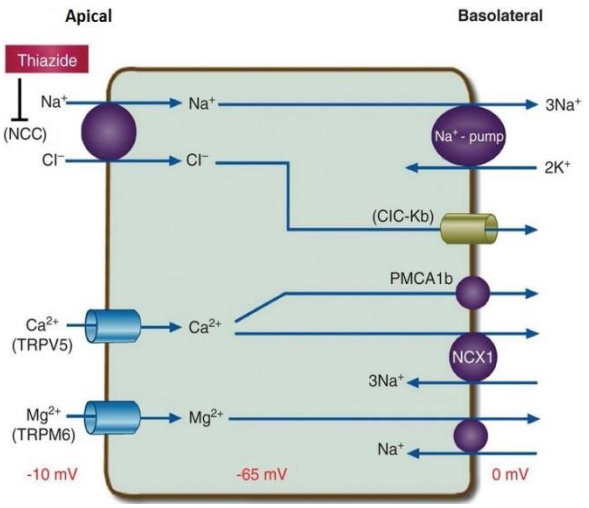
Adapted from Tamargo 2014[47]
Panel A: Thiazide diuretics with the characteristic 1,2,4-benzothiadiazine-1,1-dioxide
Panel B: Thiazide-like diuretics with the sulfonamide group but not the benzothiadiazide group

pharmacology of thiazides and the association between thiazides and QT.

A. Pharmacologic Characteristics

Thiazide diuretics increase the excretion of sodium by inhibiting the reabsorption of Na^+ in the kidneys. Thiazides are actively excreted from the proximal tubule of the renal nephron from which they then move to block the electroneutral Na^+-Cl^- cotransporter (NCC) on the apical membrane of the distal convoluted tubule (DCT) of the renal nephron (Figure 11).[40] The NCC is encoded by *SLC12A3* from the SLC family of genes.[40, 66] At the NCC, Na^+ moves down its concentration gradient using energy produced by the Na^+/K^+-ATPase on the basolateral membrane of the DCT. When Na^+ absorption is inhibited, resulting in an increased delivery of Na^+ to the DCT, K^+ excretion is also increased, which can lead to hypokalemia. An increase in Mg^{++} excretion, which can result in hypomagnesemia, is also seen with thiazide use but the mechanism underlying this phenomenon is not well understood but may result from a downregulation of the transient receptor potential cation channels on the apical membrane of the DCT, encoded by *TRPM6*.[40] Conversely, thiazide use increases

Figure 11. Transport Mechanisms of the Distal Convoluted Tubule



Adapted from Tamargo 2014[47]

Ca^{++} reabsorption, thereby reducing calcium excretion. When water and sodium excretion is increased with thiazide use, the contraction of the extracellular fluid volume triggers an increase in sodium reabsorption in the proximal tubules, which also causes passive Ca^{++} transport. Thiazides also stimulate Ca^{++} reabsorption in the DCT through the basolateral Na^{+}/Ca^{++} exchanger (NCX1) and the Ca^{++}-ATPase channel (PMCA1b).[40]

B. Indications of Use

Thiazides are most commonly used to treat hypertension and are effective in lowering blood pressure (BP) in hypertensive individuals without lowering BP in normotensive individuals.[40] The "Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure" (JNC 7) recommend thiazide diuretics as the first choice medication, either as monotherapy or as part of a combination therapy, in the treatment of hypertension.[232] Thiazides are also used to treat edema, or the accumulation of fluid in the body cavities, in patients with heart failure, although usually only in combination with a loop diuretic.[233] Similarly, thiazides are used to treat edema associated with liver cirrhosis and renal therapy.[234] Thiazides have also been found effective in treating osteoporosis, likely due to its effects on calcium reasbsorption.[235, 236] The same mechanism leads to the usage of thiazides to treat calcium-based kidney stones.[237]

B.1. Contraindications

Thiazides are contraindicated in patients with anuria, renal failure, an allergy to thiazides or other sulfonamide drugs, or hepatic coma.[234] Thiazides are also contraindicated if BP control worsens in patients with chronic kidney disease that progresses to stage 4 or 5. Pregnant women should also avoid thiazides. Furthermore, patients with impaired liver function, hypokalemia, hyponatremia, hyperuricemia, hypercalcemia, glucose intolerance, or diabetes should be closely monitored while using thiazides to prevent adverse outcomes.[40]

C. Prevalence

Thiazide diuretics are an increasingly common antihypertensive therapy. Over a quarter of the hypertensive population in the U.S. (1 in 3 U.S. adults) uses a thiazide diuretic, amounting to 13% of the total U.S. EU population, 10% of the HL population, and 23% of the AA population.[36, 37, 238] The use of thiazides increased from 2001 to 2010, with 22% of hypertensive individuals using a thiazide in 2001 to almost 28% of hypertensives using a thiazide in 2010 (Table 12).[37] The majority of those using a thiazide (>90%) were using it in conjunction with other drugs in polytherapy.[37] Thiazide use is more common in females than males (32% and 23%, respectively). These medications are also more commonly used by Blacks (34%) and least commonly by Hispanic populations (22%). Of the different types of thiazide and thiazide-like diuretics, hydrochlorothiazide is the most commonly used (11% of the U.S. adult hypertensive population).[37, 40, 239] However, despite the high prevalence of thiazide diuretic use, it is worth noting that adherence to thiazides is particularly low, with some of the lowest adherence rates among antihypertensive medications, with only 51% mean adherence, compared to 65% adherence to angiotensin receptor blockers, the drug class with the highest levels of adherence.[240, 241] However, Smith *et al.* found that hydrochlorothiazide, the most commonly used thiazide, had good agreement between reported thiazide use and serum measurements of thiazides (kappa [degree of agreement beyond chance] = 0.62, 95% C.I.: 0.53-0.91).[57]

Table 12. Prevalence of Thiazide Diuretic Use Among Hypertensive Adults Over Time in the United States

Population	2001-2002 % (SE)	2003-2004 % (SE)	2005-2006 % (SE)	2007-2008 % (SE)	2009-2010 % (SE)	*P* trend
Overall	22.4 (2.1)	24.2 (1.4)	26.3 (1.9)	26.7 (1.8)	27.6 (1.3)	0.02
Monotherapy	NA	1.6 (0.4)	4.1 (0.6)	2.1 (0.5)	2.5 (0.4)	0.09
Polytherapy	20.8 (1.9)	22.5 (1.4)	22.2 (1.7)	24.5 (1.6)	25.1 (1.3)	0.04
Male	16.4 (2.1)	21.2 (2.0)	20.9 (2.4)	21.0 (1.9)	23.3 (1.4)	0.04
Female	27.2 (2.1)	26.8 (1.7)	31.3 (2.1)	31.8 (2.0)	31.6 (2.1)	0.04
Non-Hispanic White	23.3 (2.4)	23.8 (1.4)	26.6 (2.6)	27.4 (2.1)	27.4 (1.6)	0.07
Non-Hispanic Black	26.0 (2.7)	29.0 (2.9)	32.0 (2.3)	30.7 (1.8)	34.2 (2.2)	0.02
Mexican American	13.4 (3.2)	19.2 (2.8)	10.4 (2.8)	18.4 (2.0)	22.2 (2.4)	0.06

Adapted from Gu 2012[44]

D. Thiazide-Induced QT Prolongation

Thiazide diuretic use has been linked to the development of QT prolongation and drug-induced torsades de pointes, although the underlying mechanism of this association is not as well understood as that of many of the other QT prolonging drugs, making it an excellent candidate for pharmacogenomics inquiry.[38-40] Both indapamide and hydrochlorothiazide are considered to have conditional risk of QT prolongation and TdP by the UAZ-CERT database but it is not well-established what the risk is conditional on. Pharmacogenomics work can help elucidate what potential mechanisms are underlying the risk of QT prolongation and thiazide use, aiding in identification of those at highest risk of QT prolongation and TdP due to thiazide use.

D.1. Pharmacoepidemiology

Associations between thiazide diuretics, ECG abnormalities, and arrhythmogenic death was first reported in the 1980s,[242-245] when Hollifield and Slaton found that patients taking hydrochlorothiazide were more likely to suffer SCD than patients not on a thiazide diuretic.[242] The relationship between SCD and diuretic use was also reported by Hoes *et al.* and Cooper *et al.* in the 1990s. While not restricted to thiazides, both studies found that the use of non-potassium sparing diuretics increased the risk of SCD: Cooper *et al*. found that

diuretics increased the risk of SCD 1.33-fold (95% CI: 1.05-1.69); Hoes *et al*. found an OR of 2.2 (95% CI: 1.1-4.6) if the diuretic was not taken with a beta blocker.[246, 247] It was estimated that, in 1994, thiazide use was responsible for more than 10% of all SCD in the Netherlands, totaling 120 deaths.[248] Furthermore, Siscovick and colleagues found that the relationship between cardiac arrest and thiazides was dose dependent. A high dose (100 mg) was found to increase the risk of cardiac arrest compared to a low dose (25 mg) with an OR = 3.6 (95% CI: 1.2-10.8).[249] Together, these results suggested a link between thiazide use and SCD, a correlate of QT prolongation.

In addition to the link between thiazides and SCD, a link has also been found between thiazides and ECG abnormalities. Hollifield and Slaton found that thiazide use was associated with an increased prevalence of premature ventricular contractions (PVCs) in the presence of exercise, which have been shown to precede TdP in cases of LQTS.[242, 250] Additionally, the Multiple Risk Factor Intervention Trial (MRFIT) identified an unexpected excess of CVD mortality, primarily sudden death, among hypertensive men with ECG abnormalities who received high-dose diuretic treatment (hydrochlorothiazide or chlorthalidone).[243] Porthan *et al.* also found that hydrochlorothiazide use increased the length of the T wave component of the QT interval, which suggests an increase in the repolarization heterogeneity.[251] Repolarization heterogeneity has been suggested as a marker of TdP development in the case of prolonged QT, indicating thiazide use may predispose individuals for fatal outcomes of QT prolongation.[251]

The link between thiazides and prolonged QT has been specifically tested in several studies. This was first shown by Struthers *et al.* who found that pretreatment with benzofluamethiazide was associated with prolongation of the QT interval in the presence of

adrenaline.[245] However, it was not clear if prolonged QT was the result of the thiazide usage or adrenaline. The Prevention of Atherosclerotic Complications with Ketanserin (PACK) trial was able to better separate the effects of the thiazide from that of ketanserin, the serotonin antagonist which was being tested in the trial, to get a clearer result. The PACK trial found that, at randomization and prior to the introduction of ketanserin, patients who were taking a diuretic had a longer QT than those not on a diuretic by an average of 7 ms; when ketanserin was added, participants on a diuretic had a QT interval that was an average of 12 ms longer than those who were not taking a diuretic and just taking ketanserin alone.[244] Finally, Rautaharju *et al*. examined the association between thiazide usage and QT prolongation in a population of more than 4,000 men and women in the Cardiovascular Health Study (CHS). They found that, after adjusting for the use of other QT prolonging agents, serum potassium levels, sex, gender, and other potential confounders, thiazide diuretic usage was associated with a significantly increased likelihood of QT-prolongation (OR = 1.73, 95% C.I.: 1.43-2.11).[252]

Despite the above studies, there are still several areas of research that are lacking. For example, no studies to date have examined the relationship between thiazide use and QT prolongation in a large, population-based cohort. Iribarren and colleagues did examine a single thiazide-like diuretic, indapamide, in a large cohort of almost 60,000 individuals and found indapamide increased QT by an average of 9.4 ms (95% C.I.: 4.9-14.0).[148] However, no larger studies of additional thiazide class medications has been conducted for QT prolongation. Furthermore, while both the study by Struthers and the PACK trial found that diuretic use was associated with an increased QT in the presence of other medications, few additional studies to date have specifically examined potential modifications of the thiazide-

QT relationship. Given the conditional nature of the thiazide-QT relationship, it is imperative to understand the conditions by which thiazides prolong QT, which calls for additional studies of potential modifiers, including genetics, which is the subject of this proposal.

D.2. Proposed Mechanisms

While thiazide diuretics interfere with cellular ion channels, the ion channels affected by thiazides have not yet been identified in cardiac conduction. Therefore, it has been suggested the thiazide-induced QT prolongation is the result of the electrolyte imbalances induced by thiazide usage, primarily hypokalemia. Potassium depletion is a well-known side effect of thiazides,[253-256] and is also an established risk factor for QT prolongation and TdP, suggesting that QT prolongation may be caused by the thiazide-induced hypokalemia.[111]

Further evidence supporting a potassium-mediated cause for QT prolongation in thiazide users comes from patients who suffer from Gitelman syndrome (GS). GS is a familial hypokalemia-hypomagnesemia disorder affecting approximately 1 in 40,000 individuals.[42] Most cases of GS are caused by mutations in the *SLC12A3* gene, which encodes the thiazide-sensitive NCC (Figure 11).[42] Studies of patients with GS have found that QT is significantly prolonged in these patients and they are at higher risk for cardiac arrhythmias.[41, 257]

However, there has been some evidence that thiazide diuretics interfere directly with cardiac conduction and that the mechanism of QT prolongation may not be solely through electrolyte levels. For example, Lu *et al*. found that the thiazide-like diuretic agent indapamide inhibited the sodium currents in the heart, as well as two of the potassium currents directly involved in ventricular repolarization: the I_{to} (transient outward current),

involved in phase 1 of repolarization and the I_{Ks} (slow delayed rectifying current), involved in phase 3.[43] Additionally, Fiset *et al.* demonstrated that the addition of indapamide to class III antiarrhythmic drugs, known QT prolonging medications, exacerbates the block of the I_{Ks} and can lead to excessive QT prolongation.[258] While thiazides do not have as clear or strong direct effects on cardiac ion channels, these effects may still be significant enough to prolong ventricular conduction, especially when taken in combination with other QT prolonging agents.

Pharmacogenomics

Drug efficacy and safety are highly variable between individuals and this variability in drug response poses a significant problem in the effective treatment of disease.[2-4] For example, as discussed above, many drug classes including thiazides can lead to QT prolongation but this potentially dangerous side effect does not occur in all individuals. However, it is often unclear what the underlying causes of this variability are. Genetics are believed to play a major role in determining drug response. Genetic variants are known to interfere in pharmacokinetic, or the relationship between drug dose and concentration, processes, which include absorption, distribution, metabolism, and excretion, as well as in pharmacodynamic, or the manifestation of drug action, processes, such as the interaction between the drug and drug targets.[61] This has led to the field of pharmacogenomics, which studies gene-environment interactions relating to pharmaceuticals.

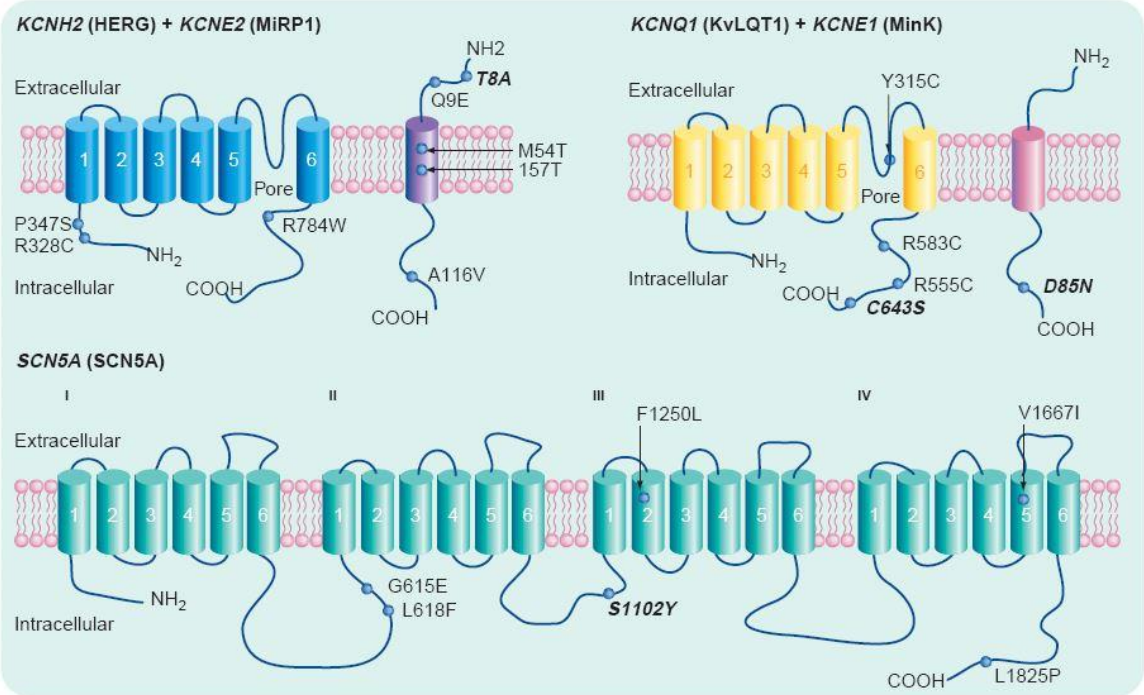
The most well-known example of applied pharmacogenomics is warfarin (a commonly used anticoagulant) dosing.[259] Genetic variants identified in the *CYP2C9* and *VKORC1* explain up to 50% of the variability in dose response to warfarin.[259] These two genes are responsible for metabolizing the pharmacologically active *S*-warfarin isomer and variants in these genes confer increased sensitivity to warfarin, resulting in a smaller

effective dose in patients with these variants.[7] However, there have also been numerous other successful applications of pharmacogenomics research. The FDA recommends genetic testing for AS populations before prescribing carbamazepine, an anticonvulsant, after a prospective trial found that variants in the *HLA-B* gene found on the Asian haplotype modified the risk of fatal toxic side effects.[7, 260] The FDA also recommends screening for variants in the *HLA-B* gene before prescribing abacavir, an antiretroviral, after a randomized clinical trial showed that genetic screening significantly reduced cases of hypersensitivity.[259, 261] These examples illustrate the potential clinical significance of pharmacogenomics research.

A. Pharmacogenomics of QT-Prolonging Drugs

As drug-induced QT prolongation is a leading cause of withdrawal or restricted marketing of drugs, identifying the genetic component of diLQTS is a critical question for pharmacogenomics researchers. There already exists a substantial body of research on this subject, although most studies have evaluated QT prolonging medications in aggregate rather than examining specific drug classes. In other words, these studies evaluate populations taking any drug or combination of drugs that have been implicated in QT prolongation. Unsurprisingly, many of the genes involved in congenital LQTS have also been implicated in diLQTS. In particular, *KCNH2, KCNE1, KCNE2, KCNQ1, and SCN5A* have been associated with diLQTS in multiple studies.[262-267] A subset of the genetic variants associated with diLQTS within these five genes and their locations within the product protein can be seen in Figure 12. The majority of variants identified are rare mutations (present in less than 1% of the population).[266, 268] However, Kaab *et al.* identified a common polymorphism in *KCNE1* (rs1805128) which was associated with a high risk of diLQTS (OR=9.0, 95% CI: 3.5-22.9).[264]

Figure 12. Structure of Ion Channel Proteins Involved in Drug-Induced Long QT Syndrome



Adapted from Aerssens 2004[263]
Mutations and Polymorphisms that have been associated with drug-induced LQTS are marked with blue dots and labeled

In addition to the genes involved in congenital LQTS, several other genes have been implicated in diLQTS. Jamshidi and colleagues identified a common variant in *NOS1AP* (rs10800397), the top gene associated with QT in GWAS, which confers a three-fold increase in risk of diLQTS (OR=3.3, 95% CI: 1.0-10.8).[269] A candidate gene analysis of antipsychotics and QT also identified *NOS1AP* as a modifier of diLQTS.[270] In addition, two genes encoding cytochrome P450 enzymes, *CYP2D6* and *CYP3A4*, enzymes involved in drug metabolism, have been associated with diLQTS.[263, 271] Mutations in these cytochrome P450 enzymes can reduce the efficiency of drug metabolism, thus increasing the concentration of QT-prolonging medications in the heart and thus induce QT prolongation.[263] Additionally, two GWAS of the antipsychotic-QT association identified multiple genes in the SLC family of genes, including *SLC22A23* and *SLCO3A1*. While these findings support the

role of genetics in determining those at risk of drug-induced QT prolongation, few loci have been replicated and studies have been underpowered. Furthermore, the reported results are often imprecise and the field likely suffers from publication bias. To confirm and expand on the above findings, larger GWAS of diLQTS are needed.

B. Pharmacogenomics of Thiazide Diuretics

Multiple genetic loci have been implicated and replicated in the antihypertensive response and risk of side effects of thiazide diuretics. Unlike other genetic studies, pharmacogenomics work on antihypertensive response has been conducted in multiple racial groups, including EU, AA, and AS descent populations, and has been replicated across populations. For example, genes involved in the renin-angiotensin-aldosterone system, including have been implicated in all three populations, including *ACE* and *CYP11B2*, in which genetic variants can lead to a reduced blood pressure response in those take a thiazide.[272-274] Additionally, multiple ligases and kinases involved in ion channels and ion handling have been identified in EU and AA populations, such as *NEDD4L*, *PRKCA*, *WNK1*, and *WNK4*.[274-276] Finally, *YEATS4*, a gene believed to be involved in RNA transcription, has been associated with blood pressure response among thiazide users in two separate studies of both EU and AA populations.[277, 278]

In addition to antihypertensive response, genetics has been implicated in the potential adverse drug reactions of thiazide diuretics. In a study of 425 EU and 342 AA participants, Del-Aguila *et al*. identified two SNPs in AAs (rs12279250 and rs4319515) in the *NELL1* gene which were associated with fasting plasma triglyceride levels among thiazide users, for which hypertriglyceridemia is a known potential side effect.[279] However, these results did not replicate in the EU cohort. Additionally, hypokalemia, another side effect of thiazide use, has been shown to be modified by genes in the HEME pathway.[280] Another study by

Vandell and colleagues identified a collection of genetic variants across five genetic loci which explained 11% of the variability in uric acid levels among AA thiazide users; a sixth region was associated with uric acid levels among an EU population of thiazide users which was not associated in AA populations.[281] In each of these cases, the identified genetic loci have brought forth potentially new pathways involved in thiazide drug reactions but have rarely been replicated. Additionally, many of these studies, as well as the studies of diLQTS in general, have often used a candidate gene approach to account for the fact that pharmacogenomic studies are often underpowered; however, as was previously discussed, candidate gene studies have not been successful in explaining the heritability of complex traits. While the findings in the above pharmacogenomic studies demonstrate the need for further pharmacogenomic studies of thiazides and the variability in drug response, future work will need to be well-powered and consider genome-wide analyses.

B.1. Thiazide Diuretics and QT Prolongation

To date, one study of the pharmacogenomics of thiazides and QT in EU populations has been conducted.[282] In this study, no SNPs reached the genome-wide significance threshold. However, the study had several notable limitations. The study was conducted with only cross-sectional data using many of the same study populations to be used in this proposal, despite the repeated ECG and medication measures available in many of the studies. Additionally, the study only evaluated EU populations. Furthermore, the analysis was significantly underpowered to detect drug-gene interaction effect sizes consistent with those observed in QT main effect GWAS studies. However, the authors noted that by including the repeat measures, such as is suggested in this proposal, the power to detect even small interaction effects would be greater than 80%. This suggests that the study proposed

here, which will incorporate both the repeated measures as well as additional populations and race/ethnic groups, has the potential to identify genetic variants which modify the potential of thiazides to prolong QT. Additionally, this work failed to consider the effects of prevalent user bias, a form of selection bias known to impact pharmacoepidemiologic studies, on study results. The work proposed here will consider the effects of this bias and interpret results accordingly.

Bias in Pharmacoepidemiologic and Pharmacogenomic Studies

It is well known that pharmacoepidemiologic studies, which seek to understand both the use of the effects of drugs in populations, are subject to a multitude of biases.[283] However, it is unclear if pharmacogenomic studies are similarly susceptible. In an era where prescription drug use continues to rise and variability in drug response posing a growing public health burden, pharmacoepidemiologic and pharmacogenomic studies continue to be important for understanding the effects of these massive population exposures. However, it is critical to consider the potential effects of bias on these studies.

Randomized controlled trials (RCTs) are considered the gold standard of pharmacoepidemiologic research although an increasing number of studies are conducted in observational settings.[45, 48, 284] Observational settings often provide larger sample sizes, greater statistical power, and better generalization to a broader population than RCTs.[284] Unfortunately, observational studies also are subject to many biases, such as selection bias.[284] Particularly concerning for pharmacoepidemiology studies is a form of selection bias sometimes called prevalent user bias, reflecting the potential enrichment for prevalent, long-term drug users who are less likely to have experienced an ADR when compared to prior users. Furthermore, prevalent user bias results from depletion of susceptibles and differential loss of follow-up, as participants at highest risk for an ADR are not observed at the measured

time points as they have died or dropped out.[284, 285] Additionally, exposure misclassification, in which short-term users have a lower chance of being seen while on therapy is another concern leading to the enrichment of prevalent users; in cases of ADRs, participants may be taken off the medication soon after therapy initiation and thus the outcome of interest is not seen in the study and the participants are classified as non-users. These issues result in the potential for bias in observational studies of pharmaceutical usage.

Pharmacogenomic studies are similarly conducted in observational settings but GxE studies which, unlike pharmacoepidemiologic studies, incorporate a third parameter, the SNP. Pharmacogenomic studies are different than studies of main effects or of non-genetic modifiers, as the modifying variable, the SNP, is assigned at conception and therefore is not affected by subsequent exposures, a difference which is paramount in determining the effects of bias, and in particular, selection bias, in pharmacogenomics studies. Supporting this assertion is prior research that has shown that interaction effects in genetic studies do not suffer from selection biases when the genotype does not influence selection other than through an association with the disease or the second exposure.[286-288] In other words, there is no bias in cases where the selection proportions are the same between populations with the same genotypes even if they differ between categories of disease or environmental exposure status, in this case, pharmaceutical use.

Another scenario distinguishing pharmacoepidemiologic studies from pharmacogenomic studies is presented by a previous study that evaluated the influence of confounding by contraindication.[47] Confounding by contraindication is a form of bias present when an outside factor is associated with an avoidance of treatment and with the outcome of interest and is a common threat to internal validity in pharmacoepidemiology.[47,

[48] However, a pharmacogenomic simulation study found that, while there may be modest bias present in the interaction term when very large SNP main effects were simulated, the amount of bias varies by study design and the magnitude of the bias may be negligible given the size of effects observed in published QT GWAS.[47] This suggests that when conducting pharmacogenomic studies, researchers must consider the potential effects of bias under different scenarios and then, if this bias is not negligible, interpret results accordingly.

Multi-Ethnic Populations

Minority populations are historically underrepresented in clinical and health research.[289-291] Because of the lack of research in multi-ethnic populations, many health policies and recommendations which were developed in EU populations do not account for differences in disease burden and etiology found in multi-ethnic populations.[292] Minority populations hold a disproportionate burden of negative health outcomes compared to the EU populations which represent the majority of healthcare research to date.[293, 294] In particular, AAs have a higher risk of diLQTS,[295] a higher risk of mortality due to QT prolongation,[296] and a higher prevalence of thiazide use compared to other race/ethnic groups.[37]

As has been previously stated, underrepresentation of minority groups is particularly prominent in genomics research, where the majority of participants included in genetic studies to date are of EU descent.[297] This poses multiple problems, which have been briefly described in the previous sections. First among these is the limited relevance of current research to medical genomics and pharmacogenomics in populations of non-EU descent.[298] As of 2014, there are over 130 pharmaceuticals with FDA-approved genetic information on their labels.[299] However, the research behind the genetic information included on these labels has been predominantly conducted in EU populations. The lack of representation of multi-ethnic populations is particularly concerning in pharmacogenomics, as Ramos and

colleagues have demonstrated that the underlying genetic architecture of multi-ethnic populations, including genetic variants associated with drug absorption, distribution, metabolism, and excretion, varies widely, indicating that it is inadequate to extrapolate genetic findings from EU populations to diverse ancestral populations.[300] Furthermore, in the pharmacogenomics of warfarin dosing, Perera *et al.* found a genetic variant, rs12777823, in the *CYP2C18* gene which is associated with warfarin dosing and is population-specific, in this case to AA populations.[15] According to this study, failing to account for this SNP accounts for 21% of the dose variability explained by the current warfarin dosing formula and results in higher doses than needed in AA populations with an A allele at rs12777823.[15] These findings emphasize the need to include minority populations in future pharmacogenomics research. The work proposed herein will make use of large AA and HL populations in addition to the EU populations in the participating cohorts, thus enabling this research to incorporate multi-ethnic populations from the onset.

Public Health Significance

Variable drug response poses a significant problem in the effective treatment of disease.[2-4] Dose response and overall therapeutic response can vary between individuals and is influenced by a variety of factors, such as age, diet, smoking status, temporal trends, chemical exposures, drug-drug interactions, genetic variants, and drug-gene interactions.[2-4, 301-313] As of 2002, the FDA's Adverse Event Reporting System, begun in 1969, had recorded 2.3 million cases of adverse events.[314] However, this number is likely low given the widespread under-reporting of ADRs.[170] Efforts to correct for this under-reporting estimate that ADRs cause 2.2 million serious health events, 106,000 deaths, and account for 6-7% of all hospital admissions annually.[5, 315] Pharmacogenomics research represents a promising step forward in both the progression of genetic knowledge from academic research to applied

public health and medical science and the potential to better understand the mechanisms of and reduce the burden of variable drug response.

The potential public health and clinical applications of this pharmacogenomics work are numerous. Thiazides are commonly used pharmaceuticals, used by almost a fifth of the U.S. population, providing a broad potential for impact of any findings from this study.[37] Furthermore, genetic information has the potential to be incorporated into drug selection and dosing in a clinical setting, as has been done with warfarin dosing[316] or clopidogrel prescribing.[317] Furthermore, pharmacogenomics findings can be used to alter medication labels, or, in extreme cases, remove drugs from the market.[318, 319] Understanding the pharmacogenomics of drug response can also provide insight into the mechanism of drug response, and consequently, ADRs, which can then be used to better understand the etiology of the disease or even to develop better, more effective medications.[17, 18] As we come to understand more about the genetic underpinnings of human health, the possibility for personalized, genomic medicine moves ever closer and pharmacogenomics is on the leading edge of this potential.

CHAPTER 4: RESEARCH PLAN

Overview

This work will be conducted in two parts. Specific Aim 1 will be a pharmacogenomics study of the thiazide-QT relationship. Specific aim 1 will be conducted using extant cohort data from a collaboration between the Women's Health Initiative (WHI), the Hispanic Community Health Study/Study of Latinos (SOL), and the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium's pharmacogenomics working group (PWG). All participating studies have extensive genotyping data and have detailed and, in many cases, repeated measurements across multiple time points on electrocardiograms (ECGs) and medication use. In specific aim 2, a simulation study will be conducted to determine the effects of prevalent user bias on pharmacogenomics studies. This simulation study will be conducted using published clinical and genome-wide association studies (GWAS) to inform specification of the relationship between the QT interval (QT), QT-prolonging medication use, and genotypes.

Specific Aim 1

Identify genetic variants that modify the association between thiazide diuretics and QT and its component parts (QRS complex [QRS]; JT interval [JT]) in European descent, African descent, and Hispanic populations.

1. Classify thiazide diuretic exposure among all cohorts using medication inventories, which have been validated in cohort studies against physiologic measurements,[55] pharmacy databases,[56] and serum measurements.[57]

2. Conduct genome-wide, race-stratified analyses to identify significant interactions between genetic variants, thiazides, and QT and its component parts (QRS; JT), leveraging longitudinal data when possible. Study and race/ethnic-stratified results will be combined across studies using fixed-effect, trans-ethnic, and cross-phenotypic meta-analytic techniques (N_{total}=78,199).
3. Characterize identified genetic variants using in silico functional characterization techniques including computer databases and pathway analysis.

A. Study Populations

This study will make use of fifteen separate population-based cohort studies (Table 13). All study participants from each participating study who have genotype data as well as ECG measurements and medication data will be eligible for inclusion in this study. Furthermore, all time points at which both ECG and medication data were measured will be eligible for inclusion in the analysis. For distributions of pertinent population characteristics across all fifteen cohorts, see Table 13.

A.1. Women's Health Initiative

The WHI is a population-based study consisted of two arms.[52] A total of 161,808 participants from 40 study centers were enrolled, 62,132 into the clinical trial arm and 93,676 into the observational study. All participants were female between the ages of 50 and 79 years at enrollment, were postmenopausal, and did not suffer from alcoholism, drug dependency, mental illness, or dementia. Participants were subsequently brought back for four additional visits at which ECGs and medication were measured.

Table 13. Study Population Characteristics

			CHARGE											
Characteristic	WHI	SOL	AGES	ARIC	CHS	FHS	RS	ERF	Health 2000	Health ABC	MESA	PROSPER	NEO	JHS
N, Total	161,808	16,479	5,764	15,792	5,888	14,518	14,926	1,503	8,028	3,075	8,313	5,804	6,673	5,301
N, Genotyped+ECG	20,395	12,456	2,587	11,132	3,856	3,168	7,196	1,503	2,124	2,802	8,313	4,556	6,673	1,962[1]
Baseline Visit	93-98	09-11	02-06	87-89	89-90[2]	48-53[3,4]	90-93[5,6]	02-05	00-01	97-98	00-02	97-99	08-12	00-04
Length of Follow-up (yrs)	9	0	5	24	11[2]	60[3,4]	20[5,6]	0	0	10	10	0	0	9
Mean Age (yrs)	64	46	77	54	72	55	65	48	50	74	62	75	56	55
% Female	100	46	58	55	61	53	58	59	52	52	54	54	57	55
Mean QT	401	415	406	398	414	414	399	398	389	413	412	414	NA	413
% Thiazides	18	NA	24	12	21	3	3	2	7	11	13	26	NA	25
% Race/Eth														
European Am	65	0	100	77	85	100	94	100	100	60	32	100	100	0
African Am	24	0	0	23	15	0	0	0	0	40	33	0	0	100
Hispanic	10	100	0	0	0	0	0	0	0	0	26	0	0	0
Other	1	0	0	0	0	0	6	0	0	0	9	0	0	0

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHARGE, Cohorts for Heart and Aging Research in Genetic Epidemiology; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; Health ABC, Health, Aging, Body, and Composition; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; NEO, the Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SOL, Hispanic Community Health Study/Study of Latinos; WHI, Women's Health Initiative

NA indicates that this data was available as of this proposal. The final dissertation project will have these numbers available.

[1]Excludes overlap of participates who are also included in the ARIC cohort

[2]The CHS African American cohort (N=687) was recruited in 1992-93 and has a length of follow-up of 7 years (4 visits)

[3]The FHS Offspring cohort (N=5,124) was recruited in 1971-75 and has a length of follow-up of 34 years (8 visits)

[4]The FHS 3rd Generation cohort (N=4,095) was recruited in 2002-05 and has a length of follow-up of 6 years (2 visits)

[5]The RS-II cohort (N=3,011) was recruited in 2000-01 and has a length of follow-up of 11 years (3 visits)

[6]The RS-III cohort (N=3,932) was recruited in 2006-08 and has a length of follow-up of 6 years (2 visits)

A.2. Hispanic Community Health Study/Study of Latinos

The SOL study collected data on approximately 16,000 Hispanic individuals from four communities: Miami, Florida; the Bronx, New York; Chicago, Illinois; and San Diego, California.[53] Participants were aged 18-74 years at baseline and were sampled from communities with large HL populations, targeting the following subgroups: Mexicans, Cubans, Puerto Ricans, Dominicans, Central Americans, and South Americans. Active duty military personnel and those physically unable to attend clinic visits were deemed ineligible. To avoid a language barrier, exams were conducted in both English and Spanish.

A.3. Cohorts for Heart and Aging Research in Genetic Epidemiology

The CHARGE consortium was formed to facilitate genome-wide association studies, meta-analyses, and replication opportunities among large, well-phenotyped longitudinal cohort studies with genetic data.[54] CHARGE has five founding cohorts but has since grown to include more than ten large cohort studies. Analyses are conducted through working groups. This project will fall under the auspices of the pharmacogenomics working group (PWG).

Age, Gene/Environment Susceptibility – Reykjavik Study

The Age, Gene/Environment Susceptibility – Reykjavík Study (AGES) is the follow-up study to the Reykjavik study in Iceland. The Reykjavik study was a longitudinal study conducted from 1967-94 of 30,795 participants born between 1907 and 1935.[320] Participants were selected from a random sample of the Reykjavik population. Between 2002-06, the AGES study recruited 5,764 participants from the surviving 11,549 members of the Reykjavik Study.

Atherosclerosis Risk in Communities Study

The Atherosclerosis Risk in Communities Study (ARIC) is a population-based cohort study of 15,792 individuals from four communities: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland.[321] Participants ranged in age from 45 to 64 years at baseline. Recruitment was concentrated on EU and AA populations. All individuals in the targeted age range residing in households identified through area sampling were considered eligible for study participation.

Cardiovascular Health Study

The Cardiovascular Health Study (CHS) collected data on 5,201 individuals sampled from Medicare eligibility lists from four communities: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania.[322] Participants were 65 years or older at study entry. In 1992-93, an additional 687 AA individuals were recruited. Participants who were home-bound, receiving hospice care, or receiving radiation or chemotherapy treatment were excluded.

Erasmus Rucphen Family Study

The Erasmus Rucphen Family study (ERF) is part of the Genetic Research in Isolated Populations (GRIP) program in the southwest Netherlands.[323, 324] The ERF identified twenty-two families with a minimum of six children baptized in the community church between 1850 and 1900 through detailed genealogical records. All living descendants of these couples and their spouses were invited to take part in this study, for which 3,200 individuals participated between 2002 and 2005.

Framingham Heart Study

The Framingham Heart Study (FHS) collected data on 5,209 participants aged 28 – 62 from residents of Framingham, Massachusetts.[325-327] In 1971, 5,214 additional participants were enrolled in the FHS Offspring Study, recruiting from children and spouses of children of the original cohort. In 2002, 4,095 additional participants were recruited from the population of children from the offspring cohort and enrolled into the 3rd Generation cohort.

Health, Aging, Body and Composition

The Health, Aging, Body and Composition (Health ABC) study is a prospective cohort study that recruited individuals aged 70 – 79 from Medicare enrollees in Pittsburgh, Pennsylvania and Memphis, Tennessee.[328] Participants were excluded if they had difficulty performing basic daily activities, difficulty walking a quarter mile or climbing steps, or had a life-threatening illness.

Health 2000

The Health 2000 study is a population-based health examination survey carried out in Finland from 2000 to 2001.[329, 330] The survey was conducted with a two-stage stratified cluster sample representative of the Finnish adult population 30 years of age or older. The Health 2000 performed a comprehensive health examination including questionnaires, clinical measurements, and physical examinations on 8,028 individuals.

Jackson Heart Study

The Jackson Heart Study (JHS) collected data on 5,301 non-institutionalized AA individuals from Jackson, Mississippi, aged 35 – 84 years.[331] Participants who were physically or mentally incompetent were excluded.

Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis (MESA) study collected data on 6,814 participants aged 45 – 84 and free of clinical cardiovascular disease from six communities: Forsyth County, North Carolina; Northern Manhattan and the Bronx, New York; Baltimore County, Maryland; St. Paul, Minnesota; Chicago, Illinois; and Los Angeles County, California.[332] Participants were recruited from a diverse range of ethnic backgrounds. Exams were conducted in English, Spanish, Cantonese, and Mandarin. Individuals undergoing active cancer treatment, who were pregnant, weighted more than 300 pounds, or were in a nursing home were excluded.

The Netherlands Epidemiology of Obesity Study

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort of 6,673 individuals from the greater Leiden area of the Netherlands.[333] Between 2008 and 2012, all individuals aged 45-65 from Leiderdorp, a municipality of Leiden, and individuals with a reported body mass index greater than 27 kg/m^2 from the greater Leiden area were recruited, resulting in an oversampling of overweight (43%) and obese (45%) individuals. Participants answered questionnaires and were administered physical and medical examinations at baseline examinations, including medical histories, medication inventories, blood sampling, and resting ECGs.

Prospective Study of Pravastatin in the Elderly at Risk

The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) study was a prospective, multicenter, randomized, placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in the elderly.[334, 335] Between 1997 and 1999, participants aged 70-82 years were screened and enrolled from Glasgow,

Scotland, Cork, Ireland, and Leiden, the Netherlands, resulting in a total cohort of 5, 804 individuals.

Rotterdam Study

The Rotterdam Study (RS) recruited 7,893 subjects over the age of 55 from the Ommoord suburb of Rotterdam in the Netherlands for baseline examination.[336, 337] In 2002, the RS-II recruited an additional 3,011 participants who were not eligible for the first round of the RS but had since turned 55 years of age or who had moved into the region since the start of the RS-I. In 2006, an additional 3,932 participants were recruited into the RS-III. The RS-III recruited participants aged 45-54 years from the same base population as the previous RS recruitments.

A.4. Exclusion Criteria

Table 14. Visit-Specific Exclusion Criteria

Poor ECG Quality
Atrial Fibrillation indicated on ECG
Pacemaker Implantation
$2^{nd}/3^{rd}$ Degree Atrioventricular Heart Block
QRS > 120 ms
Prevalent Heart Failure
Pregnant

Only study visits which measured both medication and ECGs will be considered for this analysis. Individuals from the above studies will be excluded from this analysis based on the criteria listed in Table 14, which for studies with longitudinal data are visit-specific. Furthermore, individuals who did not consent to genetic analysis or who are not of EU, AA, or HL descent based on self-report or assessment of ancestry through principal component analysis will be excluded.

B. Outcome Assessment

QT is measured, in milliseconds, using a standard 12-lead electrocardiogram (ECG). The 12-lead ECG involves the placement of electrodes on both arms, the left leg, and across the chest. In each participating study, technicians recorded resting, supine or semi-recumbent, standard 12-lead ECGs. Studies used Marquette MAC 5000, MAC 12, or MAC PC (GE Healthcare, Milwaukee, Wisconsin, USA), or ACTA (EASOTE, Florence, Italy) machines. Comparable procedures were used for preparing participants, placing electrodes, recording, transmitting, processing, and controlling quality of ECGs. QT was measured electronically using one of the following programs: Marquette 12SL, MEANS, Burdick Eclips 850i, Digital calipers, or Health 2000 custom-made software.

C. Exposure Assessment

C.1. Medication Assessment

Medication inventories were collected at examinations by each participating study except the RS on the same day as ECGs. The RS assessment medication using pharmacy databases, recording all prescriptions filled less than or equal to 30 days before examination visits. All other medication data was collected through a drug inventory. The RS has validated this method of medication data collection against pharmacy databases, showing a 94% concordance rate with pharmacy records.[56] The CHS has also validated medication inventories against physiologic measurements[55] and serum measurements.[57] Medication inventories were either conducted during clinic visits or home interviews, varying by study. In both settings, medication data was recorded directly from medication containers, rather than through participant recall. Recorded data included drug name, strength, and in some cases, dosing instructions. Using recorded data and ingredient lists provided by drug companies, all participants will be classified as thiazide users or nonusers at each study visit.

Table 13 shows the prevalence of individuals taking a thiazide diuretic at a minimum of one study visit.

C.2. Genotyping

Each study conducted genome-wide genotyping independently prior to this study. WHI participants were genotyped through four sub-studies: GWAS of Treatment Response in Randomized Clinical Trials (GARNET); Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations (MOPMAP); the SNP Health Association Resource (SHARe); and the WHI Memory Study (WHIMS). The SOL study conducted genotyping using a custom array which included 109,571 ancestry informative markers. See Table 15 for a complete list of genotyping platforms using across all fourteen participating studies. All studies excluded SNPs which failed to meet Hardy-Weinberg equilibrium, had a MAF of less than 1%, or had a low call rate (<90-97%, varied by study). To maximize genome coverage and comparisons across genotyping platforms, typed genotypes were used in each study to impute genotypes using HapMap2[338-341] or 1000 Genomes[342, 343] data.

D. Data Analysis

D.1. Genome-Wide Analysis

Pharmacogenomic analyses will be conducted using a genome-wide analysis. Each study will conduct race-stratified analyses with EU, AA, and HL populations for QT. Longitudinal data will be used whenever available and analyses will be conducted using a combination of linear regression, mixed effects models (MEM) and generalized estimating equations (GEE) depending on their study design and the availability of longitudinal data. All analyses will be adjusted for age (measured in years), sex, visit-specific RR interval, visit specific QT altering medications defined using UAZ drug list (Table 6, Table 7), and

Table 15. Genotyping Platforms

Study	Genotyping Array	# of SNPs
WHI		
GARNET	Illumina HumanOmni1 Quad	1,051,295
MOPMAP	Affymetrix Genome-wide Human SNP Array 6.0	934,940
SHARe	Affymetrix Genome-wide Human SNP Array 6.0	934,940
WHIMS	Illumina HumanOmni Express	733,202
SOL	Illumina custom array	2,536,661
CHARGE		
AGES	Illumina 370 CNV	370,404
ARIC	Affymetrix Genome-wide Human SNP Array 6.0	934,940
CHS	Illumina HumanOmni1 Quad	1,051,295
FHS	Multiple Affymetrix Mapping Arrays	>262,264
RS	Illumina Infinium II HumanHap 550	555,352
ERF	Illumina 6K/318K/370K	374,496
Health 2000	Illumina Human610-Quad BeadChip	601,273
Health ABC	Illumina 1M	1,049,348
MESA	Affymetrix Genome-wide Human SNP Array 6.0	934,940
PROSPER	Illumina 660K	557,192
NEO	Illumina HumanCoreExome-24v1_A Beadchip	361,046
JHS	Affymetrix Genome-wide Human SNP Array 6.0	934,940

study specific measures of principal components of genetic ancestry,study site or region, and relatedness when appropriate.

Cross-Sectional Studies

Studies for which only one ECG/drug measure per participant is available will conduct linear regression using robust estimates of standard errors if using populations of unrelated individuals or MEM if using populations of related individuals. Using the following model:

$$E[Y_i] = \beta_0 + \beta_E I_i + \beta_G SNP_i + \beta_{G:E} I_i SNP_i + \beta_4 C_i$$

where Y_i is our outcome of interest (QT, QRS, or JT in ms) for the ith participant, β_0 is the intercept, I_i is an indicator for thiazide use, SNP_i is the (dosage of the) genetic variant, and C_i is the vector of covariates. The primary parameter of interest is $\beta_{G:E}$, the multiplicative interaction term between genotype and thiazide use.

Longitudinal Studies

Studies for which there are two or more study visits measuring both ECG and medication use will use GEE models with independence working correlation if using populations of unrelated individuals or MEM if using populations of related individuals, in both cases using robust estimates of standard errors. All data from as many visits as possible with be used within each study. Using the following model:

$$E[Y_{ij}] = \beta_0 + \beta_E I_{ij} + \beta_G SNP_i + \beta_{G:E} I_{ij} SNP_i + \beta_4 C_{ij}$$

where Y_{ij} is our outcome of interest (QT, QRS, or JT in ms) for the ith participant at the jth timepoint, β_0 is the intercept, I_{ij} is an indicator for thiazide use, SNP_i is the (dosage of the) genetic variant, and C_{ij} is the vector of covariates. The primary parameter of interest is $\beta_{G:E}$, the multiplicative interaction term between genotype and thiazide use.

D.2. Meta-Analytic Techniques

Two separate meta-analytic techniques will be used to combine data across studies. First will be a race-stratified, fixed-effect, inverse variance weighted meta-analysis conducted using the METAL program.[344] However, the assumption that each contributing population will have the same underlying effect does not always hold across multiple race/ethnicities because of differences in patterns of LD across ancestral populations, potential allelic heterogeneity, differences in gene-environment interactions, differences in gene-gene interactions, and differences in environmental and lifestyle factors between

different race/ethnic groups. Thus, to allow for underlying differences between race/ethnic groups, we will also conduct trans-ethnic meta-analysis using a Bayesian approach developed by Morris using the MANTRA program.[345]

Fixed Effects Inverse Variance Weighted Meta-Analysis

Fixed effects meta-analysis will be conducted, stratified by race, using METAL, using a genome-wide significance level of $P < 5\text{x}10^{-8}$.[344] However, in previous pharmacogenomics work conducted by the PWG has indicated that there is a possibility for early departure of the test statistic from the null distribution. In such scenarios, a t-distribution approach will be used.[346] P-values will be recalculated by applying a t reference distribution to the drug-SNP interaction estimates of β (standard error [SE]), and then meta-analyzed using a weighted Z-statistic, with weights based on the SNP imputation quality multiplied by the estimated number of independent observations exposed to thiazides ($N_{exposed}$). $N_{exposed}$ will be estimated as follows:

1. In cross-sectional studies, $N_{exposed}$ equals the number of participants classified as thiazide users.
2. In longitudinal studies, $N_{exposed}$ will be calculated as follows:

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

where n_i is the number of observations for participant i, $\hat{\rho}$ is an estimate of the pairwise visit-to-visit correlation within participants from a GEE-exchangeable model that does not contain genetic data, and $\#\{E_{it} = 1\}$ is the number of observations in which participant i is exposed.

Ideally, the cohort- and SNP-specific degrees of freedom (df) for the t reference distribution will be estimated using Satterthwaite's method,[347] both for cross-sectional and for longitudinal analyses:

1. For cross-sectional studies, df will be calculated as follows:

$$df = 2\frac{E\left[Var\left(\hat{\beta}\right)\right]^2}{Var\left[Var\left(\hat{\beta}\right)\right]}$$

   Estimates of $E\left[Var\left(\hat{\beta}\right)\right]^2$ and $Var\left[Var\left(\hat{\beta}\right)\right]$ are calculated using an R code developed by Ken Rice, Thomas Lumley, *et al.* that is available on the CHARGE PWG wiki page: http://depts.washington.edu/chargeco/wiki/Pharmacogenetics.

2. For longitudinal studies, df will be calculated as follows:

$$df = 2\frac{E\left[Var\left(\hat{\beta}\right)\right]^2}{Var\left[Var\left(\hat{\beta}\right)\right]}$$

   where $E\left[Var\left(\hat{\beta}\right)\right]^2$ is assumed to equal $Var\left(\hat{\beta}\right)$ and the formula for estimating $Var\left[Var\left(\hat{\beta}\right)\right]$ is based on the method presented by Pan and Wall.[348] Longitudinal df estimates are calculated using the R bossWithdf package, available on the CHARGE PWG wiki page: http://depts.washington.edu/chargeco/wiki/Pharmacogenetics.

If Satterthwaite's method cannot be implemented in a particular cohort, then an approximate df will be calculated as the cohort- and SNP-specific product of the SNP imputation quality (range: 0,1), the MAF (range: 0, 0.50), and $N_{exposed}$.

Trans-Ethnic Meta-Analysis

To allow for underlying differences between race/ethnic groups while also enabling us to take full advantage of the large sample size found across all three included race/ethnic groups, we will conduct a trans-ethnic meta-analysis using alternative methods. The trans-ethnic meta-analysis will use a Bayesian approach developed by Morris and implemented in MANTRA.[345] MANTRA utilizes allele frequencies in each population to cluster studies according to genetic-relatedness and then generates a Bayes factor for each SNP. Morris has determined that a significance level of 10^5 is approximately equivalent to a genome-wide significant level of $5x10^{-8}$ and will be used as our significance level in these Bayesian analyses.[345] However, this method does not provide an over-all effect estimate and so can be used to identify significant SNPs but cannot be used to estimate an effect size across race/ethnicities.

Cross-Phenotype Meta-Analysis

Previous studies have demonstrated the potential to increase power and detect evidence of pleiotropy by conducting multi-trait analysis across correlated traits.[349, 350] To examine potential pleiotropy across ventricular depolarization and repolarization, we conducted cross-phenotype meta-analysis combining *t*-statistics across QRS and JT using an adaptive sum of powered score (aSPU) test, which tests for both concordant and discordant associations across some or all of the included traits.[351] The reference distribution for the aSPU test was calculated using 10^8 simulations.

D.3. Sensitivity Analyses

It is important to remember that QT is a measure of both ventricular depolarization *and* repolarization, two processes that, although related, are opposites and can thus, under certain conditions, be oppositely affected.[60] In the case identified by Akylbekova *et al.*, the genetic effects underlying the QT interval work in opposite directions on QRS and JT and the associations with all intervals studied were substantially enhanced in patients with hypokalemia. For example, before accounting for hypokalemia, the coded allele increased QT_c by only 3.3 ms; however, when the interaction with hypokalemia was added, the coded allele increased QT_c by 20.2 ms.[229] This makes it imperative that we consider not just QT but also QRS and JT separately in order to fully capture the effects underlying QT prolongation, particularly when studying diuretics, which are known to alter potassium levels.

To account for this, this work will conduct sensitivity analyses, examining both QRS and JT duration as outcomes. QRS, like QT, was measured in ms using a standard 12-lead ECG. For more detail, see Section Outcome Assessment. JT will be calculated from QT and QRS as follows: $JT = QT - QRS$. All analyses will be conducted using the same analysis plan that is used for QT, including exclusions, covariates, statistical analyses, and meta-analysis.

D.4. In-Silico Functional Characterization

For all SNPs identified in the above analyses as genome-wide significant, including the sensitivity analyses, I will conduct *in-silico* functional characterization. The *in-silico* characterization will be carried out in four stages (Figure 13). First, I will use race-specific LD patterns to identify SNPs that are in high HD ($r^2 > 0.5$) with the index SNPs identified in

the above analyses. LD patterns will be based off the most recent release of the 1000 Genomes[342, 343] data and linked SNPs will be determined using two online databases: SNAP[352] and HaploReg.[353] The two databases complement each other, as the SNAP database examines a larger list of SNPs but HaploReg provides a greater degree of information, including structural details on nine cell types, conservation across different mammal species, and mutation type (i.e. nonsense, missense, silent, etc.). In the second stage of characterization, I will examine the index SNPs, as well as all SNPs in high LD as determined by the first stage, in the dbSNP database[354] and the UCSC GenomeBrowser,[355] which makes use of previous functional characterization, as well as the recent ENCODE project,[356] which sought to characterize the non-coding regions of the genome. I expect most findings will occur in the non-coding regions, such as promoter regions, and thus will impact expression levels rather than protein structure and function. However, I do expect to find a subset of missense and nonsense mutations within the coding region of the genome, particularly among population-specific variants. For this subset of SNPs, I will move onto the third stage of characterization, which will utilize SIFT[357-359] and PolyPhen 2[360] to predict the functional effects of the amino acid substitutions or premature terminations. Finally, the fourth phase of characterization will examine each of the genetic loci identified in a pathway analysis using Ingenuity Pathway Analysis (IPA)[361] to identify potential linkages within the genome between the genetic loci associated with drug response.

Figure 13. Flowchart of *In-Silico* Functional Characterization

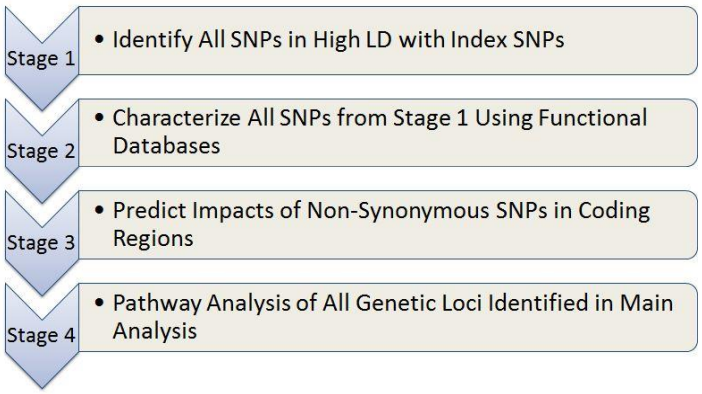
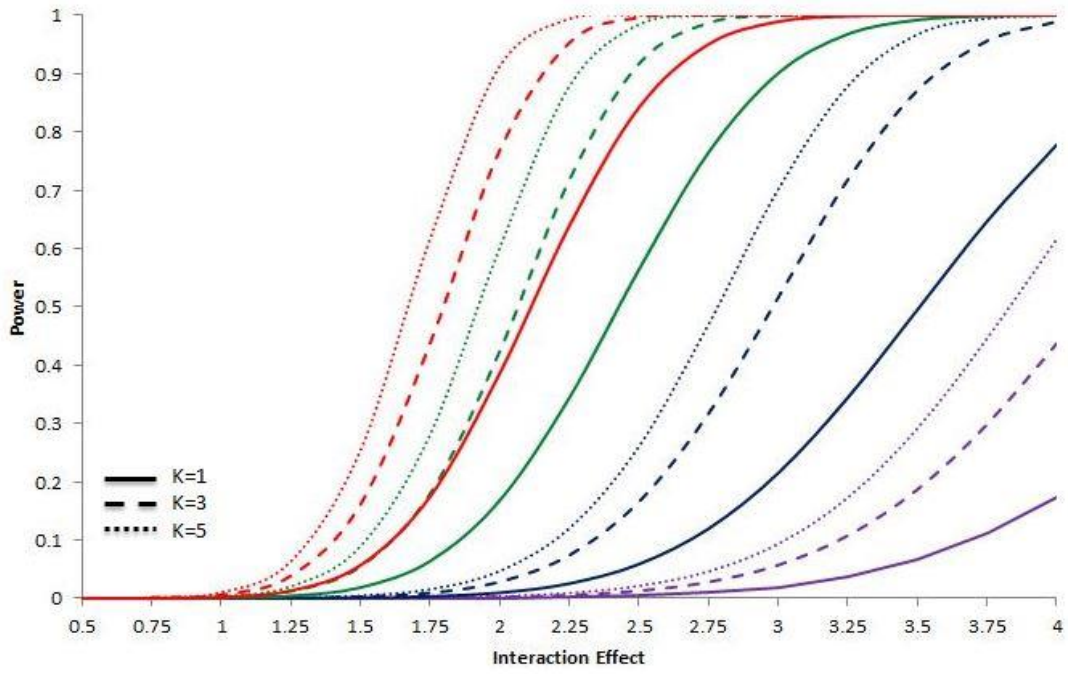


Figure 14. Statistical Power Curves, Presented for K=1, 3, and 5 Variants and a Range of Minor Allele Frequencies



K represents the number of independent SNPs modifying the thiazide-QT association. Curves represent the power to detect at least one SNP assuming K SNPs modify the association. Minor Allele Frequencies: 5% (Purple), 10% (Blue), 25% (Green), and 45% (Red)

E. Sample Size and Statistical Power

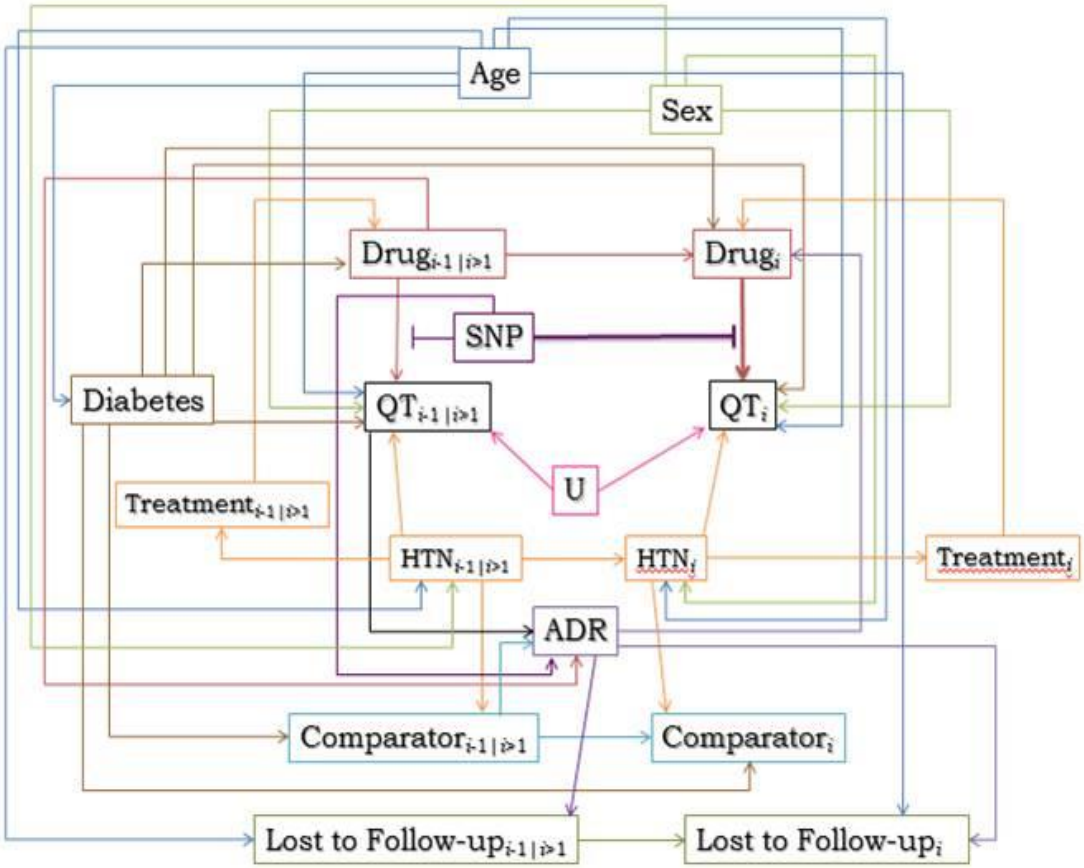
Power was calculated using Quanto[362] with a two-sided $\alpha = 5x10^{-8}$ and conservatively assuming a cross-sectional study design. It is worth noting that 69% of the total population has multiple measures of medications and QT, indicating that our actual power will be higher than that estimated here. Power estimates were calculated across a range of MAF (5-45%), an estimated 14% prevalence of thiazide use (based on Table 13), an expected main effect for both the SNP and thiazide of 2 ms, and a sample size of 94,479. The power is low at low MAFs (interaction effects of >4 ms needed to achieve 80% power at MAF = 10%). However, at more common MAFs, this study is better powered. At 25% MAF, there is 80% power to detect interaction effects of 2.8 ms and at 45% MAF, there is 80% power to detect interaction effects of 2.4 ms. However, we expect multiple independent SNPs to be modifying the thiazide-QT association. Therefore, assuming no between population variance in the interaction, I calculated the power to detect at least one association assuming that K independent SNPs modify the association between thiazides and QT (Figure 14). At powers P_0, $P_1 \ldots P_N$, the probability of detecting at least one variant of the K independent variants is $1-(1-P_0)(1-P_1)\ldots(1-P_N)$. Assuming only 3 independent SNPs modifying the thiazide-QT association, the threshold for 80% power decreases to 3.37 ms, 2.33 ms, and 2.03 ms for MAFs of 10%, 25%, and 45%, respectively. At K=5, the threshold for 80% further decreases to 3.12 ms, 2.16 ms, and 1.88 ms for MAFs of 10%, 25%, and 45%, respectively. As has been pointed out, these thresholds are expected to be lower given the inclusion of longitudinal data.

Specific Aim 2

Examine the influence of prevalent user bias and exposure misclassification caused by prevalent user bias on a pharmacogenomics study conducted in an observational setting.

a. Using simulations, evaluate bias, power, and type I error in the drug-SNP interaction caused by prevalent user bias and exposure misclassification.
b. Compare the results of aim 2a under different study designs (e.g. whole cohort, active comparator, new-user).

Figure 15. Conceptual Model of Relationship Between Study Variables



→ Indicates a directed relationship between two variables
┬ Indicates effect measure modification by one variable on the relationship between two other variables
i indicates visit 1-4, ADR indicates the occurrence of an adverse drug reaction, U represents unknown/unmeasured correlation between QT between visits, Comparator represents an active comparator drug class, Drug represents the drug of interest, in this scenario modeled after a thiazide diuretic, SNP represents the genetic variant of interest that modifies the drug-QT relationship, and QT represents the QT interval

A. Simulation Overview

The simulation will begin with a literature review to design the relationships between QT, QT-prolonging medication use, and the genetic modification (Figure 15). Using the results of this literature review, I will determine plausible effect sizes for thiazide, SNP, and drug-SNP effects on QT and determine important risk factors for QT prolongation. The conceptual model includes seven covariates besides the SNP: age, sex, U, which represents unknown/unmeasured confounders of the drug-QT relationship, hypertension (HTN),

treatment of hypertension, diabetes, adverse drug reaction (ADR), and loss to follow-up. Furthermore, an active comparator is included in the model. For our simulation analysis, we will simulate four visits. The correlation between variables at Visit *i* and Visit *i-1* will calculated based on correlation between visits 1 and 2 in the Atherosclerosis Risk in Communities (ARIC) study. Furthermore, we will compare results across three study designs: whole cohort (WC), active comparator (AC), and new-user (NU).

B. Simulation Parameters and Values

For a summary of all variables to be simulated in this analysis, see Table 16.

B.1. Covariates

Age for each observation will be simulated using a normal distribution with a mean value determined using the ARIC baseline visit. Sex will be simulated as a uniform random variable with a defined probability of being male. Unknown/Unmeasured confounders (U) will be simulated using a normal distribution with a set mean and SD determined by the correlation seen between QT in visit 1 and visit 2 of the ARIC study. Diabetes, hypertension, adverse events, and loss to follow-up will be simulated using binomial distributions as defined in Table 16.

B.2. Genotype

The genotype of the SNP will be simulated with a uniform distribution using a specified minor allele frequency (MAF), which will be varied across simulation runs and range between 5% and 45%. The probability of an observation being heterozygous or homozygous for the major or minor allele will be calculated under the assumption of Hardy-Weinberg equilibrium.

B.3. Drug Use

QT-prolonging drug use at visit *i* will be predicted conditional on age and sex using a logit function:

$$if \begin{Bmatrix} Treatment_i = 0\ then\ pr(Drug_i) = 0 \\ Treatment_i = 1\ then\ Logit\big(pr(Drug_i = 1)\big) = \alpha_0 + \alpha_1 Diabetes_i \\ +\alpha_2 Drug_{i-1|i>1} + \alpha_3 ADR_i + \varepsilon \end{Bmatrix}$$

where α_0 will correspond to a preset prevalence of QT-prolonging drug use, which will then be assigned using a binomial distribution and this calculated probability. An active comparator drug will be simulated as:

$$if \begin{Bmatrix} Treatment_i = 0\ then\ pr(Comparator_i) = 0 \\ Treatment_i = 1\ then\ Logit(pr\big((Comparator_i)\big) = 1 - pr(Drug_i) + \varepsilon \end{Bmatrix}$$

B.4. QT Interval

QT at the *i*th visit will be calculated using a linear model:

$$QT_i = \beta_0 + \beta_1 Drug_i + \beta_2 SNP + \beta_3 Drug_i \times SNP + \beta_4 Age_i + \beta_5 Sex + \beta_6 Diabetes \\ +\beta_7 HTN + \beta_8 U + \varepsilon$$

where the mean and SD of QT will be set based on ARIC's baseline visit.

C. Simulation Models and Analyses

Multiple simulation models will be run to compare different conditions. The effect of age, sex, heart rate, HTN, diabetes, and U will not vary across models. The SNP main effect, drug-SNP interaction, and the MAF will be varied across models. Furthermore, to test the effect of prevalent user bias on the interaction effect, different levels of informative missingness (through adverse events and loss to follow-up) will be tested. Figure 17 presents different scenarios which could lead to prevalent user bias in a longitudinal study with two visits, spaced two years apart. Scenario 1 represents a prevalent user. Scenario 2 represents an incident user. Scenarios 3-6 represent possible scenarios which could lead to prevalent

Table 16. Simulation Parameters and Scenarios

Parameter	Simulation Scenario
Age	Normal distribution, mean determined using ARIC data
Sex	Uniform random variable with defined probability of being male
Unmeasured/Unknown Confounders (U)	Normal distribution, mean determined by the correlation between QT in visits 1 and 2 in ARIC study
SNP	Uniform distribution with defined minor allele frequency, which will vary across models, and with probability of being heterozygous/homozygous calculated under Hardy-Weinberg equilibrium
Diabetes (Diabetes)	Binomial distribution with probability of Diabetes=1 defined as follows: $Logit\big(pr(Diabetes = 1)\big) = \alpha_0 + \alpha_1 Age + \varepsilon$
Hypertension at visit *i* (HTN_i)	Binomial distribution with probability of HTN=1 defined as follows: $Logit\big(pr(HTN_i = 1)\big) = \alpha_0 + \alpha_1 Age + \alpha_2 Sex + \alpha_3 HTN_{i-1\|i>1} + \varepsilon$
Treated HTN at visit *i* ($Treatment_i$)	Binomial distribution with probability of Treatment=1 defined as follows: $if \begin{cases} HTN_i = 0 \; then \; pr(Treatment_i) = 0 \\ HTN_i = 1 \; then \; Logit\big(pr(Treatment_i = 1)\big) = \alpha_1 R + \varepsilon \\ where \; R \; represents \; a \; random \; number \end{cases}$
QT Prolonging Drug Use at Visit *i* ($Drug_i$) or Alternative Drug at Visit *i* ($Comparator_i$)	Binomial distribution with probability of Comparator=1 defined as follows: $if \begin{cases} Treatment_i = 0 \; then \; pr(Drug_i) = 0 \; and \; pr(Comparator_i) = 0 \\ Treatment_i = 1 \; then \; Logit\big(pr(Drug_i = 1)\big) = \alpha_0 + \alpha_1 Diabetes_i \\ +\alpha_2 Drug_{i-1\|i>1} + \alpha_3 ADR_i + \varepsilon \\ and \; Logit(pr\big((Comparator_i)\big) = 1 - pr(Drug_i) + \varepsilon \end{cases}$
QT at Visit i (QT_i)	$QT_i = \beta_0 + \beta_1 Drug_i + \beta_2 SNP + \beta_3 Drug_i \times SNP + \beta_4 Age_i + \beta_5 Sex + \beta_6 U + \beta_7 Diabetes_i + \beta_8 HTN_i + \varepsilon$
Adverse Event (ADR)	Binomial distribution with probability of ADR defined as follows: $if \begin{cases} Drug_{i-1} = 0 \; then \; pr(ADR) = 0 \\ Drug_{i-1} = 1 \; then \; Logit\big(pr(ADR_i = 1)\big) = \alpha_0 + \alpha_1 QT_{Long} \\ where \; QT_{Long} \; is \; defined \; using \; QT_{i-1} + \varepsilon \end{cases}$
Loss to Follow-up at Visit *i* ($Loss_i$)	Binomial distribution with probability of Loss=1 defined as follows: $if \begin{cases} Loss_{i-1\|i>1} = 1 \; then \; pr(Loss_i) = 1 \\ Loss_{i-1\|i>1} = 0 \; then \; Logit\big(pr(Loss_i = 1)\big) = \alpha_0 + \alpha_1 Age \\ +\alpha_2 ADR + \varepsilon \end{cases}$

user bias through differential misclassification (scenarios 3-4) or depletion of susceptibles (scenarios 5-6). The simulation of $Drug_i$ accounts for scenario 3 by accounting for the probability that an ADR occurred. Scenarios 4-6 will be accounted for by allowing ADRs and loss to follow-up between visits 1 and 4.

Figure 16. Flowchart of Simulation Analysis Process

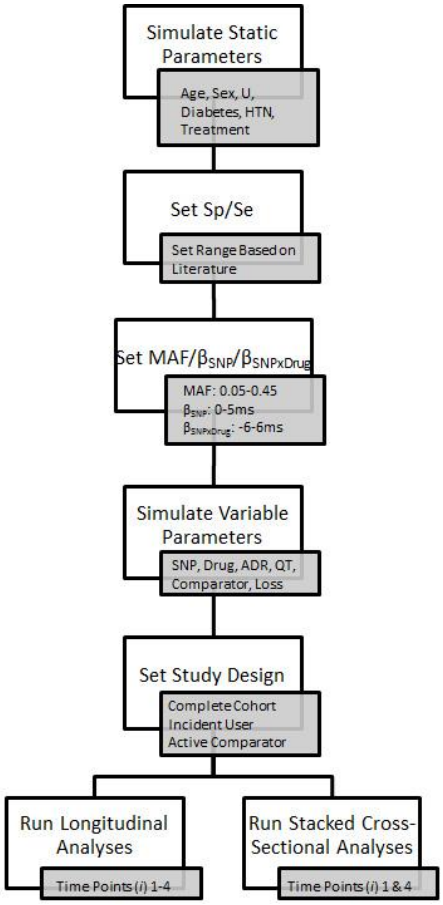
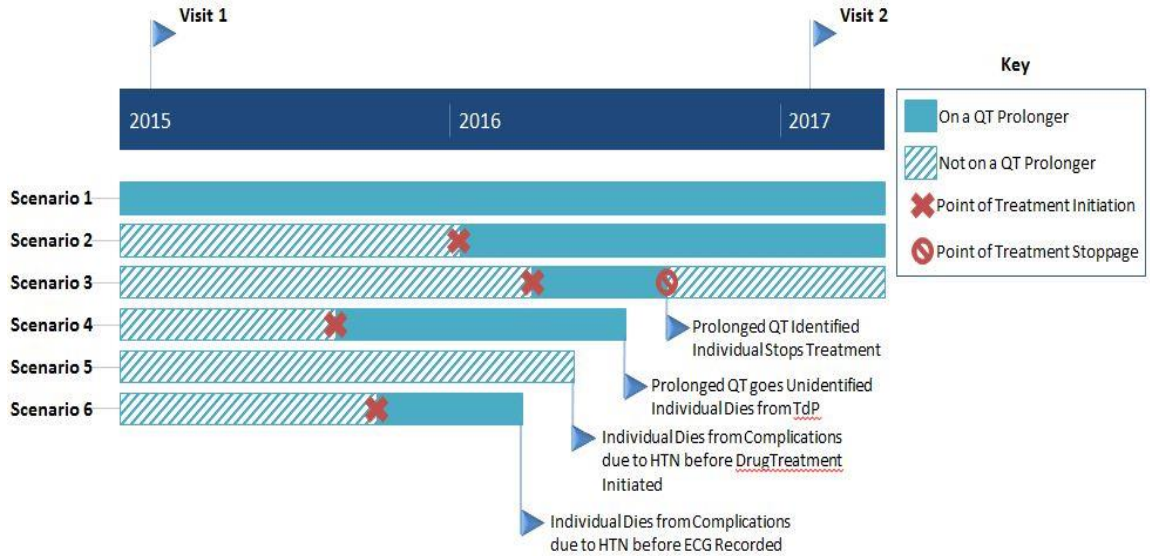


Figure 17. Scenarios Leading to Prevalent User Bias in Pharmacoepidemiology Studies



The following parameters will be varied across models: MAF, probability of an ADR/loss to follow-up, SNP main effect, and SNP-drug interaction effect. Variable MAF will cause the SNP parameter to vary across models. The variable ADR will cause the $Drug_i$ parameter to vary across models. Variation in the SNP and $Drug_i$ parameters will subsequently cause the QT_i parameter to vary across models.

I will then use the simulations to contrast three different study designs: whole cohort (WC), active comparator (AC), and new-user (NU). For WC simulations, no cohort observations will be excluded. For AC simulations, analyses will be restricted to those on a QT-prolonging drug or those on the simulated alternative drug. Finally, for NU simulations, prevalent QT-prolonging drug users at visit 1 will be excluded from analyses. For each study design, I will evaluate all model conditions as discussed above. For a breakdown of the simulation process, see Figure 16. All models will estimate the QT-prolonging drug-SNP interaction using generalized estimating equations with an independence working correlation matrix and be run for both longitudinal data (visits 1-4) and a stacked cross-sectional approach (visit 1 and 4 only).

Integration of Specific Aims 1 and 2

I will use the results of Specific Aim 2 to inform the interpretation of results from Specific Aim 1. In an ideal scenario, the simulation study from the first aim will indicate that the potential impacts of prevalent user bias on a pharmacogenomics study are negligible under the given parameters. However, in the event that Aim 2 indicates that prevalent user bias is a cause for concern, there are several potential steps which can be taken, depending on the level of concern. For example, if the potential bias is only of concern in SNPs with small MAF, these SNPs can be excluded from the analysis on a study-by-study basis. Furthermore, we can filter on effect size for bias associated with effect size. If there is a greater potential

for prevalent user bias to affect the results of the pharmacogenomics study, results will be interpreted conditional on the level of concern indicated. While I appreciate that probabilistic sensitivity analysis or inverse-probability-of-treatment-weighting are potential steps for handling bias in single-exposure studies, both the scale and the scope (i.e. 2.5 million SNPs examined in fifteen studies) of this interaction study make the potential burden of these methods unreasonable. Therefore, I will instead consider the results of Specific Aim 1 conditional on the results of Specific Aim 2.

Strengths and Limitations

This study represents the first large, multi-ethnic pharmacogenomics study of thiazide diuretics, a commonly used antihypertensive medication, and QT, the prolongation of which is a leading cause of the withdrawal or restricted marketing of pharmaceuticals. This work will also make use of the deep phenotyping and genotyping available in the participating cohorts (the Women's Health Initiative [WHI], the Hispanic Community Health Study/Study of Latinos [SOL], and the Cohorts for Heart and Aging Research in Genetic Epidemiology [CHARGE] consortium). By bringing together these fourteen study populations (WHI, SOL, and twelve member studies in the CHARGE consortium), many of which have longitudinal measures of thiazide use and QT, this study will have a significantly larger population than previous pharmacogenomics studies, substantially increasing the power to detect a much greater range of interaction effects than was possible in previous studies. Furthermore, I will take advantage of the large AA and HL populations available in the participating cohorts, broadening generalizability and allowing me to leverage the unique genetic architecture that characterizes AA and HL populations. Finally, I will incorporate recently developed techniques for including functional annotations in genetic analyses, which will allow this

work to prioritize findings based on the probability of causality, something which much of the early genetics and pharmacogenomics work has failed to accomplish.

However, there are several limitations to this work. First, I am relying on observational cohort studies, which are known to be potentially biased in pharmacoepidemiologic studies. Of particular concern is the chance of medication inventories to miss cases of short term medication use and acute ADRs. To avoid this, pharmacy-linked databases with more complete medication-use data are preferable. Unfortunately, at this stage, these databases have no mechanism for linking genetic data or deep phenotyping data to individual records, making large, population based cohorts the best alternative. Furthermore, it is unclear if these potential biases are a concern in pharmacogenomic studies, which study the drug-gene interaction. This work will also run simulations to determine the potential issues with selection bias on pharmacogenomics work and the best study designs for handling this data, which can then be used to inform the subsequent work.

Additionally, results of the trans-ethnic meta-analysis will be driven by the contributing EU population, which is more than 3.5 times larger than the next largest race/ethnic group (HL). Furthermore, the power of this study is a concern, given that this is both a GxE interaction study and a genome-wide study, both of which negatively impact power. However, this is the largest pharmacogenomics work on QT to date and has the largest populations of non-EU participants. There have been extensive efforts to identify and include any studies with ECG, medication, and genotype data, with an emphasis on identifying cohorts with multi-ethnic populations. In addition, this work leverages the longitudinal data available in many of the participating cohorts, further increasing our power

to detect interaction associations. Finally, given the extensive efforts to identify and include all available studies with the needed data, there is no replication sample for this work. However, given the size of the populations (78,199), results are more robust to the potential of winner's curse, a form of bias that results in the over-estimation of effect sizes and consequently results in false positives. While most GWAS use replication samples to protect against winner's curse, the large sample size in this work will aid in protecting this analysis from the selection that results in winner's curse.

CHAPTER 5: RESEARCH PAPER 1-PHARMACOGENOMICS STUDY OF THIAZIDE DIURETICS AND QT INTERVAL IN MULTI-ETHNIC POPULATIONS: THE COHORTS FOR HEART AND AGING RESEARCH IN GENOMIC EPIDEMIOLOGY (CHARGE)[1]

Introduction

Over the past decade, the use of prescription drugs has skyrocketed, with nearly half of all Americans now taking at least one prescription drug.[1] Accompanying the increased prevalence of drug use is a high burden of adverse drug reactions (ADRs), which account for approximately 100,000 deaths and 2.2 million serious health effects annually.[2-4] QT interval (QT) prolongation, which can trigger fatal ventricular arrhythmias, is a long-recognized adverse effect[185] of numerous common medications such as antipsychotics, antibiotics, antiarrhythmics, and antihypertensives.[146] Within the past ten years, QT prolongation has represented the most common cause for withdrawal of a drug from the market (or relabeling) after approval by the U.S. Food and Drug Administration (FDA).[39, 145] However, drug-induced QT prolongation remains difficult to predict.[38]

Genetic variants are known to mediate both pharmacokinetic and pharmacodynamic processes, thereby playing a major role in drug response. [61] Pharmacogenomics, which

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evaluates the role of genetics in drug response, offers a promising avenue for understanding variation in drug response,[7] illuminating novel pathways, informing drug development and selection,[8-10] optimizing dosing regimens,[11-15] and avoiding ADRs.[16-18] QT is highly heritable (35-40%).[24-27, 363] Previous pharmacogenomics studies of drugs associated with QT prolongation, including thiazide diuretics, a common antihypertensive therapy used by over a quarter of the U.S. hypertensive population,[37] identified multiple loci associated with anti-hypertensive response and ADRs.[272, 273, 275-278] Therefore, the pharmacogenomics of thiazide-induced QT prolongation represents an excellent but understudied candidate for pharmacogenomic inquiry.

We previously examined evidence for common single nucleotide polymorphisms (SNPs) that modified the association between thiazide use and QT and failed to identify any genome-wide significant ($P<5x10^{-8}$) loci.[282] However, our previous study was limited to European descent populations and cross-sectional analyses, despite many of the contributing studies having longitudinal drug and electrocardiographic data.[282] Here, we expand upon that work, applying recent statistical innovations to leverage longitudinal data and including an additional 44,418 participants of European, African American, and Hispanic/Latino descent to perform the first trans-ethnic genome-wide association study (GWAS) to examine genetic associations that modify the association between thiazides and QT, as well as the component parts of QT (JT interval [JT], QRS interval [QRS]).

Materials and Methods

A. Study Populations

Fourteen cohorts participated in this analysis from in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE)[54] Pharmacogenomics Working Group (PWG), contributing 78,199 participants: European descent (51,601), African American

(11,482), and Hispanic/Latino (15,116) participants (Table 19, Supplementary Text). Among the fourteen cohorts, six (55% of the total population) had repeated measurements of medication use and electrocardiogram (ECG) assessments and contributed longitudinal data to the analysis: Age, Gene/Environment Susceptibility – Reykjavik Study (AGES), Atherosclerosis Risk in Communities (ARIC) Study, Cardiovascular Health Study (CHS), Rotterdam Study (RS), Multi-Ethnic Study of Atherosclerosis (MESA), and Women's Health Initiative (WHI). The remaining eight cohorts contributed cross-sectional data to the analysis: Framingham Heart Study (FHS), Erasmus Rucphen Family (ERF) Study, Health 2000, Health, Aging, and Body Composition (Health ABC), Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), Jackson Heart Study (JHS), Netherlands Epidemiology of Obesity (NEO) Study, and Hispanic Community Health Study/Study of Latinos (HCHS/SOL).

B. Study Design

Participants with electrocardiogram (ECG) measurements, medication assessment, and genome-wide genotype data were eligible for inclusion. The following exclusion criteria were applied: poor ECG quality, atrial fibrillation detected by ECG, pacemaker implantation, second or third degree atrioventricular heart block, QRS greater than 120 milliseconds (ms), prevalent heart failure, pregnancy, missing ECG, missing medication assessment, missing genotype information, or race/ethnicity other than European descent, African American, or Hispanic/Latino. For studies with longitudinal data, exclusion criteria were applied on a visit-specific basis.

C. Medication Assessment

Medication use was assessed through medication inventories conducted during clinic visits or home interviews or through pharmacy databases (Table 20). Six studies captured medication used on the day of the study visit. A further six of the 14 participating cohorts captured medications used one to two weeks preceding ECG assessment. HCHS/SOL ascertained medications used within four weeks preceding ECG measurement and the RS captured medication used within 30 days preceding ECG assessment. Participants were classified as thiazide diuretic users if they took a thiazide or thiazide-like diuretic in a single or combination preparation, with or without potassium (K)-sparing agents, and with or without K-supplements.

For cross-sectional studies, the number of exposed participants ($N_{exposed}$) was defined as the number of participants classified as thiazide users. For studies with longitudinal data, $N_{exposed}$ was calculated as follows:

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

where n_i is the number of observations for participant *i*, $\hat{\rho}$ is an estimate of the pairwise visit-to-visit correlation within participants from a Generalized Estimating Equation (GEE)-exchangeable model that does not contain genetic data, and $\#\{E_{it}=1\}$ is the number of observations for which participant *i* was exposed.[346]

D. ECG Interval Measurement

QT and QRS were digitally recorded by each participating study using resting, supine or semi-recumbent, standard 12-lead ECGs (Table 21). Comparable procedures were used for preparing participants, placing electrodes, recording, transmitting, processing, and controlling quality of ECGs. Studies used Marquette MAC 5000, MAC 12, MAC 1200, or MAC PC (GE Healthcare, Milwaukee, Wisconsin, USA), University of Glasgow (Cardiac Science, Manchester, UK), or ACTA (EASOTE, Florence, Italy) machines. Recordings were processed using one of the following programs (Marquette 12SL, MEANS, University of Glasgow, digital calipers, or Health 2000 custom-made software. JT was calculated by the formula: JT=QT–QRS.

E. Genotyping and Imputation

Each study conducted genome-wide genotyping independently using either Affymetrix (Santa Clara, CA, USA) or Illumina (San Diego, CA, USA) arrays (Table 22). Sex mismatches, duplicate samples, and first-degree relatives (except in ERF, FHS, HCHS/SOL, and JHS) were excluded. DNA samples with call rates less than 95-98% were excluded as were SNPs with SNP call rates less than 90-98%, minor allele frequencies (MAF) less than 1%, or that failed Hardy-Weinberg equilibrium. To maximize genome coverage and comparisons across genotyping platforms, genotypes were imputed using HapMap2,[338-340] 1000 Genomes Phase 1, or 1000 Genomes Phase 3 reference panels.[342, 343] Genotypes imputed using build 37 were lifted over to build 36[364, 365] to enable comparisons between imputation platforms and results were restricted to SNPs present in HapMap2.

F. Statistical Analyses

Genome-wide pharmacogenomic analyses were performed by each cohort independently across approximately 2.5 million SNPs for QT, QRS, and JT separately. Drug-SNP interactions were estimated assuming an additive genetic model, using mixed effect models, GEE, or linear regression with robust standard errors. The analytic model varied based on study design and the availability of longitudinal data (Table 23). All analyses were adjusted for age (years), sex when applicable, study site or region, principal components of genetic ancestry, visit-specific RR interval (ms), and visit-specific QT altering medications defined using the University of Arizona Center for Education and Research on Therapeutics (UAZ CERT) QT-prolonging drug classification.[146] Furthermore, ERF, FHS, and HCHS/SOL incorporated estimates of relatedness into all analyses. Study-specific results were corrected for genomic inflation (λ).

Previous simulations demonstrated that models using robust standard errors underestimate the variance of coefficient estimates for SNPs with low MAFs.[346] To account for this underestimation, corrected standard errors were calculated using a (Student's) *t*-reference distribution.[346] The degrees of freedom (df) for the *t*-reference distribution were estimated using Satterthwaite's method.[347] When cohorts were unable to implement Satterthwaite's method, an approximate df was calculated as twice the cohort- and SNP-specific product of the SNP imputation quality (range: 0,1), the MAF (range: 0.0,0.50), and $N_{exposed}$. Standard errors were then "corrected" by assuming a normal reference distribution that yielded the *t*-distribution based *P*-values from the beta estimates.[346] Furthermore, because simulations demonstrated that corrected standard errors were unstable when minor allele counts among the exposed were low, a cohort-specific df filter of 15 was applied across all SNPs.[346]

For each trait, race-stratified and trans-ethnic betas and corrected standard errors were combined with inverse-variance weighted meta-analysis conducted in METAL.[344] We used a genome-wide significance threshold of $P<5x10^{-8}$ and a suggestive threshold of $P<5x10^{-6}$. However, the assumptions of a fixed-effects meta-analysis do not always hold between race/ethnicities due to differences in patterns of linkage disequilibrium (LD) across ancestral populations, potential allelic heterogeneity, differences in gene-environment and gene-gene interactions, and differences in environmental and lifestyle factors.[300, 366] Therefore, trans-ethnic meta-analysis was also conducted using the Bayesian MANTRA approach and a genome-wide threshold of $\log_{10}$(Bayes Factor [BF])>6 and a suggestive threshold of $\log_{10}$(BF)>5.[345] Additionally, previous studies have demonstrated the potential to increase power and detect evidence of pleiotropy by conducting multi-trait analysis across correlated traits.[349, 350] To examine potential pleiotropy across ventricular depolarization and repolarization, we conducted cross-phenotype meta-analysis combining *t*-statistics across QRS and JT using an adaptive sum of powered score (aSPU) test, which tests for both concordant and discordant associations across some or all of the included traits.[351] The reference distribution for the aSPU test was calculated using 10^8 simulations.

Genome-wide significant and suggestive meta-analysis results were examined for gene or pathway enrichment. Previous work has shown that it is beneficial to apply multiple methods of gene-set analysis (GSA) when the underlying etiology of the genetic mechanism is unclear.[367-369] We therefore used two methods of GSA. We performed a multiple regression gene analysis approach followed by a self-contained GSA using gene-level regression as implemented in MAGMA.[370] Post-meta-analysis *P*-values were used as input in the analysis and gene-sets were collected from Ingenuity,[371] Panter,[372] KEGG,[373] and

ConsensusPathDB[374, 375] and restricted to biologically motivated pathways involved in the following: ion transport and homeostasis, transcription and translation, renal and cardiac development and function, and pharmacokinetic/dynamic pathways. Additionally, we selected all SNPs with $P<1x10^{-5}$ for analysis with DEPICT, which searches for gene, gene-set, and tissue enrichment among 14,461 reconstituted gene-sets, eliminating the need to select candidate gene-sets.[376] To account for multiple testing, we applied a false discovery rate (FDR) threshold of 5% for both GSA approaches.

G. Statistical Power Simulations

Power to detect drug-SNP interactions using cross-sectional and longitudinal modeling approaches was estimated via simulation studies. Assumptions, which were informed by European ancestry populations, included: (1) 50,000 participants; (2) a two-sided, per-SNP $\alpha=5x10^{-8}$; (3) a mean heart rate-corrected QT (standard deviation)=400 (30) ms; (4) $N_{exposed}$=8,100; (5) a mean drug effect for those with zero copies of the minor allele=5 ms; (6) a mean SNP effect for those not exposed to drug=0 ms; (7) a MAF=0.05 or 0.25; (8) an additive model of inheritance; (9) two study visits for longitudinal simulations; (10) within-person QT correlation=0.80; (11) an attrition rate between visits for longitudinal simulations=0.13; (12) random missingness rate across study visits=0.09; and (13) an independent GEE correlation structure for longitudinal simulations. For longitudinal simulations, drug use was either temporally constant or variable. When variable, drug exposure was assumed to be completely random at both visits.

Results

A. Study Characteristics

A total of 78,199 participants were included in the analysis, of which 13,730 (18%) were exposed to thiazides (Table 19). Thiazide use was most common among African Americans (36%), compared with 16% and 9% among European descent and Hispanic/Latino populations, respectively. Mean age ranged from 40 (FHS) to 75 years (PROSPER) and the percentage of females ranged from 47% (NEO, PROSPER) to 100% (WHI). Average QT was between 389 ms (H2000) and 416 ms (HCHS/SOL).

B. Genome-Wide Analysis of Interaction Between Thiazides and QT Interval

Q-Q plots for individual study results, as well as for meta-analyzed results, demonstrated adequate calibration of study specific test statistics (Figure 18, Appendix 2). However, the family-based studies (ERF, FHS, HCHS/SOL) showed modest evidence of over-dispersion (λ=1.07 – 1.16).

No genome-wide significant thiazide-SNP interaction effects were detected in any race/ethnic group (Figure 19). However, suggestive interaction effects ($P<5x10^{-6}$) were found for 22 loci in at least one race/ethnic group: European descent (seven loci), African American (six loci), Hispanic/Latino (six loci), or trans-ethnic (nine loci) (Figure 19, Table 24). Only the *DNAH8/BTBD9* locus was suggestively significant in more than one race/ethnic group (rs862433 in African Americans, rs1950398 in Hispanic/Latinos). Only two of the suggestive SNPs were heterogeneous across populations with $P_{het}<0.05$ (rs4890550 and rs13223427).

Additionally, examination of 35 loci previously associated with QT in a published main effects GWAS[209] found no significant associations in European descent populations using a Bonferroni corrected threshold of $P<0.001$ (0.001=0.05/35; Table 25). The

magnitude of the interaction effect was close to zero for all but six of the 35 SNP, which had interaction effects greater than 0.50 ms.

Similarly, while no locus showed genome-wide significance in our trans-ethnic MANTRA analysis (Figure 20), one SNP (rs2765279) were above the suggestive threshold, with a $\log_{10}$(BF) of 5.2. Rs2765279, located in *RGSL1*, a gene involved in G-protein signaling regulation, was also the most significant SNP in the fixed-effects trans-ethnic analysis (P=3x10^{-7}).

C. Genome-Wide Analysis of Interaction Between Thiazides and QRS Interval or JT Interval

Results for QRS showed a similar pattern to those for QT (Figure 21, Table 26). Whereas no results achieved genome-wide significance, 28 loci showed suggestive evidence of modifying the thiazide-QRS association (four loci in European descent populations, 11 in African Americans, eight in Hispanic/Latinos, and seven in trans-ethnic populations) and only one SNP had a P_{het}<0.05 (rs11591185). The most significant SNP, rs7638855 (P=2x10^{-7}), located upstream from *GAP43*, was also suggestively significant after trans-ethnic analysis in MANTRA ($\log_{10}$(BF)=5.4; Figure 20).

Similarly, no SNPs showed genome-wide significant interaction for JT, although 19 loci were suggestively associated (five loci in European descent populations, four in African Americans, five in Hispanic/Latino, and seven in trans-ethnic populations; Figure 21, Table 27). No SNPs showed significant heterogeneity between populations. Moreover, MANTRA analysis identified two SNPs that achieved suggestive significance (Figure 20). The rs1264878 variant near *KCNIP4*, a voltage-gated potassium channel interacting protein was the most significant SNP in our fixed-effects meta-analyses (P=3x10^{-7}) and had a

$\log_{10}$(BF)=5.1. However, most significant SNP in MANTRA meta-analyses was rs9303589, in *CA10*, with a $\log_{10}$(BF)=5.1.

D. Cross-Phenotype Meta-Analysis

Cross-phenotype meta-analysis found no genome-wide significant evidence of pleiotropy across QRS and JT (Figure 22, Figure 23). However, eight loci had a suggestive evidence of thiazide-SNP interaction after meta-analyzing QRS and JT results (Table 28). These included three loci that were nominally associated with QRS and JT (P<0.05), but whose effects did not reach the suggestive association threshold in either univariate analysis (rs1295230 [*PIK3R6*], rs6931354 [*ADGRB3*], and rs8119517 [*PREX1*]).

E. Gene and Pathway Enrichment Analysis

Although analysis with DEPICT found no gene or tissue enrichment, gene-set enrichment analysis in European descent populations found enrichment in the *ATXN3* subnetwork for the interactive effect of genotype and thiazide use on QT (P=1x10^{-6}). There was no enrichment found in QRS or JT analyses. MAGMA analyses found significant enrichment in six genes among African Americans in the interactive effect of genotype and thiazide use on QRS: *CNTRL, CPN1, FAM65B, RAB14, ISY1, NELL1* (Table 29). No other analyses found gene enrichment. MAGMA GSA for QT and JT analyses found significant enrichment for transcription and translational pathways, although no gene-set enrichment was found in QRS analyses (Table 30).

F. Statistical Power

Given the biologic plausibility of the suggestive results for all three traits, we examined statistical power for our analysis to assess our ability to detect interaction effects. Simulations demonstrated that all analyses were underpowered to detect thiazide-SNP interaction effects less than 3 ms (e.g. 15% power to detect an interactive effect of 2 ms;

Figure 24). However, even with time-varying drug exposure (i.e. observed QT measurement on and off drug within an individual), which demonstrated the greatest power, analyses for SNPs with MAF=5% did not achieve 80% power until the thiazide-SNP interaction effect reached 6 ms.

Discussion

In this study, we examined 78,199 participants of European, African American, or Hispanic/Latino descent for evidence of thiazide-SNP interactions influencing QT. Although we used a comprehensive approach that considered multi-ethnic populations, leveraged pleiotropy, accommodated population heterogeneity, and examined QT as well as its component parts (QRS, JT), we did not identify any genome-wide significant SNPs modifying the association between thiazides and these ECG intervals. However, we identified 74 loci with suggestive evidence of association through both univariate and cross-phenotype analyses as well as evidence of enrichment in pathways involved in transcription and translation.

Interestingly, our suggestive results included multiple loci involved in ion transport and handling, the disruption of which is believed to be an underlying mechanism in drug-induced QT prolongation,[111] supporting the hypothesis that common SNPs modify the thiazide-QT relationship. For example, the *NELL1* locus was previously associated with changes in fasting plasma triglyceride levels in response to hydrochlorothiazide use.[279] Other interesting suggestive results include the *PITX2* and *RYR3* QRS loci identified in Hispanic/Latinos, which may directly regulate ion channel genes and genes involved in calcium handling.[377] Moreover, we found suggestive evidence of thiazide-SNP interactions on QT, QRS, or JT in other genes involved in ion transport and handling, including *STC2*,[378]

EDN1,[379] *TRPC7*,[380] *PKP2*,[381] and *DISC1*,[382] as well as a voltage-gated potassium channel gene (*KCNQ3*).

Despite these intriguing results, our power simulations suggested there was limited power to detect interaction effects of 2 ms, sizes consistent with QT main effects analyses.[209] The low power suggests that larger sample sizes and/or innovative statistical methods may be required to study gene-environment interactions given the stringent genome-wide significance threshold.[383-385] Furthermore, our power simulations demonstrated insufficient power to detect interaction effects of 5 ms or less for less common SNPs (MAF=5%). Therefore, future work should utilize larger sample sizes, particularly studies with longitudinal data, if available.

Another limitation of our work was that medication use data were collected infrequently, e.g. years apart. Particularly, medication assessments covered only one to two weeks of medication use in most participating cohorts and variables such as medication dosage and duration of use were not available universally across studies. Previous work has demonstrated a dose-dependent relationship between thiazide use and cardiac arrest, a potential outcome of QT prolongation.[249] However, we were unable to identify participants using high dose thiazides because medication dosage data was unavailable in all cohorts. Furthermore, K^+ measurements and information on K^+ supplements was not obtained across all cohorts so we were unable to adjust for K^+ levels in our analyses, despite the known role of thiazide diuretics in inducing hypokalemia and the role of hypokalemia in causing QT prolongation.[40, 252]

Additionally, observational cohort studies are known to be susceptible to selection biases, such as prevalent user bias, whereby long-term medication users are least likely to

suffer from ADRs and users with ADRs often stop therapy and therefore have a lower chance of being seen while on therapy.[284, 285] Unfortunately, without duration of use metrics, it remains difficult to assess the effects of prevalent user bias on study results. Indeed, it is unclear if these biases are of concern in pharmacogenomic studies.[286, 287] Additional work is needed to assess whether selection bias requires more consideration in pharmacogenomic research and to assess possible advantages of alternative designs, such as active comparator designs (whereby the control group contains participants using a different class of medications with similar indications to the medication of interest) or new user designs (whereby prevalent users are excluded). Moreover, medication inventories may be associated with non-negligible measurement error. For example, while Smith *et al*. reported good agreement between thiazide use measured using medication inventories and serum thiazide measurements, specificity remained moderate. [57]

Given the challenges associated with assembling an adequately powered pharmacogenomics study, electronic medical records (EMRs) represent a potential untapped resource that may merit evaluation. Strengths of EMRs include the potential to provide a more complete medication history, which could enable sensitivity analyses examining variables such as medication dose and duration of use. Furthermore, consortia such as eMERGE have demonstrated the feasibility of linking EMRs to genetic data for use in genetic research,[386] and have successfully identified genetic variants modifying drug response.[387] However, EMRs have limitations. Investigators using EMR data cannot control participant recruitment, timing and accuracy of data collection, or population representativeness.[388] Considering ECG research specifically, cohort studies administer ECGs to all participants at study visits, whereas EMRs may capture ECGs for patients with

medical indications, providing an inherently different population. EMRs therefore have the potential to greatly advance pharmacogenomic research but warrant further evaluation.

In conclusion, our findings suggest that additional work is needed to fully elucidate potential pharmacogenomic effects influencing the thiazide-QT relationship. Our suggestive results support a possible role of genetics in modifying the association between thiazides and QT. However, these findings can inform the biology of thiazide-induced QT-prolongation and do not preclude the possibility of common variants with small effects or rare variants with larger effects. Future work that leverages larger sample sizes, such as those available in EMRs, and innovative statistical methods to validate these suggestive findings is needed. The FDA considers further regulation of drugs that prolong QT by as little as 5 ms, a small increment easily achieved by the combination of genetic and pharmaceutical effects,[148, 282] making it critical that we unravel the complex etiology of drug-induced QT prolongation.[44] Pharmacogenomics remain a promising avenue for understanding variability in drug response and for utilizing genetics to improve public health but innovative solutions are needed to overcome inherent challenges.

Tables and Figures

Table 17. Study Population Characteristics of 25 Contributing Study Populations

Population	$N_{exposed}$	N_{total}	QT in ms, mean (SD)	QRS in ms, mean (SD)	JT in ms, mean (SD)	Age in years, mean (SD)	Female, %
European Descent							
AGES	435	2,256	405 (34)	90 (10)	316 (33)	75 (5)	64.2
ARIC	1,449	8,567	399 (29)	91 (10)	308 (29)	54 (6)	52.6
CHS	1,003	3,004	414 (32)	88 (10)	322 (30)	72 (5)	62.5
ERF	29	1,792	398 (28)	NA	NA	48 (14)	59.0
FHS	83	3,168	415 (30)	88 (10)	328 (30)	40 (9)	52.5
H2000	104	1,973	389 (30)	NA	NA	50 (11)	52.0
Health ABC	217	1,560	414 (32)	90 (11)	324 (32)	74 (3)	49.4
MESA	453	2,216	412 (29)	93 (9)	320 (29)	62 (10)	52.1
NEO	609	5,366	406 (29)	93 (10)	313 (29)	56 (6)	47.0
PROSPER	1,175	4,556	414 (36)	94 (11)	320 (35)	75 (3)	47.0
RS I	523	4,805	397 (29)	97 (11)	300 (28)	69 (9)	60.2
RS II	161	1,889	403 (28)	98 (11)	305 (28)	65 (8)	56.6
RS III	93	1,950	401 (26)	98 (11)	304 (26)	56 (6)	54.1
WHI GARNET	431	1,981	401 (29)	86 (9)	315 (29)	66 (7)	100
WHI MOPMAP	268	1,383	402 (30)	86 (8)	316 (30)	63 (7)	100
WHI WHIMS	1,106	5,135	401 (30)	86 (9)	315 (29)	68 (6)	100
Summary	8,139	51,601					
African American							
ARIC	916	2,169	400 (33)	90 (10)	310 (32)	53 (6)	62.3
CHS	351	666	409 (35)	88 (11)	317 (36)	73 (6)	64.4
Health ABC	268	1,151	411 (35)	88 (11)	322 (34)	73 (3)	57.6
JHS	463	1,862	410 (32)	92 (10)	319 (30)	50 (12)	60.9
MESA	467	1,464	410 (32)	91 (10)	319 (31)	62 (10)	54.4
WHI SHARe	1,661	4,170	401 (34)	85 (9)	316 (33)	61 (7)	100
Summary	4,215	11,482					
Hispanic/Latino							
HCHS/SOL	941	12,024	416 (28)	91 (10)	325 (29)	46 (14)	59.5
MESA	211	1,316	409 (30)	91 (10)	318 (30)	61 (10)	51.8
WHI SHARe	224	1,776	401 (30)	86 (9)	316 (30)	60 (6)	100
Summary	1,376	15,116					

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; HCHS/SOL, Hispanic Community Health Study/Study of Latinos; Health ABC, Health, Aging, and Body Composition Study; JHS, Jackson Heart Study; JT, JT interval; MESA, Multi-Ethnic Study of Atherosclerosis; MOPMAP, Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; NEO, the Netherlands Epidemiology of Obesity; $N_{exposed}$, Number of participants exposed to thiazides; N_{total}, Total number of participants in study population after exclusions; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; QRS, QRS interval; QT, QT interval; RS, Rotterdam Study; SD, Standard deviation; SHARe, The SNP Health Association Resource; WHI, Women's Health Initiative; WHIMS, the WHI Memory Study

Table 18. Description of Medication Assessment Methods for 14 Participating Studies Included in the Pharmacogenomic Analysis of QT, QRS, and JT in N=78,199 Participants

Study	Method of Medication Assessment*	Time Period	Number of Visits Included in Analysis
AGES	Medication Inventory	At time of visit	2
ARIC	Medication Inventory	2 weeks before visit	≤4
CHS	Medication Inventory	2 weeks before visit	up to 10
ERF	Medication Inventory	At time of visit	1
FHS	Medication Inventory	At time of visit	1
Health ABC	Medication Inventory	2 weeks before visit	1
Health 2000	Medication Inventory	1 week before visit	1
HCHS/SOL	Medication Inventory	4 weeks before visit	1
JHS	Medication Inventory	At time of visit	1
MESA	Medication Inventory	2 weeks before visit	2
NEO	Medication Inventory	At time of visit	1
PROSPER	Medication Inventory	At time of visit	1
RS1	Pharmacy database	Prescriptions filled ≤ 30 days before visit	5
RS2	Pharmacy database	Prescriptions filled ≤ 30 days before visit	3
RS3	Pharmacy database	Prescriptions filled ≤ 30 days before visit	1
WHI GARNET	Medication Inventory	2 weeks before visit	≤4
WHI MOPMAP	Medication Inventory	2 weeks before visit	≤4
WHI WHIMS	Medication Inventory	2 weeks before visit	≤4
WHI CT SHARe	Medication Inventory	2 weeks before visit	≤4

*Medication inventory defined as a structured interview where trained staff member reviewed and recorded all medications with participant.

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; 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; MOPMAP, Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; NEO, the Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SHARe, The SNP Health Association Resource; WHI CT, Women's Health Initiative Clinical Trial; WHIMS, the WHI Memory Study

Table 19. ECG Measurement Methods for 14 Participating Studies Included in the Pharmacogenomic Analysis of QT, QRS, and JT in N=78,199 Participants

Study	ECG Machine	Measurement System
AGES	Marquette / MAC 5000 Resting ECG	Marquette 12SL
ARIC	Marquette MAC PC	Marquette 12SL
CHS	Marquette MAC PC	Marquette 12SL
ERF	ACTA electrocardiographs (EASOTE, Florence, Italy)	MEANS[389, 390]
FHS	GE MAC 5000	Digital calipers
Health ABC	Marquette MAC PC	Marquette 12SL
Health 2000	Marquette MAC 5000	Custom-made software
HCHS/SOL	GE MAC 1200	Marquette 12SL
JHS	Marquette MAC PC	MEANS[389, 390]
MESA	Marquette MAC 1200	Marquette 12SL
NEO	University of Glasgow	University of Glasgow
PROSPER	University of Glasgow	University of Glasgow
RS1	ACTA electrocardiographs (EASOTE, Florence, Italy)	MEANS[389, 390]
RS2	ACTA electrocardiographs (EASOTE, Florence, Italy)	MEANS[389, 390]
RS3	ACTA electrocardiographs (EASOTE, Florence, Italy)	MEANS[389, 390]
WHI GARNET	Marquette MAC PC	Marquette 12SL
WHI MOPMAP	Marquette MAC PC	Marquette 12SL
WHI WHIMS	Marquette MAC PC	Marquette 12SL
WHI CT Share	Marquette MAC PC	Marquette 12SL

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; 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; MOPMAP, Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; NEO, the Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SHARe, The SNP Health Association Resource; WHI CT, Women's Health Initiative Clinical Trial; WHIMS, the WHI Memory Study

Table 20. Genotyping Characteristics for the 14 Studies Included in the Pharmacogenomic Analysis of QT, QRS, and JT in N=78,199 Participants

Study	Genotyping array	Genotype calling algorithm	Sample call rate filter	SNP call rate filter	SNP MAF filter	HWE p-value filter	Imputation software	Imputation Platform	N. autosomal SNPs passing QC
AGES	Ilumina Hu370CNV	BeadStudio	>95%	>97%	<1%	10^{-6}	MACHv.1.16	HapMap2	308,340
ARIC	Affymetrix 6.0	Birdseed	<95%	<90%	<1%	$<10^{-5}$	MACH v1.16	HapMap2 Build 36	669,450
CHS	Illumina 370CNV	GenomeStudio	≤95%	<97%	NA	$<10^{-5}$	BIMBAM 0.99	HapMap2	306,655
ERF	Illumina 6K/318K/370K	Bead Studio	< 98%	< 98%	< 1%	$<10^{-6}$	MACH v 1.0.15	HapMap2	487,573
FHS	Affymetrix 500K + 50 MIP	BRLMM	<97%	<97%	<1%	$<10^{-6}$	MACH v 1.0.15	HapMap2	378,163
Health ABC	Illumina 1M	Beadstudio	<97%	<97%	<1%	$<10^{-6}$	MACH v1.16	HapMap2	914,263
Health 2000	Illumina Human610-Quad BeadChip	GenCall	<95%	<95%	<1%	$<10^{-6}$	MACH	HapMap2	558,388
HCHS/SOL	Illumina Omni 2.5M + Custom	GenomeStudio	<98%	<98%	NA	$<10^{-5}$	IMPUTE2	1000 Genomes Phase 3	2,294,032
JHS	Affymetrix 6.0	Birdseed	<95%	<95%	NA	NA	IMPUTE v2.1.0	HapMap P2.r22.b36, CEU+YRI	868,969
MESA	Affymetrix 6.0	Birdseed	<95%	<90%	<1%	$<10^{-4}$	IMPUTE v2.1.0	HapMap2	730,000
NEO	Illumina HumanCoreExome-24v1_A Beadchip	GenCall	<98%	<98%	NA	<10E-5	IMPUTE2	1000 Genomes 2011 v3	361,046
PROSPER	Illumina 660K	Bead studio	<90%	<97.5 %	NA	$<10^{-6}$	MACH v1.15	HapMap2	557,192
RS1	Illumina 550k,	BeadStudio	<98%	<98%	<1%	$<10^{-6}$	MACH1 v 1.0.15,	HapMap2	512,349
RS2	Illumina 550K Duo, 610KQuad	GenomeStudio	<98%	<95%	<1%	$<10^{-6}$	MACH 1 v 1.0.16	HapMap2	537,405
RS3	Illimina 610 Quad	Beadstudio	<98%	<95%	<1%	<10E-6	MACH v1.0.16	HapMap2	466,389
WHI GARNET	Illumina Human Omni1-Quad v1-0 B	BeadStudio v3.1.3.0	NA	≤98%	NA	<10E-4	BEAGLE v3.3.1	1000G v3 3/2012	NA
WHI	Affymetrix Axiom	Birdseed	NA	≤90%	<0.5	<10E-6	MaCH	Hapmap 2 Build	NA

MOPMAP	Genome-Wide Human CEU I				%		minimac	36	
WHI WHIMS	Human OmniExpress Exome-8v1_B Genome-Wide Human	Birdseed	NA	≤98%	<1%	<10E-4	MaCH minimac	Hapmap 2 Build 36	NA
WHI CT SHARe	Affymetrix GeneChip SNP Array 6.0	Birdseed	NA	≤95%	<1%	$<10^{-6}$	MaCH v1.0.16	Hapmap 2 Build 36 (1:1 CEU:YRI)	NA

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; 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; MOPMAP, Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; NEO, the Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SHARe, The SNP Health Association Resource; WHI CT, Women's Health Initiative Clinical Trial; WHIMS, the WHI Memory Study

Table 21. Statistical Analysis Methods for 14 Participating Studies Included in the Pharmacogenomic Analysis of QT, QRS, and JT in N=78,199 Participants

Study	Statistical Analysis (Linear Regression, Mixed Model, GEE)	GWAS Statistical Analysis Software
AGES	GEE	R bosswithdf
ARIC	GEE	R bosswithdf
CHS	GEE	R bosswithdf
ERF	Mixed Model	GenABEL/ProbABEL
FHS	GEE	geepack
Health ABC	GEE	R bosswithdf
Health 2000	Linear Regression	ProbABEL v.0.1-6
HCHS/SOL	Mixed Model	R
JHS	GEE	R bosswithdf
MESA	GEE	R bosswithdf
NEO	Linear Regression	Probabel v0.4.3
PROSPER	Linear Regression	Probabel v0.4.3
RS1	GEE	R bosswithdf
RS2	GEE	R bosswithdf
RS3	Linear Regression	Probabel v0.4.3
WHI GARNET	GEE	R bosswithdf
WHI MOPMAP	GEE	R bosswithdf
WHI WHIMS	GEE	R bosswithdf
WHI CT Share	GEE	R bosswithdf

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; 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; MOPMAP, Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; NEO, the Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SHARe, The SNP Health Association Resource; WHI CT, Women's Health Initiative Clinical Trial; WHIMS, the WHI Memory Study

Table 22. Loci with Suggestive Evidence of Association with the Thiazide-SNP Interaction Effect on QT Interval

Locus	SNP	Chr	Position[a]	CA	CAF	Interaction Effect in ms (SE)	*P*	P_{het}
European Descent								
KIAA2013	rs17367934	1	11890791	A	0.89	2.4 (0.5)	$2x10^{-6}$	0.9
SLC14A2	rs4890550	18	41409189	C	0.44	-1.4 (0.3)	$3x10^{-6}$	0.01
RPS29	rs10143493	14	47999650	A	0.01	-10.6 (2.3)	$3x10^{-6}$	0.4
NELL1	rs12225793	11	21057283	T	0.12	2.3 (0.5)	$4x10^{-6}$	1.0
STC2	rs10079004	5	172704698	A	0.71	-1.5 (0.3)	$4x10^{-6}$	0.4
LCLAT1	rs7608507	2	30447424	A	0.75	1.6 (0.3)	$4x10^{-6}$	0.7
PPP1R3A	rs13223427	7	113199332	T	0.56	1.4 (0.3)	$4x10^{-6}$	0.02
African American								
ZBTB16	rs10789991	11	113424299	T	0.03	12.3 (2.4)	$5x10^{-7}$	0.6
DNAH8	rs862433	6	38968057	A	0.25	-2.6 (0.5)	$7x10^{-7}$	0.2
Intergenic	rs9376483	6	140352934	T	0.94	7.2 (1.4)	$7x10^{-7}$	0.5
CASP8AP2	rs7753194	6	90597484	A	0.02	-11.4 (2.4)	$3x10^{-6}$	0.2
EBF1	rs11135035	5	157833407	A	0.41	2.1 (0.5)	$4x10^{-6}$	0.9
LAMA4	rs6926485	6	112630302	T	0.64	2.4 (0.5)	$5x10^{-6}$	0.5
Hispanic/Latino								
SPDYA	rs12475612	2	28883510	T	0.48	-3.5 (0.7)	$1x10^{-6}$	0.9
BTBD9	rs1950398	6	38666897	T	0.97	9.6 (2.0)	$2x10^{-6}$	0.05
TDRP	rs6558894	8	480495	C	0.14	-4.9 (1.0)	$2x10^{-6}$	0.3
COLCA2	rs10749974	11	110696967	A	0.09	-6.0 (1.3)	$3x10^{-6}$	0.2
CRYGGP	rs17868255	2	51884417	A	0.97	10.3 (2.2)	$3x10^{-6}$	0.5
RYR3	rs16968694	15	31376213	A	0.18	4.5 (1.0)	$3x10^{-6}$	1.0
Trans-Ethnic								
RGSL1	rs2765279	1	180693520	T	0.28	1.4 (0.3)	$3x10^{-7}$	0.4
ZBTB16	rs10789991	11	113424299	T	0.03	12.3 (2.4)	$5x10^{-7}$	0.6
PPP1R3A	rs17619887	7	113142601	A	0.47	1.2 (0.3)	$2x10^{-6}$	0.07
KIAA2013	rs17367934	1	11890791	A	0.89	2.3 (0.5)	$2x10^{-6}$	1.0
LCLAT1	rs6756908	2	30446501	A	0.65	1.3 (0.3)	$2x10^{-6}$	0.5
FAR1	rs7130476	11	13711632	C	0.90	2.0 (0.4)	$3x10^{-6}$	0.5
CASP8AP2	rs7753194	6	90597484	A	0.02	-11.4 (2.4)	$3x10^{-6}$	0.2
SMARCA2	rs1886261	9	2163590	A	0.75	1.5 (0.3)	$3x10^{-6}$	0.9
ZKSCAN8	rs13205911	6	28232093	T	0.09	-2.5 (0.5)	$5x10^{-6}$	0.6

[a]Build 36 Base-Pair Position

CA, Coded allele; CAF, Coded allele frequency; Chr, Chromosome; *P*, *P*-value; P_{het}, *P*-value of heterogeneity; SE, Standard error; SNP, Single nucleotide polymorphism

Table 23. P-value of Association for Thiazide-SNP Interaction Effect on QT Interval in European Descent Populations at 35 Loci Previously Associated with QT Interval in Main Effects Genome-Wide Association Studies[209]

Index SNP*	**Locus**	**CA**	**Main Effect in ms (SE)**[209]	**Interaction Effect in ms (SE)**	***P***
rs10040989	*GFRA3*	A	-0.85 (0.13)	0.87 (0.44)	0.05
rs1052536	*LIG3*	T	-0.98 (0.10)	-0.56 (0.31)	0.07
rs10919070	*ATP1B1*	A	1.68 (0.14)	-0.14 (0.44)	0.7
rs11153730	*SLC35F1-PLN*	T	-1.65 (0.10)	-0.15 (0.30)	0.6
rs11779860	*LAPTM4B*	T	0.61 (0.10)	0.16 (0.31)	0.6
rs12143842	*NOS1AP*	T	3.5 (0.11)	0.29 (0.35)	0.4
rs1296720	*CREBBP*	A	-0.83 (0.13)	0.57 (0.40)	0.2
rs12997023	*SLC8A1*	T	1.69 (0.22)	0.09 (0.72)	0.9
rs1396515	*KCNJ2*	C	-0.98 (0.09)	0.02 (0.30)	0.9
rs16936870	*NCOA2*	A	0.99 (0.16)	1.06 (0.51)	0.04
rs174583	*FEN1-FADS2*	T	-0.57 (0.09)	0.30 (0.32)	0.3
rs17784882	*C3ORF75*	A	-0.54 (0.10)	-0.18 (0.30)	0.6
rs1805128	*KCNE1*	T	7.42 (0.85)	-1.16 (1.21)	0.3
rs1961102	*AZIN1*	T	0.57 (0.10)	-0.41 (0.33)	0.2
rs2072413	*KCNH2*	T	-1.68 (0.11)	0.26 (0.34)	0.4
rs2273905	*ANKRD9*	T	0.61 (0.09)	-0.20 (0.34)	0.5
rs2298632	*TCEA3*	T	0.7 (0.09)	-0.11 (0.31)	0.7
rs2363719	*SLC4A4*	A	0.97 (0.16)	0.33 (0.50)	0.5
rs246185	*MKL2*	T	-0.72 (0.10)	-0.96 (0.35)	0.005
rs246196	*CNOT1*	T	1.73 (0.11)	-0.05 (0.34)	0.9
rs2485376	*GBF1*	A	-0.56 (0.10)	0.13 (0.32)	0.7
rs295140	*SPATS2L*	T	0.57 (0.09)	-0.40 (0.30)	0.2
rs3026445	*ATP2A2*	T	-0.62 (0.09)	-0.29 (0.31)	0.4
rs3105593	*USP50-TRPM7*	T	0.66 (0.10)	0.25 (0.30)	0.4
rs3857067	*SMARCAD1*	A	0.51 (0.08)	0.05 (0.30)	0.9
rs6793245	*SCN5A-SCN10A*	A	-1.12 (0.10)	-0.25 (0.33)	0.4
rs7122937	*KCNQ1*	T	1.93 (0.12)	-0.38 (0.40)	0.3
rs728926	*KLF12*	T	0.57 (0.10)	-0.13 (0.33)	0.7
rs735951	*LITAF*	A	-1.15 (0.10)	0.29 (0.33)	0.4
rs7561149	*TTN-CCDC141*	T	0.52 (0.09)	0.10 (0.30)	0.7
rs7765828	*GMPR*	C	-0.55 (0.09)	0.03 (0.31)	0.9
rs846111	*RNF207*	C	1.73 (0.13)	0.27 (0.38)	0.5
rs938291	*SP3*	C	-0.53 (0.09)	0.26 (0.31)	0.4
rs9892651	*PRKCA*	T	0.74 (0.10)	-0.04 (0.30)	0.9
rs9920	*CAV1*	T	-0.79 (0.14)	-0.44 (0.51)	0.4

*Index SNP as identified in a GWAS of QT main effects in European descent populations

CA, Coded allele; *P*, *P*-value; SE, Standard error

Table 24. Loci with Suggestive Evidence of Modifying the Thiazide-SNP Interaction Effect on QRS Interval

Locus	SNP	Chr	Position*	CA	CAF	Interaction Effect in ms (SE)	*P*	P_{het}
European Descent								
L3MBTL2	rs139461	22	39941406	T	0.34	0.8 (0.2)	$2x10^{-6}$	0.8
CD200R1	rs9864286	3	114147601	A	0.92	1.4 (0.3)	$2x10^{-6}$	0.7
EDG1	rs10874488	1	101640388	A	0.95	2.1 (0.4)	$3x10^{-6}$	0.6
HM13	rs6088592	20	29615942	A	0.15	-2.4 (0.5)	$4x10^{-6}$	0.2
African American								
DDX1	rs2080798	2	15789836	T	0.76	-1.4 (0.3)	$1x10^{-6}$	0.1
FAM84A	rs6711956	2	14204168	T	0.53	1.2 (0.2)	$2x10^{-6}$	0.4
GAP43	rs7638855	3	116617968	T	0.08	-2.8 (0.6)	$2x10^{-6}$	0.2
GUSBP1	rs7706102	5	21438324	C	0.92	3.1 (0.7)	$3x10^{-6}$	0.3
SV2B	rs886144	15	89518356	T	0.47	-1.1 (0.2)	$3x10^{-6}$	0.5
SLC35B3	rs850177	6	8517446	T	0.05	-3.3 (0.7)	$3x10^{-6}$	0.8
RFP4AP7	rs4363212	8	50466496	A	0.94	2.5 (0.5)	$4x10^{-6}$	1.0
KCNQ3	rs2469514	8	133218116	T	0.83	1.5 (0.3)	$4x10^{-6}$	0.3
ZCWPW2	rs6777813	3	28527314	A	0.36	1.1 (0.2)	$4x10^{-6}$	0.7
FAM65B	rs10946735	6	24980918	A	0.16	-1.5 (0.3)	$4x10^{-6}$	0.9
RASD2	rs2009681	22	34306117	A	0.76	1.3 (0.3)	$5x10^{-6}$	1.0
Hispanic/Latino								
AK2	rs11591185	1	33274771	A	0.08	3.5 (0.7)	$9x10^{-7}$	0.04
PKP2	rs12578228	12	33030528	T	0.10	-3.1 (0.7)	$2x10^{-6}$	0.4
TRPC7	rs12658104	5	135939584	A	0.03	5.2 (1.1)	$2x10^{-6}$	0.4
GATA3	rs10508356	10	8603852	T	0.42	1.7 (0.4)	$3x10^{-6}$	0.1
MYRIP	rs4557094	3	39690549	T	0.74	2.0 (0.4)	$3x10^{-6}$	0.4
TOX2	rs8120207	20	41928841	T	0.04	-3.7 (0.8)	$4x10^{-6}$	0.2
PIGM	rs2185214	1	158243375	A	0.88	-2.7 (0.6)	$5x10^{-6}$	0.5
PITX2	rs4834601	4	112190427	A	0.39	1.8 (0.4)	$5x10^{-6}$	0.6
Trans-Ethnic								
GAP43	rs7638855	3	116617968	T	0.07	-2.7 (0.5)	$2x10^{-7}$	0.6
CSMD1	rs17066601	8	3463355	T	0.08	3.0 (0.6)	$7x10^{-7}$	0.09
CD200R1	rs16860242	3	114125777	A	0.88	1.0 (0.2)	$2x10^{-6}$	0.4
TSGA10	rs720228	2	99070979	A	0.42	0.6 (0.1)	$2x10^{-6}$	0.5
ASCL1	rs2176822	12	102115378	T	0.94	-1.3 (0.3)	$3x10^{-6}$	0.6
DISC1	rs16856677	1	230389995	T	0.20	-1.2 (0.3)	$4x10^{-6}$	0.06
EDN1	rs7767845	6	12536024	T	0.97	2.3 (0.5)	$4x10^{-6}$	0.5

*Build 36 Base-Pair Position

CA, Coded allele, CAF, Coded allele frequency; Chr, Chromosome; *P*, *P*-value; P_{het}, *P*-value of heterogeneity; SE, Standard error; SNP, Single nucleotide polymorphism

Table 25. Loci with Suggestive Evidence of Modifying the Thiazide-SNP Interaction Effect on JT Interval

Locus	SNP	Chr	Position*	CA	CAF	Interaction Effect in ms (SE)	*P*	P_{het}
European Descent								
LCLAT1	rs6733641	2	30444156	T	0.27	-2.0 (0.4)	$1x10^{-6}$	0.2
TEDDM1	rs171980	1	180693220	A	0.20	1.9 (0.4)	$3x10^{-6}$	0.4
ZNF659	rs6806788	3	21450644	T	0.07	3.0 (0.6)	$3x10^{-6}$	0.7
FZD8	rs1219593	10	36587628	T	0.83	-2.0 (0.4)	$4x10^{-6}$	0.9
NELL1	rs7106157	11	21035410	A	0.55	1.5 (0.3)	$4x10^{-6}$	0.2
African American								
AP4E1	rs7176764	15	49045259	A	0.04	7.3 (1.5)	$2x10^{-6}$	0.4
LAMA4	rs6926485	6	112630302	T	0.64	-2.2 (0.5)	$3x10^{-6}$	0.4
ASH1L	rs11264369	1	153626454	T	0.72	2.5 (0.5)	$4x10^{-6}$	0.9
DCC	rs7236483	18	49367477	A	0.11	3.5 (0.8)	$4x10^{-6}$	0.6
Hispanic/Latino								
GALNT13	rs17553946	2	155055407	A	0.23	2.4 (0.5)	$9x10^{-7}$	0.6
NFKBIZ	rs1672383	3	103114652	T	0.80	-2.4 (0.5)	$3x10^{-6}$	0.7
SH3BGRL2	rs12208969	6	80370016	T	0.73	2.0 (0.4)	$4x10^{-6}$	0.2
SMARCA2	rs12339569	9	2117762	T	0.96	-4.7 (1.0)	$5x10^{-6}$	0.2
TGFBR2	rs7632716	3	30332431	T	0.83	-2.3 (0.5)	$5x10^{-6}$	0.6
Trans-Ethnic								
KCNIP4	rs12648787	4	21225700	C	0.67	-1.3 (0.2)	$3x10^{-7}$	0.6
SEL1L	rs17116425	14	81712421	A	0.89	-1.9 (0.4)	$1x10^{-6}$	0.8
ECEL1	rs2741279	2	233053235	T	0.49	-1.3 (0.3)	$1x10^{-6}$	0.3
LCA5	rs1485371	6	80235061	T	0.47	-1.1 (0.2)	$1x10^{-6}$	0.1
AP4E1	rs7176764	15	49045259	A	0.04	7.3 (1.5)	$2x10^{-6}$	0.4
IMPG1	rs6905415	6	76949519	A	0.09	2.0 (0.4)	$3x10^{-6}$	0.8
DPP10	rs9308717	2	116212012	A	0.48	1.1 (0.2)	$3x10^{-6}$	0.1

*Build 36 Base-Pair Position

CA, Coded allele, CAF, Coded allele frequency; Chr, Chromosome; *P*, *P*-value; P_{het}, *P*-value of heterogeneity; SE, Standard error; SNP, Single nucleotide polymorphism

Table 26. Loci with Suggestive Evidence Modifying the Effect Thiazide Diuretics on QRS and JT Intervals After Cross-Phenotype Meta-Analysis

Locus	SNP	Chr	Position[a]	CA	CAF	*P*	Univariate P-value QRS	Univariate P-value JT
European Americans								
PIK3R6	rs1295230	17	8682305	T	0.02	$3x10^{-6}$	0.008	0.001
African American								
ADGRB3	rs6931354	6	69527128	A	0.21	$1x10^{-7}$	0.005	0.0002
ADCY8	rs10108730	8	131767803	T	0.79	$2x10^{-6}$	$1x10^{-5}$	0.0003
PREX1	rs8119517	20	46464282	A	0.94	$3x10^{-6}$	0.0005	0.02
CDH13	rs11649358	16	81415652	A	0.75	$5x10^{-6}$	$9x10^{-6}$	0.001
Hispanic/Latino								
AK2	rs11591185	1	33274771	A	0.07	$2x10^{-6}$	$7x10^{-7}$	$3x10^{-5}$
ASS1P14	rs12578228	12	33030528	T	0.10	$2x10^{-6}$	$2x10^{-6}$	$2x10^{-5}$
GALNT13	rs17553946	2	155055407	A	0.23	$4x10^{-6}$	0.005	$9x10^{-7}$

[a]Build 36 Base-Pair Position

CA, Coded allele; CAF, Coded allele frequency; Chr, Chromosome; JT, JT interval; *P*, *P*-value; QRS, QRS interval; SE, Standard error; SNP, Single nucleotide polymorphism

Table 27. Results of Gene Enrichment Analysis with MAGMA for the Association with the Interactive Effect of Thiazide Diuretics on QRS Interval Among African Americans (N=11,482)

Gene	Chr	Start BP	Stop BP	N SNPs	P	FDR
CNTRL	9	122885395	122981207	69	$1x10^{-6}$	0.01
CPN1	10	101790555	101836632	33	$2x10^{-6}$	0.01
FAM65B	6	24946066	24990562	55	$2x10^{-6}$	0.01
RAB14	9	122978737	123008913	31	$5x10^{-6}$	0.02
ISY1	3	130329425	130367719	7	$1x10^{-5}$	0.03
NELL1	11	20642712	21555077	1227	$3x10^{-5}$	0.04

BP, Basepair; Chr, Chromosome; FDR, False Discovery Rate; N SNPs, Number of SNPs from Analysis within Gene Interval; P, P-value

Table 28. Gene-Sets with Enrichment for Genotype-Thiazide Interaction Effects Following Analysis with MAGMA

Trait	Population	Gene-Set	P	FDR
QT	Hispanic/Latino	Nucleotide Binding	$5x10^{-6}$	0.004
		Metal Ion Binding	$6x10^{-6}$	0.004
		tRNA Adenine-N1 Methyltransferase Activity	$6x10^{-5}$	0.03
		Transcription Coactivator Activity	$8x10^{-5}$	0.03
		Transcriptional Activity of SMAD2, SMAD3, SMAD4, Heterotrimer	0.0001	0.03
		Zinc Ion Binding	0.0002	0.04
		Other RNA Binding Protein	0.0002	0.04
		Insulin-like Growth Factor-2 mRNA Binding Proteins (IGF2BPS/IMPS/VICKZS)	0.0003	0.05
	Trans-Ethnic	General RNA Polymerase II Transcription	$4x10^{-6}$	0.006
		Transcription	$4x10^{-5}$	0.03
JT	African American	Transcription Factor TFIID Complex	$7x10^{-5}$	0.05
		Aminoacyl-tRNA Synthetase Multienzyme Complex	0.0001	0.05
		tRNA Aminoacylation for Protein Translation	0.0001	0.05
		Transcription Factor TFTC Complex	0.0001	0.05
	Trans-Ethnic	Transcription	$3x10^{-5}$	0.03
		General RNA Polymerase II Transcription Factor Activity	$4x10^{-5}$	0.03

FDR, False discovery rate; JT, JT interval; P, P-value; QT, QT interval

Figure 18. Quantile-Quantile Plots of P-values for Thiazide-SNP Interaction Estimates for QT Interval, QRS Interval, and JT Interval Analyses After Inverse-Variance Weighted Meta-Analysis in METAL

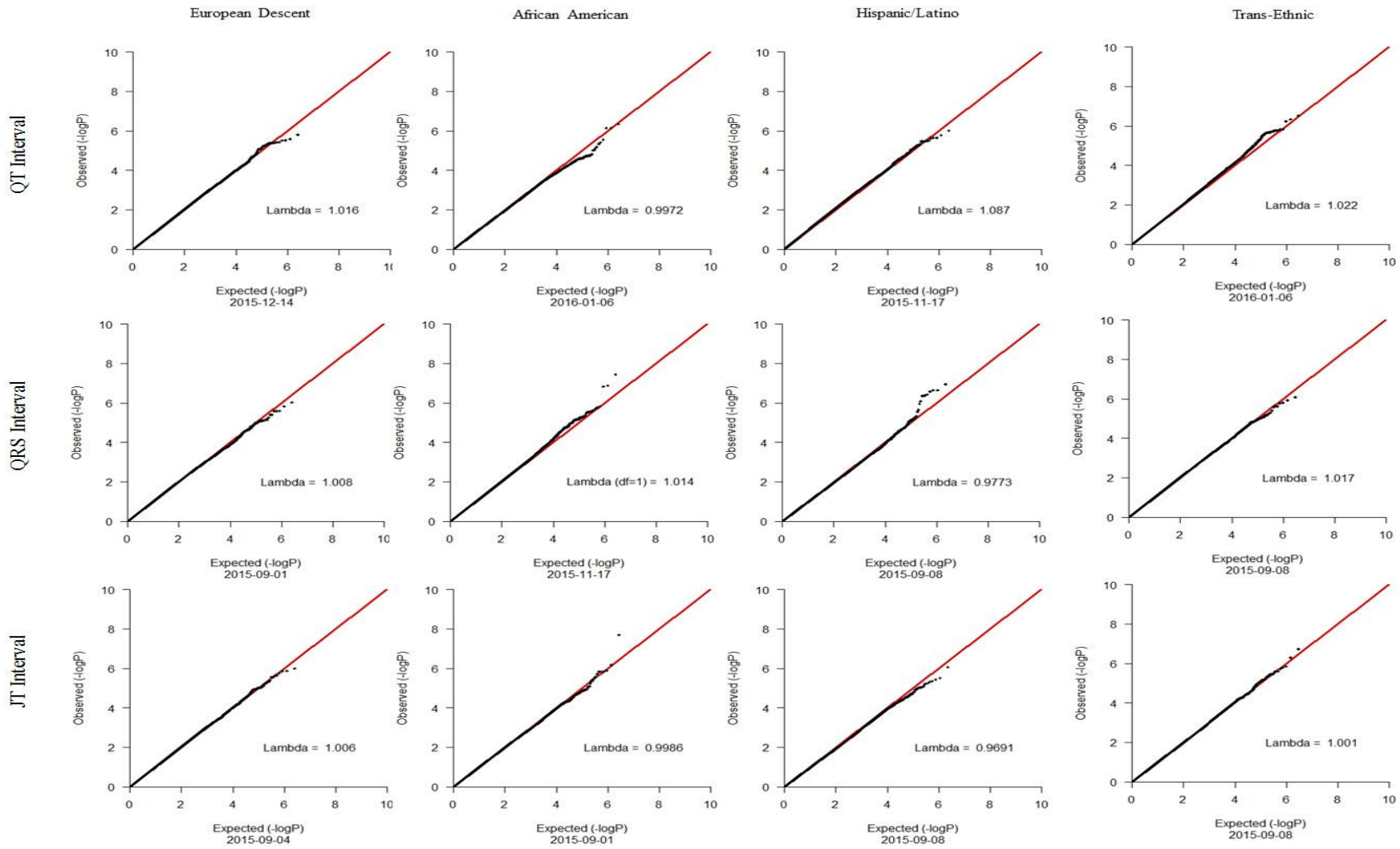
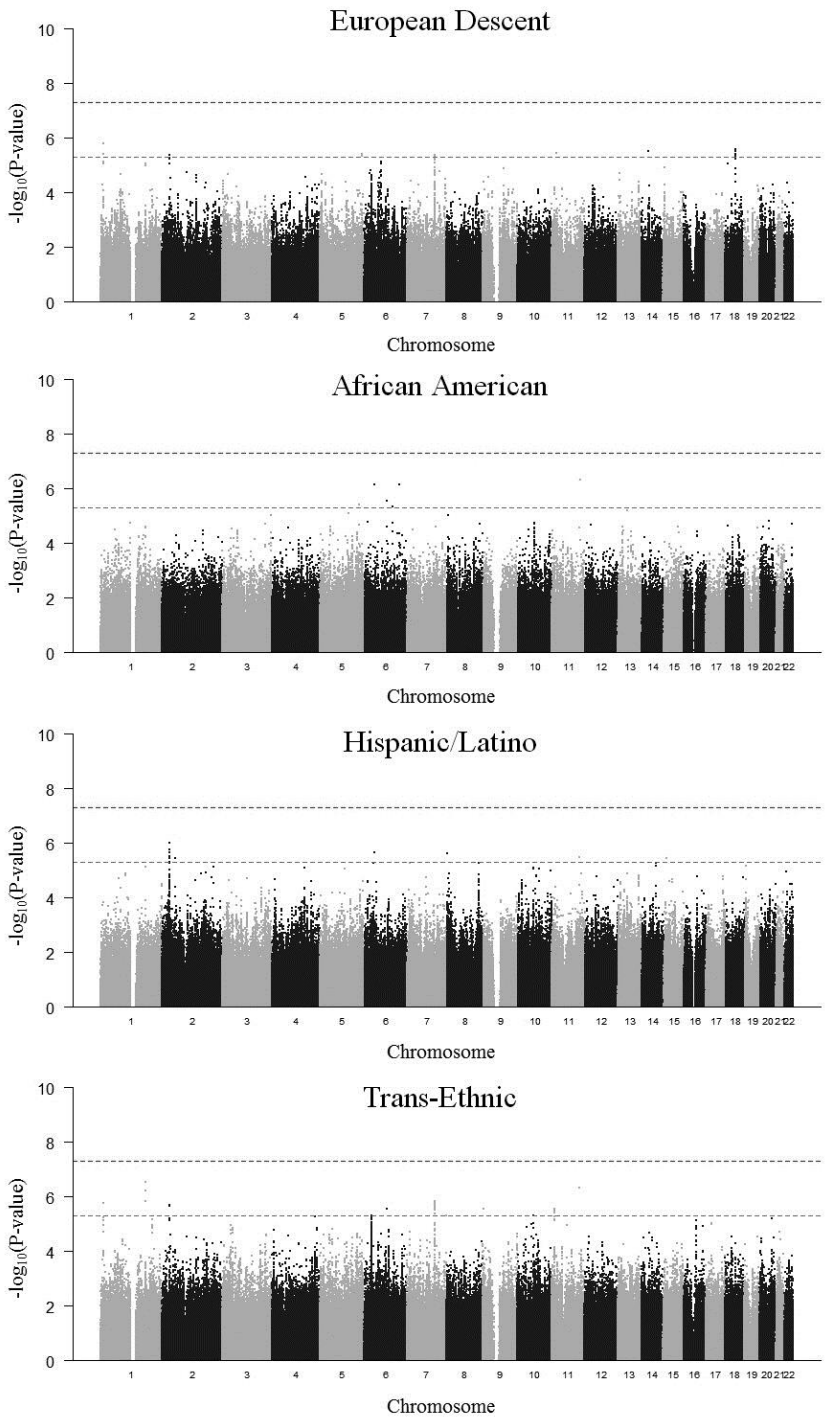
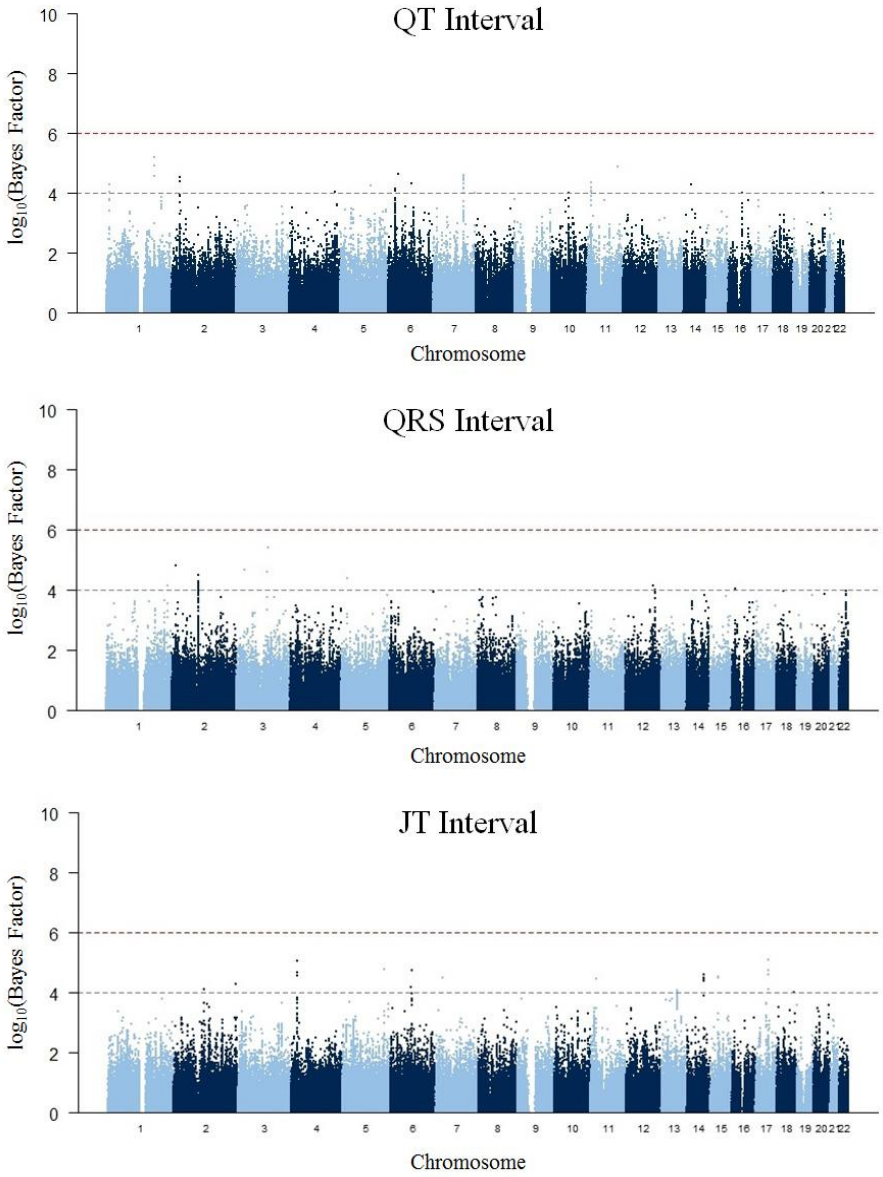


Figure 19. Manhattan plots of *P*-values for thiazide-SNP interaction estimates for QT interval analyses after fixed effects meta-analysis among European descent populations, African American populations, Hispanic/Latino populations, and all populations (trans-ethnic)



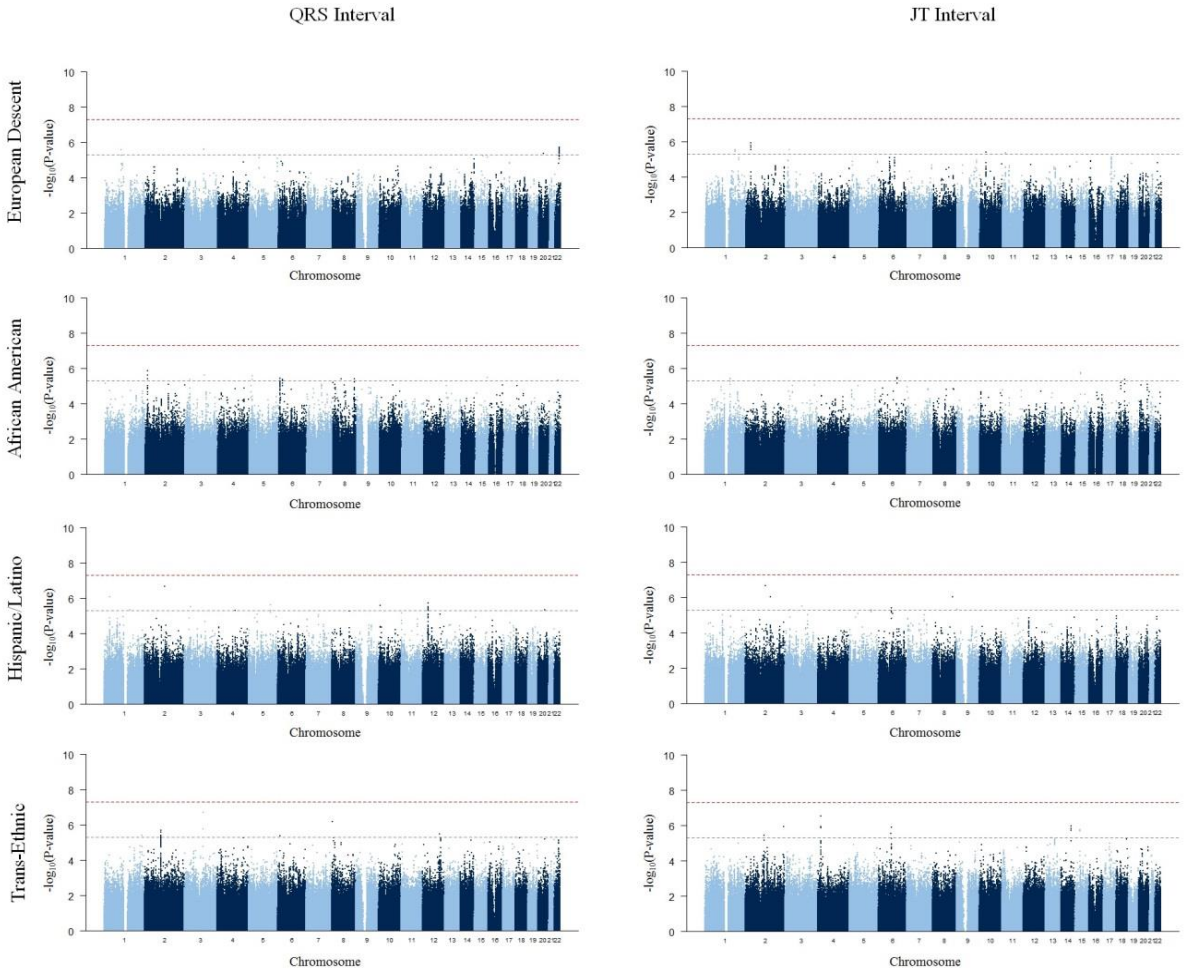
On each plot, genome wide significance ($P < 5x10^{-8}$) and suggestive significance ($P < 5x10^{-6}$) are denoted with dashed lines.

Figure 20. Manhattan Plots of Bayes Factors for Thiazide-SNP Interaction Estimates After Bayesian Trans-Ethnic Meta-Analysis in MANTRA Across European Descent, African American, and Hispanic/Latino Populations



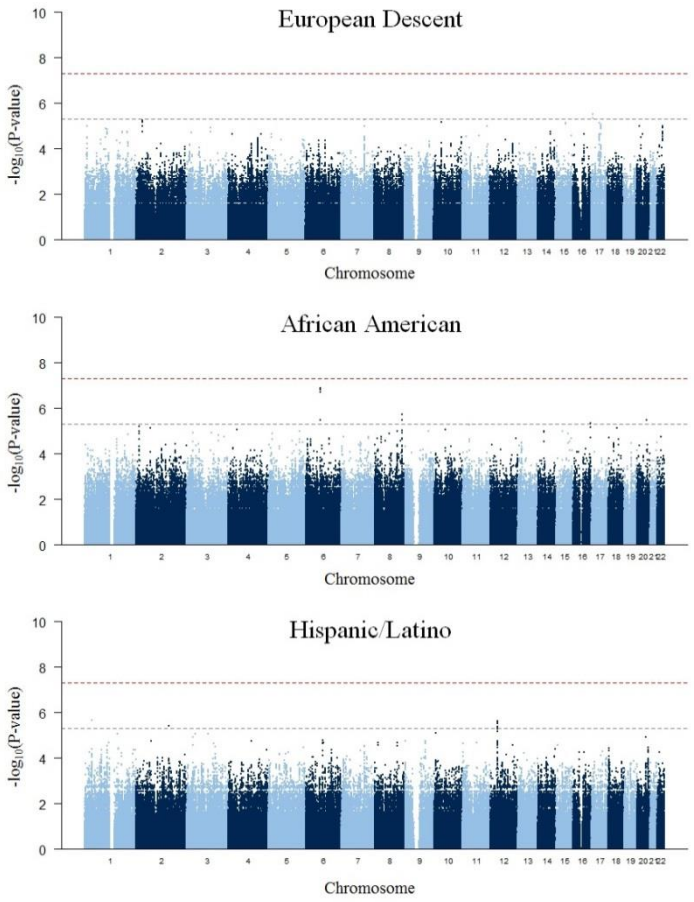
Manhattan plots of Bayes factors for thiazide-SNP interaction estimates after Bayesian trans-ethnic meta-analysis in MANTRA across European descent, African American, and Hispanic/Latino populations. On each plot, genome-wide significance (BF > 10^6) and suggestive significance (BF > 10^4) are denoted with lines.

Figure 21. Manhattan Plots of *P*-values for Thiazide-SNP Interaction Estimates for QRS, and JT Intervals



Manhattan plots of *P*-values for thiazide-SNP interaction estimates for QRS and JT interval analyses after fixed effects meta-analysis among European descent populations, African American populations, Hispanic/Latino populations, and all populations (trans-ethnic). On each plot, genome-wide significance ($P < 5x10^{-8}$) and suggestive significance ($P < 5x10^{-6}$) are denoted with dashed lines

Figure 22. Manhattan Plots of *P*-values for Thiazide-SNP Interaction Estimates After Cross-Phenotype Meta-Analysis (QRS Interval, JT Interval)



Manhattan plots of *P*-values thiazide-SNP interaction estimates after cross-phenotype meta-analysis (QRS interval, JT interval) using aSPU among European descent populations, African American populations, and Hispanic/Latino populations. On each plot, genome wide significance ($P < 5x10^{-8}$) and suggestive significance ($P < 5x10^{-6}$) are denoted with dashed lines.

Figure 23. Quantile-Quantile Plots of P-values for Thiazide-SNP Interaction Estimates After Cross-Phenotype Meta-Analysis with aSPU in European Descent, African American, and Hispanic/Latino Populations

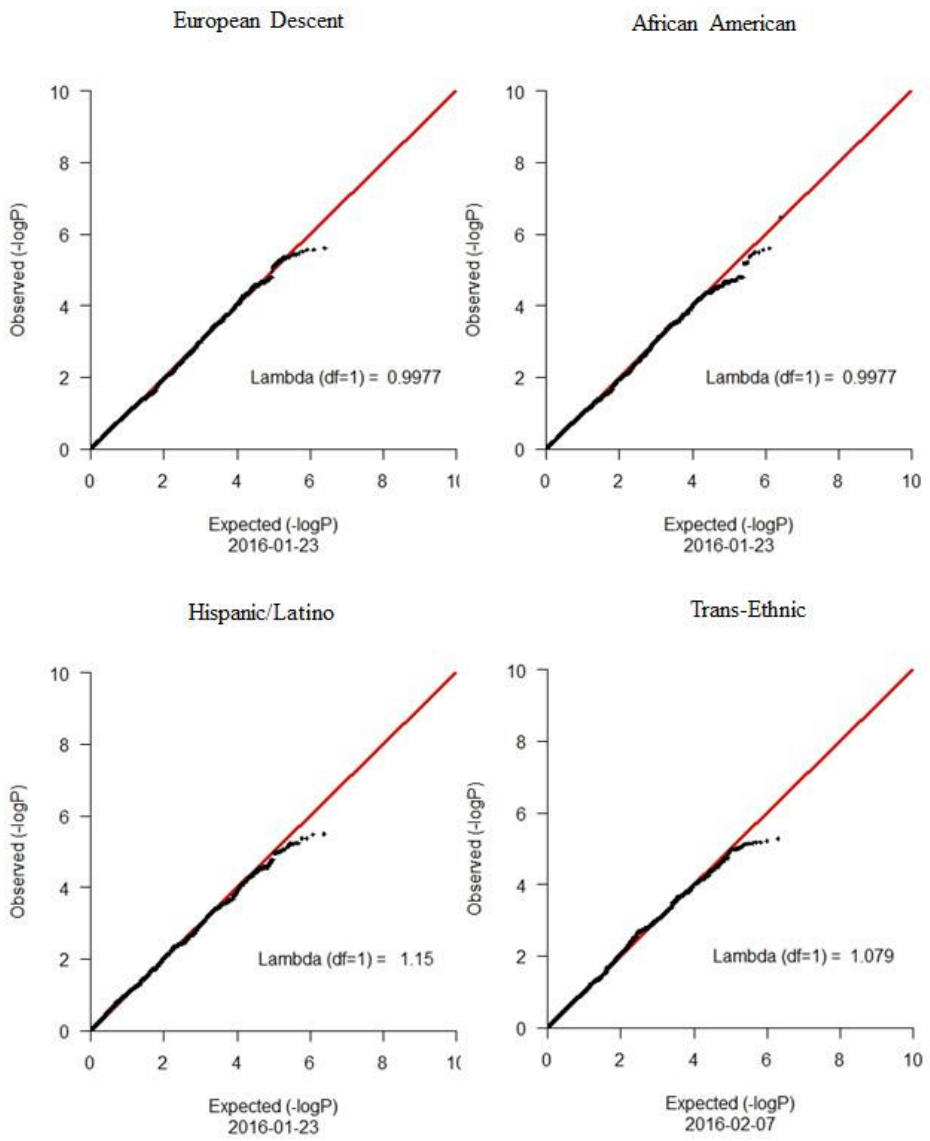
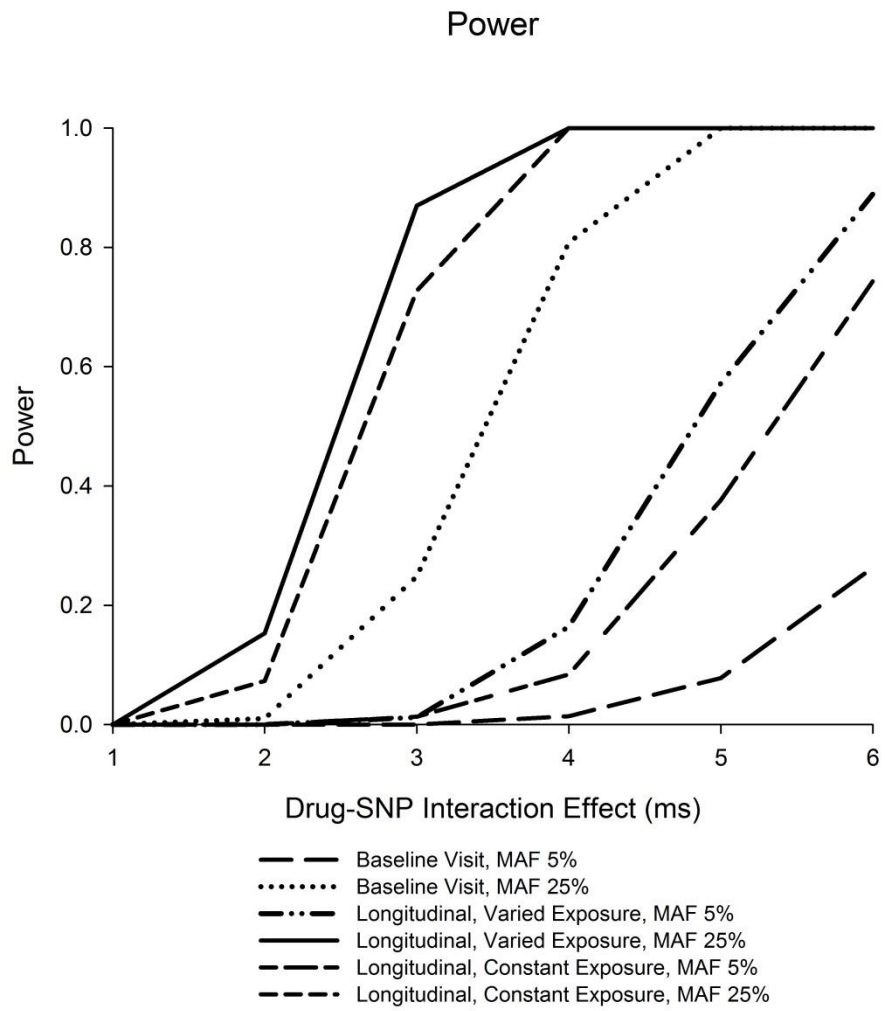


Figure 24. Statistical Power of a Simulated Pharmacogenomics Study of QT



The following assumptions were used for the calculations: 2 serial visits measuring electrocardiograms (ECGs) and drug exposure, N=50,000 participants, a single-nucleotide polymorphism (SNP) minor allele frequency (MAF) of 5% or 25%, and the $N_{exposed}$ = 8,100. Simulation analyses were run using only the baseline visit (cross-sectional) and a longitudinal model. Under the longitudinal model, simulations were run with all participants having constant drug exposure across visits or having varied drug exposure across visits.

Supplementary Material

Age, Gene/Environment Susceptibility – Reykjavik Study (AGES): The Reykjavik Study cohort originally was composed of a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967.[320] A total of 19,381 attended, resulting in 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow-up and was examined in all stages. Another group was designated a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES-Reykjavik study re-examined 5,764 survivors of the original cohort who had participated before in the Reykjavik Study.

Atherosclerosis Risk in Communities (ARIC) Study: The ARIC study is an ongoing population-based cohort of 15,792 predominantly Caucasian and African-American males and females aged 45-64 years at baseline and selected using probability sampling from four United States communities (Forsyth County NC, Jackson MS, suburban Minneapolis MN, and Washington County MD).[321] Participants were recruited in 1987-1989 to examine cardiovascular and pulmonary disease, patterns of medical care, and disease variation over time. Standardized physical examinations and interviewer-administered questionnaires were conducted at baseline (1987-1989), and at three triennial follow-up examinations (1990-1998). Eligible participants for this effort were from the NC, MN, and MD field centers, as only Caucasian participants were examined in this analysis and the MS center only recruited African American participants.

Cardiovascular Health Study (CHS): The CHS is a population-based cohort study of risk factors for CHD and stroke in adults ≥65 years conducted across four field centers.[322] The original predominantly Caucasian cohort of 5,201 persons was recruited in 1989-1990

from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African-American cohort of 687 persons was enrolled for a total sample of 5,888. DNA was extracted from blood samples drawn on all participants at their baseline examination in 1989-90. In 2007-2008, genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai using the Illumina 370CNV BeadChip system on 3,980 CHS participants who were free of CVD at baseline, consented to genetic testing, and had DNA available for genotyping.

Erasmus Rucphen Family (ERF) study: The Erasmus Rucphen Family study (http://www.erasmusmc.nl/klinische_genetica/research/genepi/?lang=en) is a family-based cohort embedded in the Genetic Research in Isolated Populations (GRIP) program in the southwest Netherlands. The aim of this program is to identify genetic risk factors for the development of complex disorders. In ERF, twenty-two families that had a minimum of six children baptized in the community church between 1850 and 1900 were identified with the help of detailed genealogical records. All living descendants of these couples, and their spouses, were invited to take part in the study. Comprehensive interviews, questionnaires, and examinations were completed at a research center in the area; approximately 3,200 individuals participated. Data collection started in June, 2002 and was completed in February, 2005. In the current analyses, 1503 participants for whom complete phenotypic, genotypic and genealogical information was available were studied.

Framingham Heart Study (FHS)**:** The FHS is a prospective, community based cohort study that was initiated in 1948 and now spans 3 generations, including the original cohort, their offspring and spouses of the offspring (Offspring Cohort, enrolment- beginning in 1971), and children from the largest offspring families (Generation 3 Cohort, enrolment

beginning in 2000). Details regarding study recruitment and design have been reported previously.[325, 391] Generation 3 cohort individuals with ECGs were used for this study. QT intervals were measured using digital calipers on scanned electrocardiograms in leads II (two cardiac cycles), V2 and V5; the average across all measurements was taken as the QT trait for analysis. All study protocols were approved by the Institutional Review Board for Boston University Medical Center. All study participants provided informed written consent.

Health, Aging, and Body Composition Study (Health ABC): The Health ABC Study is a NIA-sponsored cohort study of the factors that contribute to incident disability and the decline in function of healthy older persons, with a particular emphasis on changes in body composition in old age. Between 4/15/97 and 6/5/98 the Health ABC study has recruited 3,075 70-79 year old community-dwelling adults (41% African-American), who were initially free of mobility and activities of daily living disability. The key components of Health ABC include a baseline exam, annual follow-up clinical exams, and phone contacts every 6 months to identify major health events and document functional status between clinic visits. Provision has been made for banking of blood specimens and extracted DNA (Health ABC repository).

Health 2000: The Health 2000 Study is a population-based health examination survey carried out in Finland in 2000 – 2001.[329] A detailed description of the implementation and methodology of the survey is available online: http://www.terveys2000.fi/doc/methodologyrep.pdf . The study involved a two-stage stratified cluster sample representative of the whole adult Finnish population aged > 30 years. The Health 2000 sample comprised 8,028 individuals, of whom 79% (6,354 individuals; 2,876 men and 3,478 women) participated in a comprehensive health examination including

questionnaires, clinical measurements (e.g. resting ECG) and doctor's physical examination. DNA samples were collected from 6,597 persons and digital EGCs were available from 6,295 persons. QT-interval measurements in the Health 2000 Study have been described elsewhere.[329]

Hispanic Community Health Study / Study of Latinos (SOL): The Hispanic Community Health Study (HCHS)/Study of Latinos (SOL) is a community based cohort study of 16,415 self-identified Hispanic/Latino persons aged 18-74 years from randomly selected households in four U.S. field centers (Chicago, IL; Miami, FL; Bronx, NY; San Diego, CA) with baseline examination (2008 to 2011) and yearly telephone follow-up assessment for at least three years.[53] The two-stage sampling design selected households within census block groups. Households with Hispanic/Latino surnames and individuals over 45 years of age were oversampled to achieve increased representation of Hispanic/Latino individuals with a uniform age distribution. Due to this study design, sampling weights that reflect the probability of sampling individuals to the study were calculated for all individuals. These sampling weights were used in downstream analyses to protect against potential selection bias arising from the sampling scheme. The HCHS/SOL cohort includes participants who self-identified as having Hispanic/Latino background, the largest groups being Central American, Cuban, Dominican, Mexican, Puerto-Rican, and South American. The HCHS/SOL study was approved by institutional review boards at participating institutions, and written informed consent was obtained from all participants. 12,803 individuals were successfully genotyped on an Illumina Omni 2.5M array, and the genotype and phenotype data are posted on dbGaP (accession numbers phs000880.v1.p1 and phs000810.v1.p1)

Jackson Heart Study (JHS): The JHS is a single-site, prospective, population-based study designed to explore the environmental, behavioral, and genetic factors that influence the development of cardiovascular disease (CVD) among African Americans. A total of 5,301 women and men between the ages of 21 and 94 were recruited between September 2000 and May 2004 from a tri-county area of Mississippi: Hinds, Madison, and Rankin Counties. Participants were recruited from four sources, including (1) randomly sampled households from a commercial listing; (2) ARIC study participants; (3) a structured volunteer sample that was designed to mirror the eligible population; and (4) a nested family cohort. Of the enrolled participants, 3,630 were recruited uniquely to JHS and did not participate in ARIC. Overviews of the JHS including the sampling and recruitment, sociocultural, and laboratory methods have been described previously.[331] All of the participants provided written informed consent. Participants were between 35 and 84 years old at first visit, and members of the family cohort were ≥ 21 years old when consent for genetic testing was obtained and blood was drawn for DNA extraction. The details of first clinic visit procedures, including supine 12-lead digital electrocardiography (ECG), venipuncture, and other testing, have been previously described. The definitions of co-morbidities as well as the details of ECG measurements and medication collection and coding have also been reported.[24, 392]

Multi-Ethnic Study of Atherosclerosis (MESA): MESA is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. MESA researchers study a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. 38 percent of the recruited participants are white, 28 percent African-

American, 22 percent Hispanic, and 12 percent Asian, predominantly of Chinese descent.[332] Participants were recruited from six field centers across the United States. Two physical examinations (at baseline-1st and at 5th time points) were conducted since the electrocardiography was taken only at baseline and at 5th time points. The tenets of the Declaration of Helsinki were followed and institutional review board approval was granted at all MESA sites. Written informed consent was obtained from each participant.

The Netherlands Epidemiology of Obesity (NEO) study: The NEO study was designed for extensive phenotyping to investigate pathways that lead to obesity-related diseases. The NEO study is a population-based, prospective cohort study that includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. At baseline, information on demography, lifestyle, and medical history have been collected by questionnaires. In addition, samples of 24-h urine, fasting and postprandial blood plasma and serum, and DNA were collected. Genotyping was performed using the Illumina HumanCoreExome chip, which was subsequently imputed to the 1000 genome reference panel. Participants underwent an extensive physical examination, including anthropometry, electrocardiography, spirometry, and measurement of the carotid artery intima-media thickness by ultrasonography. In random subsamples of participants, magnetic resonance imaging of abdominal fat, pulse wave velocity of the aorta, heart, and brain, magnetic resonance spectroscopy of the liver, indirect calorimetry, dual energy X-ray absorptiometry, or accelerometry measurements were performed. The collection of data started in September 2008 and completed at the end of September 2012. Participants are currently being followed for the incidence of obesity-related diseases and mortality.

Prospective Study of Pravastatin in the Elderly at Risk (PROSPER): All data come from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the study has been published elsewhere.[334, 335] PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo. A large number of prospective tests were performed including Biobank tests and cognitive function measurements.

Rotterdam Study (RS): The RS is a prospective population based cohort study comprising 7,983 participants aged 55 years or older (RS1), which started in 1990. In 2000-2001, an additional 3,011 individuals aged 55 years or older were recruited (RS2). Furthermore, in 2006-2008, an additional 3,932 individuals aged 45 years or older were recruited (RS3).[393] At baseline, participants were interviewed at home and were examined at the research center, which included a 10 second, 12-lead electrocardiogram (ECG). Since then, participants are followed continuously and re-examined during several follow-up examination rounds. Medical information is available of all participants by collaboration with the general practitioners and with the pharmacies in the area of Ommoord. The Rotterdam Study has been approved by the medical ethics committee according to the "Wet Bevolkingsonderzoek: ERGO" (Population Study Act Rotterdam Study), executed by the

Ministry of Health, Welfare and Sports of the Netherlands and written informed consent was obtained from all study participants.

Women's Health Initiative Clinical Trials (WHI CT): The WHI is a long-term national health study focused on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. Between 1993 and 1998, it randomized 68,132 women aged 50-79 years into one or more clinical trials of hormone therapy, dietary modification, or calcium/vitamin D supplementation.[52] In this context, white WHI CT women were controls drawn from the Genome-wide Association Research Network into Effects of Treatment (GARNET),[394] controls drawn from the Modification of PM-Mediated Arrhythmogenesis in Populations (MOPMAP),[395] or participants in the Women's Health Initiative Memory Study (WHIMS) .[396] Black and Hispanic WHI CT women were participants in the single nucleotide polymorphism (SNP) Health Association Resource project (SHARe).[397]

CHAPTER 6: RESEARCH PAPER 2-EFFECT OF PREVALENT USER BIAS AND EXPOSURE MISCLASSIFICATION ON PHARMACOGENOMICS STUDIES CONDUCTED IN OBSERVATIONAL COHORT SETTINGS: A SIMULATION STUDY[2]

Introduction

Drug response and efficacy demonstrate high intra- and inter-individual variability, posing a significant problem for effective treatment,[2-4] with adverse drug reactions (ADRs) accounting for approximately 100,000 deaths and 2.2 million serious health effects annually.[5] Genetics may influence variability in drug response by affecting pharmacokinetic and pharmacodynamics pathways.[61] For example, single nucleotide polymorphisms (SNPs) in *CYP2C9* and *VKORC1* explain up to 50% of the variability in warfarin response,[259] and *HLA-B* modifies the risk of toxic side effects of carbamazepine[7, 260] and abacavir.[261] Given that more than half of all American adults take at least one prescription medication, it is critical to understand the genetics of drug response across broad, diverse populations.[1]

Despite the public health and clinical significance of research evaluating the genetic bias of drug response, very few studies have evaluated the merits of alternative pharmacogenomics study designs. Efforts examining pharmacogenomics study designs are warranted because, while pharmaco*epidemiologic* studies are subject to a multitude of biases, including selection bias and confounding by indication,[283] it remains unclear if pharmaco*genomics* studies of gene-drug interactions are similarly susceptible. Indeed, pharmacogenomics studies represent a specific subset of pharmacoepidemiologic studies that

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incorporate a third parameter, the SNP. Unlike other environmental modifiers, an individual's genotype is assigned at conception and therefore not affected by subsequent exposures, which may make SNP interaction effects less susceptible to the same biases affecting other modifiers.[287, 288] For example, previous work examining bias from confounding by contraindication in observational pharmacogenomics settings demonstrated that the degree of bias varied by study design.[47]

Yet, no studies have yet examined how other common threats to the validity of pharmacoepidemiologic studies affect pharmacogenomics studies conducted in observational settings, despite the emerging use of observational pharmacogenomics studies that offer large sample sizes, a diverse range of phenotypes, numerous medication exposures, and improved external validity when compared to clinical trials.[284] For example, no study to the best of our knowledge has evaluated the degree to which pharmacogenomics studies are subject to prevalent user bias, whereby the cohort is enriched for prevalent long-term drug users who are less likely to have experienced an ADR when compared to new users.[284, 285] The influence of drug misclassification remains similarly understudied in pharmacogenomics studies.[398-400] We therefore conducted a series of simulations examining the influence of prevalent user bias and drug misclassification on pharmacogenomics studies by evaluating three observational designs (longitudinal, cross-sectional, and new user), two control groups (whole cohort and active comparator), and two scenarios (extreme and modest drug effects). Results of this study will help guide the design of future pharmacogenomics studies conducted in observational settings.

Materials and Methods

A. Simulation Overview

We simulated a population with four study visits using QT interval (QT), a heritable measure of ventricular depolarization and repolarization,[24-28] and QT-prolonging drug use as our pharmacogenomic model. QT prolongation, a risk factor for highly fatal ventricular arrhythmias,[68, 163] is most commonly caused by prescription drugs.[133]

We simulated four time points (visits 0–3 [V0–V3], Figure 25), as informed by a literature review. V0 represented a wash-out period where the prevalence of drug use was 0%. Published clinical and genome-wide association studies (GWAS),[209] along with empirical data from the Atherosclerosis Risk in Communities (ARIC) Study,[321] informed assigned parameter estimates. For the QT-prolonging medication we simulated a thiazide diuretic, a commonly used anti-hypertensive agent with previously reported QT-prolonging effects,[146] which is henceforth referenced to as the index drug. Participants with treated hypertension who were not treated by the index drug were assigned treatment on a comparator hypertension medication with no QT-prolonging effects. In addition to these time-varying parameters measured at each visit, age, sex, diabetes status, and SNP genotype were simulated at V0, the latter we simulated as the causal SNP (i.e. not in linkage disequilibrium with the unobserved causal SNP). Diabetes status at V0 was used as a determinant of index or comparator drug use among those with treated hypertension, where those with diabetes were more likely to use the comparator drug (Figure 26).

To introduce prevalent user bias, whereby the cohort was enriched for long-term drug users, we simulated an ADR, which could occur between V1 – V3 (i.e. a maximum of two times). For participants on the index drug, the probability of having an ADR was affected by QT at the previous visit. The probability of remaining on the index drug at the subsequent

visit was then affected by the probability of an ADR (Figure 27). Having an ADR also increased the probability that a participant was lost to follow-up. Correlation between serial QT measurements was simulated as a function of unmeasured covariates (i.e. 'U', which represents heart rate, congenital heart disease, other medication use, other SNPs, hypokalemia, myocardial infarction, etc.).[68]

B. Simulation Parameters and Values

We simulated a population size of 120,000, the sample size in a cross-sectional pharmacogenomics study necessary to achieve 80% power to detect a 1.6 ms drug-SNP interaction on QT (minor allele frequency [MAF] = 25%; 17% drug prevalence; 20 ms standard deviation [SD]), an effect estimate similar in magnitude to published SNP main effects on QT.[209] In sensitivity analyses we increased the simulated population size to a maximum of 300,000 participants.

Age was simulated using a normal distribution with a mean (54.4 years) and SD (5.7 years) equal to that observed at the ARIC baseline visit among white participants. Sex was simulated as a uniform random variable with a defined probability of being male (47.3%). A uniform distribution also was used to simulated SNP genotype according to a prespecified MAF (MAF=0.05, 0.25, and 0.45). Participant genotype was calculated under the assumption of Hardy-Weinberg equilibrium. Diabetes was predicted conditional on age using the logit function:

$$Logit\big(pr(Diabetes = 1)\big) = \alpha_0 + \alpha_1 Age + \varepsilon$$

Unknown/unmeasured confounding (U) was simulated using a normal distribution with a mean (0) and SD (20) to approximate the correlation between serial visits observed in ARIC (r =0.75).

Hypertension status at each visit was predicted conditional on age and sex and, for V1-V3, hypertension status as the previous visit, using the logit function:

$$Logit\big(pr(HTN_i = 1)\big) = \alpha_0 + \alpha_1 Age + \alpha_2 Sex + \alpha_3 HTN_{i-1|i\geq 1} + \varepsilon$$

The probability of having treated hypertension at V1 – V3 was randomly simulated among participants with hypertension with a defined probability of treatment (91%). The prevalence of the index drug at V1 was assigned at 17%. Among participants with treated hypertension at V1 – V3, drug treatment (index or comparator) was predicted conditional on diabetes status and, for V2 – V3, drug use at the previous visit and occurrence of an ADR between visits as:

$$Logit\big(pr(Drug_i = 1)\big) = \alpha_0 + \alpha_1 Diabetes + \alpha_2 Drug_{i-1|i\geq 2} + \alpha_3 ADR_{i|i\geq 2} + \varepsilon$$

The probability of using the comparator medication was modeled as 1 – pr(Drug=1).

Previous work has suggested the potential for measurement error in medication assessment conducted in observational settings.[57] We therefore simulated an alternate scenario assuming a reduced sensitivity (97%) and specificity (79%) in the ascertainment of the index drug.[57]

QT was simulated as a linear function of the SNP, age, sex, U, hypertension, diabetes, drug treatment and the interaction between SNP and drug treatment:

$$QT = \beta_0 + \beta_1 Drug + \beta_2 SNP + \beta_3 Drug \times SNP + \beta_4 Age + \beta_5 Sex + \beta_6 U + \beta_7 HTN + \beta_8 Diabetes + \varepsilon$$

where mean QT was simulated as 389 ms when all other variables equaled 0. A one-year increase in age, male sex, one unit increase in U, hypertension status and diabetes status were associated with 0.39, -3.84, 2.00, 4.50, and -4.90 ms changes in QT, respectively (Table 29).

The occurrence of an ADR between visits among those on drug treatment was predicted using a logit model conditional on QT > 450 ms (QT_{Long}) at the previous visit:

$$Logit\big(pr(ADR_i = 1)\big) = \alpha_0 + \alpha_1 QT_{Long,i-1} + \varepsilon$$

Loss-to-follow-up was predicted using a logit model conditional on age and the occurrence of an ADR between visits:

$$Logit\big(pr(Loss_i = 1)\big) = \alpha_0 + \alpha_1 Age + \alpha_2 ADR_i + \varepsilon$$

Simulations assumed both extreme index drug effects on QT (i.e. the index drug prolonged QT by 30 ms, termed the extreme scenario, Table 29) and modest index drug effects on QT (i.e. the index drug prolonged QT by 5 ms, termed the modest scenario).[148] Simulations also were performed varying the SNP main effect on QT (range: 0–10 ms), the drug-SNP interactive effect on QT (range: -6–6 ms), the log-odds of drug continuation given an ADR (range: -50–0), the effect of drug treatment on QT (range: 0–30 ms), and the log-odds of an ADR given prolonged QT (range: 0–50). A total of 10,000 iterations were simulated for each setting across which results were averaged. All analyses were performed using the statistical programming package SAS (Cary, North Carolina, USA).

C. Analysis of Drug-SNP Interactions

We used our simulations to contrast 12 settings that evaluated in combination three study designs (new user, longitudinal, and cross-sectional), two control groups (whole cohort and active comparator), and two scenarios (extreme and modest drug effects). The new user design was restricted to V1, where all participants on drug treatment were new initiators (Figure 25). The longitudinal design included V2 and V3 and the cross-sectional design was restricted to V3 (Figure 25). In each design, simulations were run for both the whole cohort and the active comparator control groups, the control group for the latter being restricted to

participants on the comparator treatment. Finally, we examined two scenarios, the extreme scenario (index drugs with QT-prolonging effects of 30 ms) and modest (index drugs with QT-prolonging effects of 5 ms), representing 12 total settings.

In the new user and cross-sectional designs, the SNP-drug interaction effect was estimated using linear regression with QT_i as the dependent variable and the SNP, drug, SNP-drug interaction, age, sex, diabetes, and HTN_i as independent variables, as follows:

$$QT = \beta_0 + \beta_1 Drug_i + \beta_2 SNP_i + \beta_3 Drug_i \times SNP_i + \beta_4 Age_i + \beta_5 Sex_i + \beta_6 HTN_i + \beta_7 Diabetes_i$$

where β_3 represents the SNP-drug interaction effect. For the longitudinal designs, the SNP-drug interaction effect was estimated using generalized estimating equations with an independence working correlation[346] as follows:

$$E[QT_{ij}] = \beta_0 + \beta_1 Drug_{ij} + \beta_2 SNP_i + \beta_3 Drug_{ij} \times SNP_i + \beta_4 Age_i + \beta_5 Sex_i + \beta_6 HTN_{ij} + \beta_7 Diabetes_i$$

where QT_{ij} is our outcome for the ith participant at the jth timepoint conditional on the SNP, drug, SNP-drug interaction, age, sex, diabetes, and HTN_i and β_3 represents the SNP-drug interaction effect. Our simulations used a genome-wide α level ($5x10^{-8}$).

Results

The mean (SD) of QT was 409 ms (26 ms) at V0 for both the modest and extreme scenarios and increased to 410 (26) ms and 414 (29) ms at V1 for the modest and extreme scenarios, respectively (Table 30). Exposure to the index drug increased across visits under the modest scenario, as participants initiated drug use and few discontinued due to ADRs (1.5% of those exposed at V2 and 1.4% of those exposed at V3 discontinued) or switched to the comparator drug (6-7% across V1-V3). As expected, index drug exposure was lower at

V2 and V3 in the extreme versus modest scenario (17% at V2 and V3 in the extreme scenario), as a larger proportion of drug users were susceptible to an ADR and subsequently discontinued drug use (e.g. 33% index drug users before V3; 25% index drug users before V4) or were switched to the comparator drug (7-9% across V1-V3).

We first tested the performance of our models by examining bias in the index drug effect on QT, expecting minimal bias in the new user design.[401] Our simulations demonstrated negligible bias in the drug effect using the new user design both when a 5 ms (modest scenario) and a 30 ms (extreme scenario) drug effect were simulated, suggesting that our models performed as expected (Figure 28).

A. Simulations with Drug-SNP Interaction = 0 and Varied SNP Main Effect

We then evaluated the influence of prevalent user bias by contrasting the performance of the three study designs, two control groups, and two scenarios in the absence of a simulated drug-SNP interaction (i.e. drug-SNP interaction=0) but in the presence of a SNP main effect (range: 0 to 10 ms), which represented the effect of the SNP on QT independent of its interactive effect with the index drug (Figure 28-31). Under the modest scenario, all estimates were minimally biased across simulated SNP main effects (mean bias range: -0.02 ms to -0.06 ms, Figure 29A). Under the extreme scenario, the new user designs remained unbiased for both control groups (Figure 29B). However, bias was observed among the remaining cohorts in the extreme scenario, particularly for the active comparator control group (maximum bias at simulated SNP main effect=10 ms: longitudinal active comparator = 1.34 ms; cross-sectional active comparator = 1.14 ms). Simulated results with MAF of 5% and 45% showed similar patterns of bias (Figure 30, Figure 31).

Under both the modest and extreme scenarios, estimates of the false-positive proportion (FPP) for the drug-SNP interaction effect remained under 5% for all study settings

except the longitudinal active comparator setting under the extreme scenario (Figure 29C-D), for which the FPP ranged from 5% at a SNP main effect of 2 ms to 46% at a SNP main effect of 10 ms.

B. Simulations with SNP Main Effect = 0 and Varied Drug-SNP Interaction Effect

We next evaluated the performance of the12 settings in the presence of a simulated drug-SNP effect (range: -6 ms–6 ms), but the absence of a SNP main effect (i.e. SNP main effect = 0). As with the previous results, the modest scenario showed negligible bias in the drug-SNP interaction effect across all designs and control groups (maximum bias = -0.09 ms, Figure 32A). However, under the extreme scenario, while the new user design remained unbiased, the whole cohort and active comparator control groups in the context of the longitudinal or cross-sectional designs demonstrated opposite patterns of bias, with the magnitude of bias being approximately 120% greater in the active comparator compared to whole cohort control groups (e.g. 0.77 vs 0.25 ms at a simulated drug-SNP interaction effect of 6ms; Figure 32B). Patterns of bias remained the same when MAF was varied (Figure 33).

Despite greater bias in the extreme scenario, power to detect the drug-SNP interaction effect was similar under both scenarios (Figure 32C-D). For example, power to detect the drug-SNP interaction effect reached 80% at drug-SNP interaction effects of 2 or 3 ms for all study settings except the new user or cross-sectional active comparator settings. For the new user active comparator setting, power exceeded 80% at a drug-SNP interaction effect of 6 ms. Power for the cross-sectional active comparator setting never exceeded 50% for the modest scenario but reached 80% at 6 ms for the extreme scenario. For MAFs of 5%, power was best for the longitudinal whole cohort setting but only exceeded 80% when a drug-SNP interaction effect of 4 ms was simulated. Power to detect drug-SNP interaction effects ranging from -6 to 6 ms remained below 80% in the new user or cross-sectional active

comparator setting when MAF was 5% under both scenarios (Figure 34). As expected, power was improved when evaluating SNPs with MAF of 45%, particularly for the active comparator control groups.

C. Simulations with Varied Drug and ADR Effects

To test how medications with different degrees of QT-prolongation and the probability of ADRs effected bias and power, we then varied the effect of the drug on QT (0–30 ms), the proportion of participants with a prolonged QT (QT>450 ms) who experienced an ADR (log-odds of an ADR given prolonged QT: 0–50), and the probability of medication discontinuation given an ADR (log-odds of medication continuation given an ADR: -50–0). For these simulations we evaluated a SNP main effect of 0 ms and a drug-SNP interaction effect of 5 ms. There was no bias in the drug-SNP interaction effect for new user designs under any of the three scenarios examined (Figure 35). However, as the simulated effect of the drug on QT increased, bias in the drug-SNP interaction effect was observed for the longitudinal and cross-sectional designs, which varied based on the control group and simulated drug effect (longitudinal whole cohort bias range = -0.53– -0.24 ms; longitudinal active comparator = -0.16– 0.62 ms, Figure 35A).

Varying the log-odds of an ADR following QT prolongation or the log-odds of medication discontinuation given an ADR demonstrated a distinct pattern of bias. When the log-odds of an ADR given QT prolongation was small bias in the drug-SNP interaction was negligible (Figure 35B). However, once the log-odds of having an ADR given QT prolongation exceeded 10, corresponding to an ADR probability of 60%, the bias above (range: 0.54–0.62 ms in active comparator control groups) and below (range: -0.24– -0.14 ms in whole cohort control groups) in the drug-SNP interaction effect was observed for all designs except the new user design. The bias also remained the same across increasing log-

odds of an ADR (>10). When the log-odds of medication continuation was varied, we observed an opposite pattern of bias (i.e. bias[range above: 0.54–0.62 ms in active comparator control groups; range below: -0.24– -0.14 ms in whole cohort control groups] increased across decreasing log-odds of drug continuation, but remained uniform after a simulation log-odds of -10; Figure 35C).

D. Simulations with Reduced Specificity and Sensitivity

Because medication use may be misclassified,[57] we evaluated the influence of reduced sensitivity (97%) and specificity (79%) of medication use assessment. Reduced sensitivity and specificity were associated with a considerable increase in bias (Figure 36) compared to perfect medication assessment (Figure 32). For example, in both the extreme and modest scenarios, reduced sensitivity and specificity biased estimated drug-SNP interaction effects toward the null by as much as 59% (e.g. true effect size=6 ms; estimated effect size in the presence of drug measurement error=2.49 ms in longitudinal whole cohort setting under extreme scenario).

Power to detect the drug-SNP interaction effect also was expectedly reduced. Under the extreme scenario, perfect sensitivity and specificity resulted in 80% power to detect the drug-SNP interaction effect between 2 and 6 ms, depending on study setting (Figure 32D). However, reduced sensitivity and specificity lowered the power so that 80% power was not achieved until 3 ms in the longitudinal designs and 4 ms in the new user and cross-sectional whole cohort settings (Figure 36D). The new user cross-sectional active comparator settings did not achieve 80% power under any tested drug-SNP interaction effect tested here (range: -6 to 6 ms). The modest scenario demonstrated similar power to detect interaction effects as the extreme scenario (Figure 36C).

E. Power Across Varying Sample Sizes

Lastly, given the reduced power when simulations were extended to accommodate reduced sensitivity and specificity, we evaluated the population size needed to achieve 80% power to detect a 2 ms drug-SNP interaction (Figure 37), power estimates consistent with study designs that assumed perfect sensitivity and specificity. With reduced sensitivity and specificity, the smallest sample size that achieved 80% power to detect a 2 ms drug-SNP interaction was 150,000 for the longitudinal whole cohort setting. For the cross-sectional design, the smallest sample size that achieved 80% power to detect a 2 ms drug-SNP interaction was 260,000 (whole cohort control group). For the new user designs, power to detect a 2 ms drug-SNP interaction never exceeded 70% despite a sample size of 300,000 participants.

Discussion

In these simulations, we examined the influence of prevalent user bias and exposure misclassification in pharmacogenomic studies conducted in observational cohort settings by contrasting three designs (new user, cross-sectional, and longitudinal), two control groups (whole cohort and active comparator) and two scenarios (modes and extreme drug effects on QT). Our simulations identified settings where prevalent user bias caused moderate bias on the drug-SNP interaction effect. Yet, the greatest bias, as well as the largest reductions in power were detected when simulations were extended to examine exposure misclassification. Given that the amount of bias and potential for reduced power varied by the design, control group, and the strength of the drug-induced QT prolongation, these results have broad implications for pharmacogenomics studies conducted in observational settings.

To date, numerous pharmacogenomics GWAS with likely insufficient power have been published, indicating the difficulty in obtaining sample sizes sufficient to detect

interactions at stringent GWAS significance thresholds.[282, 402-404] Indeed, such "disappointing" results have prompted the speculation that with noted exceptions,[7, 259-261] there might be fewer instances of large and clinically significant pharmacogenomics effects than previously expected.[282, 403, 405] Yet, our results show that even at sample sizes four times larger than QT pharmacogenomics studies published to date,[282] the influence of exposure misclassification makes it difficult to rule-out clinically significant results, generally defined as interactions with effects of 5 ms or greater.[44] Further, the influence of other sources of measurement error (e.g. outcome measurement error) and the inability to accommodate other important covariates like medication dose in most observational cohort studies likely reduced power further. Indeed, a strength of pharmacogenomics studies of QT is the degree to which QT is reliably measured.[74, 406] However, other phenotypes of interest (e.g. blood pressure and glycemic traits) show greater variability in measurement, which warrants evaluation in future efforts.[407-409]

Given the massive statistical testing penalty of genome-wide association studies and the potential for bias in study designs that optimize power (i.e. longitudinal), it is tempting to consider hypothesis-based genomic analyses such as a candidate gene studies as an alternative to genome-wide analyses. There has been some success in identifying genetic variants in genes associated with congenital long QT syndrome that modify drug-induced QT-prolongation.[410] However, efforts evaluating loci from main effects QT GWAS as candidate genes in pharmacogenomic studies of QT-prolonging drugs have not yielded positive results.[282] The lack of positive results is not unique to drug-induced QT-prolongation. Efforts by Bis *et al*. to identify genetic variants that modified the association between antihypertensives and cardiovascular disease in genes previously associated with

coronary heart disease were similarly unsuccessful.[403] Lessons from main effects analyses have also demonstrated a higher success of GWAS over candidate gene studies, where few biologically motivated association studies were consistently replicated[411-413] while GWAS have successfully replicated thousands of associations across populations.[414, 415]

Although drug misclassification had the greatest influence on power and bias, prevalent user bias also exerted non-negligible effects, which varied by the magnitude of the drug-outcome association. Our simulations suggest that, even with exposure misclassification, longitudinal designs optimize the power to detect effects in discovery analyses over the other designs considered here but also demonstrated the largest potential for bias when the drug-outcome association was large. Iribarren *et al*. identified drugs with QT-prolonging effects greater than 25 ms,[148] five times the standard for regulation by the U.S. Food and Drug Administration (FDA).[44] These results demonstrate the necessity of considering bias from extreme drug effects not only in studies of QT, but also in studies of other drugs with large effects on the outcome, including pharmacogenomics studies of statins and low-density lipoprotein levels[416] or thiazides and antihypertensive response.[272-274] In the context of large drug effects on the outcome, our results suggest the use of a longitudinal design with a whole cohort control group to increase power to detect drug-SNP interaction effects but acknowledging the potential for a drug-SNP interaction estimate moderately biased toward the null. Our simulations suggested that when the drug had a modest effect on the outcome (i.e. indapamide, a thiazide-like diuretic that prolongs QT by under 10 ms),[148] bias in the drug-SNP effect was minimal but drugs with stronger effects or studies with exposure misclassification can result in substantial bias in the drug-SNP interaction effect with up to half the true effect lost to bias.

It is important to note that the conclusions of this study are limited by the scope of the simulations presented herein. For example, our choice of active comparator encompassed any antihypertensive medication other than a thiazide diuretic, which was modeled as our drug of interest. Thus, if a participant had treated hypertension but had an ADR, the participant was automatically changed to the active comparator control group. Our simulations therefore do not indicate how prevalent user bias would affect an active comparator control group that was chosen from a different group of medications that had a different indication of treatment from the drug of interest. Furthermore, we only investigated a single scenario of medication misclassification. Given the strong effects reduced specificity and sensitivity had on prevalent user bias, future work examining how different levels of specificity and sensitivity affect prevalent user bias as well as other biases potentially affecting pharmacogenomic studies, such as confounding by contraindication, is warranted.

In conclusion, our simulations suggested that when medication is well assessed and the underlying drug effect on the outcome is modest, prevalent user bias may be negligible. However, prevalent user bias scales with increasing drug effects, although the bias had only moderate effects on study power. The most striking effects were estimated for exposure misclassification, which caused sizable bias in the drug-SNP interaction and large reductions in power. Researchers must carefully weigh different sources of bias and misclassification against power considerations when designing their pharmacogenomic analyses in order to optimize efforts to identify and characterize SNPs that modify drug-outcome associations.

Tables and Figures

Table 29. Parameter Values for the Extreme, Modest, and Alternative Scenarios

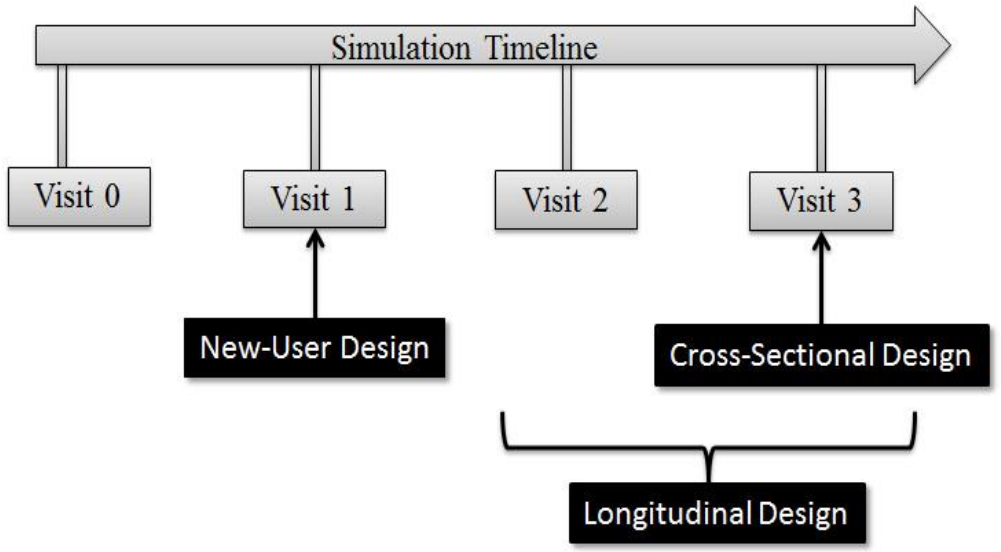
			Parameter Values		
Variable	**Parameter**	**Meaning**	**Extreme Scenario**	**Modest Scenario**	**Alternative Scenarios**
Diabetes	α_0	Value chosen for 13% prevalence of diabetes	-6.00	-6.00	--
	α_{Age}	Log odds of diabetes for 1-year increase in age	0.075	0.075	--
Hypertension	α_0	Value chosen for 33% prevalence of HTN	-5.25	-5.25	--
	α_{Age}	Log odds of hypertension for 1-year increase in age	0.08	0.08	--
	α_{Sex}	Log odds of hypertension for men versus women	0.16	0.16	--
	α_{HTN}	Log odds of hypertension for those with previous hypertension versus those without	-4.74	-4.74	--
Drug Treatment	α_0	Value chosen for 17% prevalence of drug treatment	1.55	1.55	--
	α_{Diab}	Log odds of drug treatment for those with diabetes versus those without	-90	-90	--
	α_{Drug}	Log odds of drug treatment for those previously on treatment versus those not previously on treatment	4.80	4.80	--
	α_{ADR}	Log odds of drug treatment for those who suffered an ADR versus those who did not	-50	-3.75	-50 – 0
QT	β_0	Mean QT for women with 0 copies of the minor allele and the mean amount of unmeasured confounding	389	389	--
	β_{Drug}	Drug effect on QT	30	5	0 – 30
	β_{SNP}	SNP effect on QT	0	0	0 – 10
	$\beta_{Drug \times SNP}$	Drug-SNP interaction effect on QT	0	0	-6 – 6
	β_{Age}	Age effect on QT	0.39	0.39	--
	β_{Sex}	Sex effect on QT	-3.84	-3.84	--
	β_U	Effect of unknown/unmeasured correlates of QT	2.00	2.00	--
	β_{HTN}	Hypertension effect on QT	4.50	4.50	--
	β_{Diab}	Diabetes effect on QT	-4.90	-4.90	--
ADR	α_0	Value chosen for a 2.5% prevalence of ADR among those on drug treatment	-4.55	-4.55	--
	α_{QTlong}	Log odds of ADR for those with QT > 450 ms versus those without	50	2.05	0 – 50
Loss-to-Follow-up	α_0	Value chosen for a 4% prevalence of loss-to-follow-up	-2.62	-2.62	--
	α_{Age}	Log odds of loss-to-follow-up for 1-year increase in age	-0.01	-0.01	--
	α_{ADR}	Log odds of loss-to-follow-up for those with ADR versus those without	2.89	2.89	--

ADR, Adverse drug reaction; HTN, Hypertension; QT, QT interval; SNP, Single nucleotide polymorphism

Table 30. Descriptive Statistics Across Visits in Simulation Studies Using the Modest Scenario and Extreme Scenario

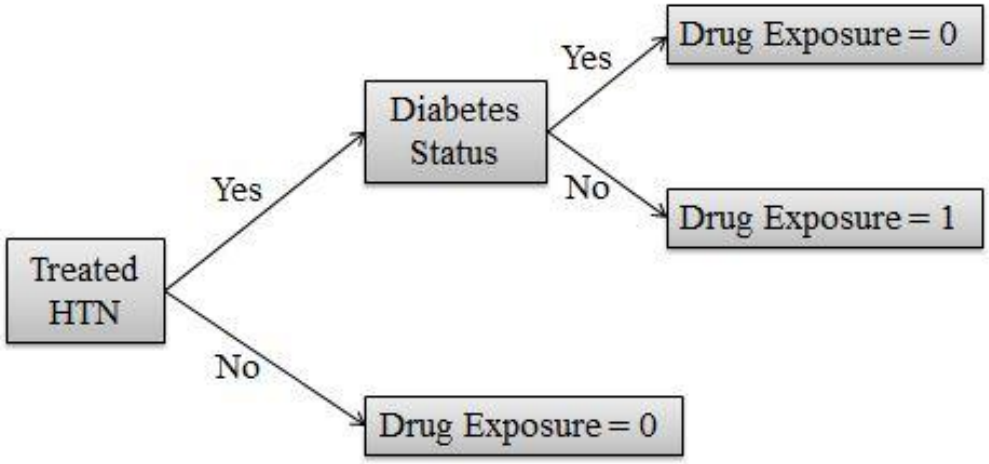
	Modest Scenario				Extreme Scenario			
	Visit 0	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 0**	**Visit 1**	**Visit 2**	**Visit 3**
N	120,000	120,000	115,016	110,093	120,000	120,000	111,685	104,546
Mean Age (SD)	54 (6)	54 (6)	54 (6)	54 (6)	54 (6)	54 (6)	54 (6)	54 (6)
Mean QT (SD)	409 (26)	410 (26)	410 (26)	411 (26)	409 (26)	414 (29)	414 (28)	414 (28)
% Male	47%	47%	47%	47%	47%	47%	47%	47%
% Diabetic	14%	14%	14%	14%	14%	14%	14%	14%
% Hypertensive	31%	36%	40%	43%	31%	36%	39%	41%
% Exposed to Drug	0%	17%	21%	23%	0%	17%	17%	19%
% Exposed to Comparator Drug	0%	7%	6%	6%	0%	7%	9%	9%
% ADR	--	--	0.4%	0.5%	--	--	8%	6%

Figure 25. Simulation Study Timeline and Study Designs



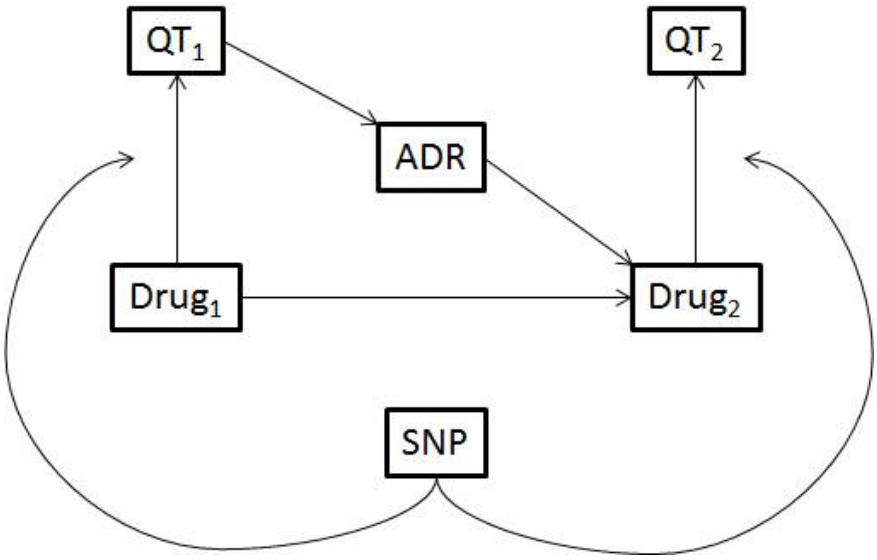
Visit 0 represents a wash-out period where no participants are on medications of interest. At visit 1, all users are new initiators, allowing a new-user study design to be applied to this visit. By visit 3, users have been on medication across time and there has been multiple opportunities for individuals to initiate, have adverse reactions, and stop drug use, enriching visit 3 for participants who are long-term drug users. For longitudinal analyses, visit 2 is included, along with visit 3, as both are enriched for prevalent users.

Figure 26. Decision Tree to Classify Drug Exposure in a Simulation Study



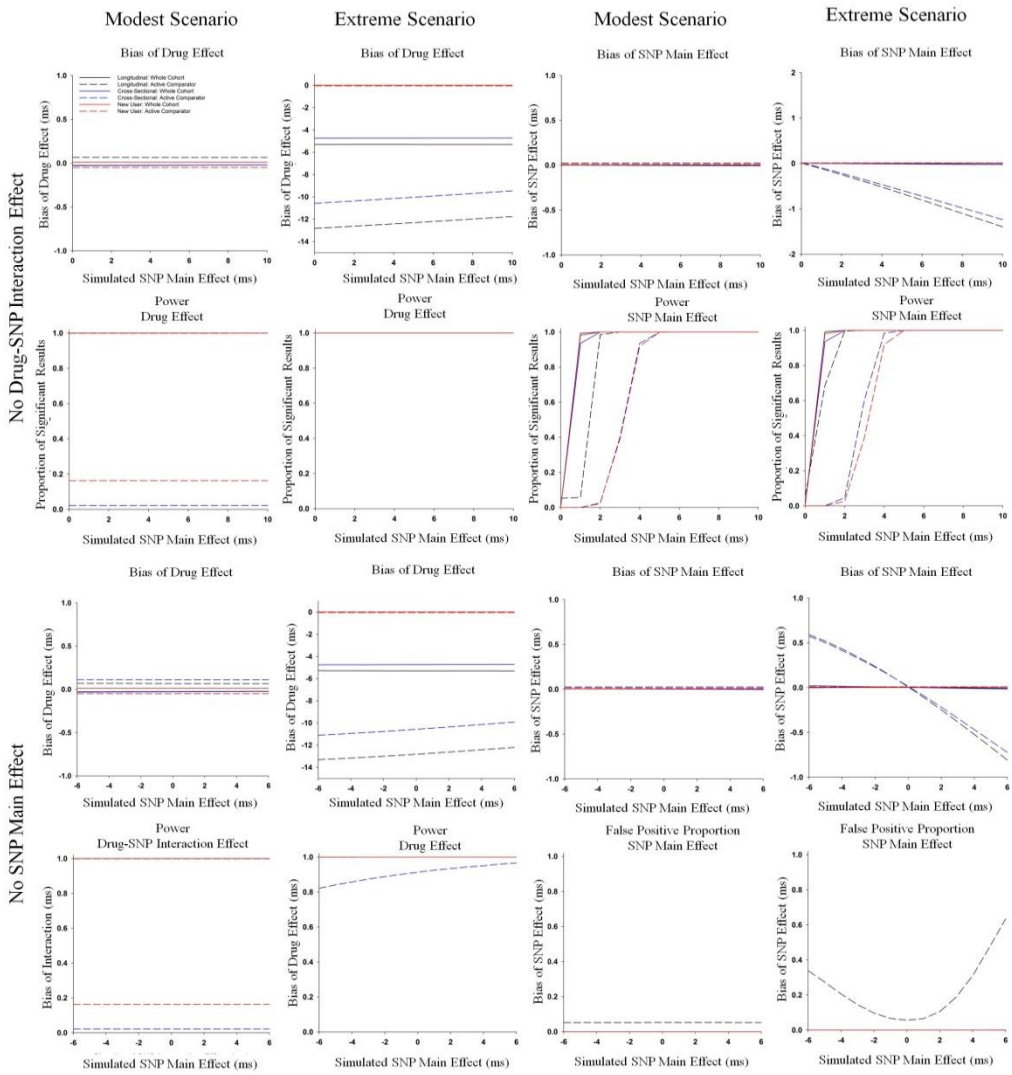
When participants do not have treated hypertension (HTN), they are unexposed to the drug of interest. If they have treated HTN, their exposure status is conditional on their diabetes status.

Figure 27. Conceptual Model of Relationship Between QT-Prolonging Drug Use, QT Interval, and Adverse Drug Reactions



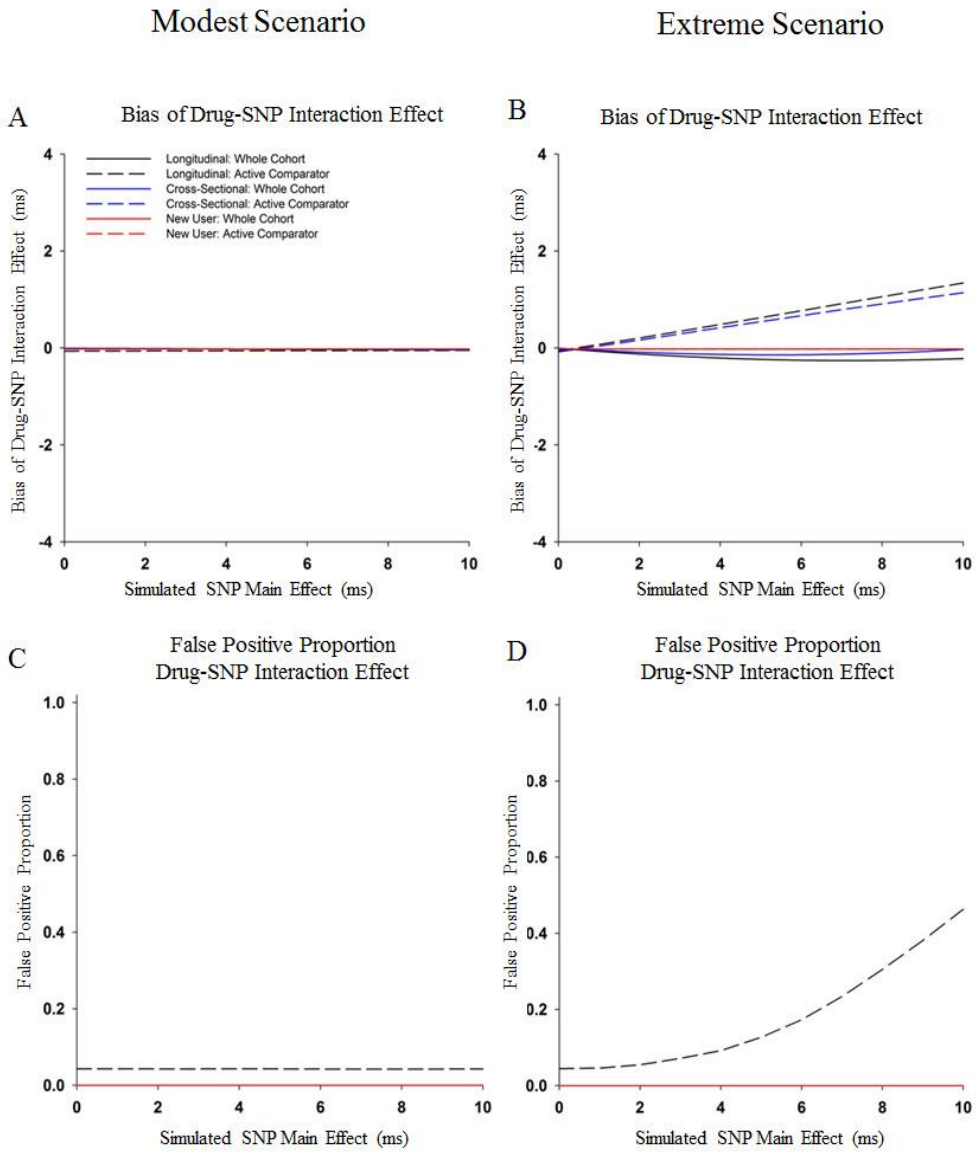
Single nucleotide polymorphism (SNP) is an effect measure modifier on the Drug-QT association. Drug effects QT at visit 1, modified by SNP, which in turn affects ADR, which then effects drug status at visit 2.

Figure 28. Bias, False Positive Proportion, and Power in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a Drug Main Effect or the Absence of a Drug-SNP Interaction Effect



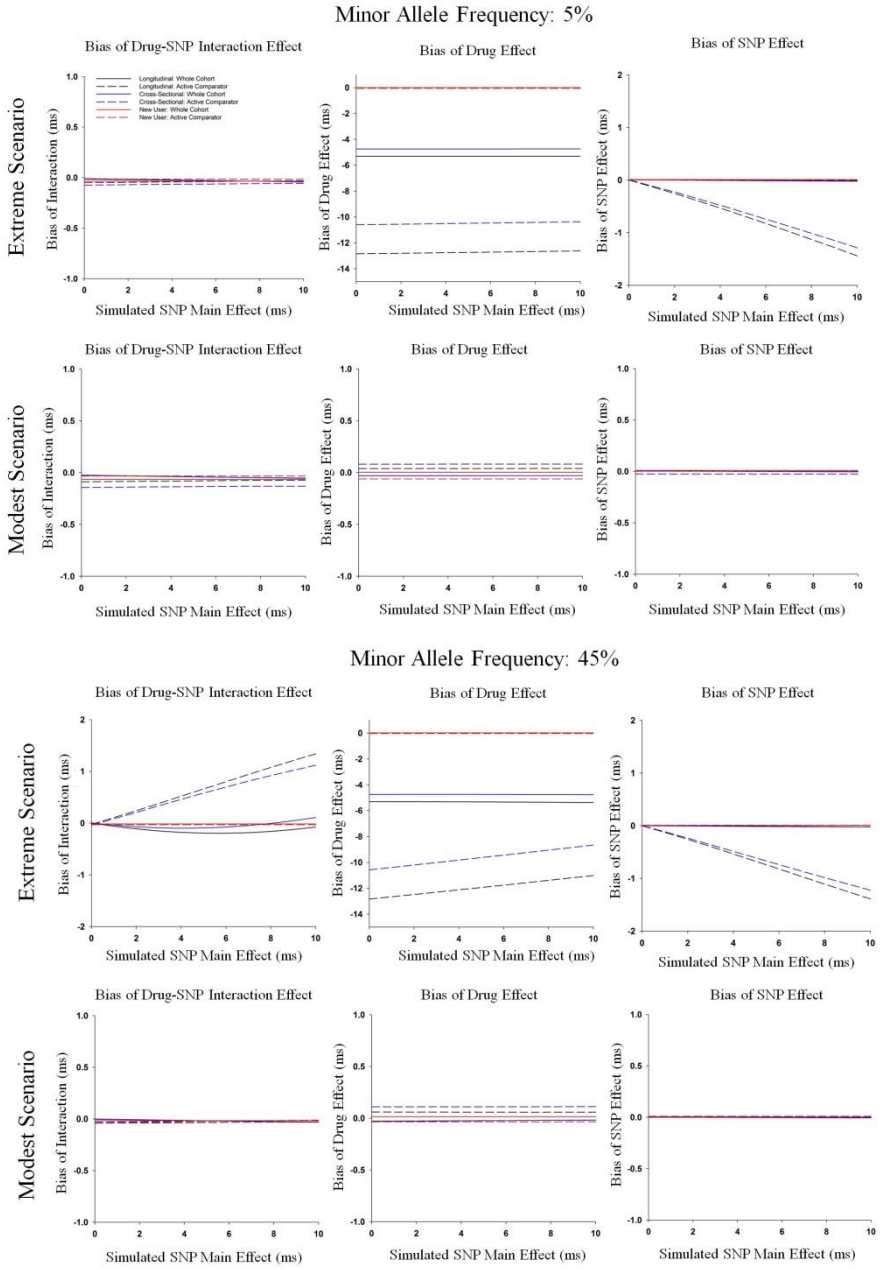
The bias in drug or SNP effect estimated for a pharmacogenomics study of QT in the absence of a simulated drug-SNP interaction effect over a varied main effect and the bias estimated in the absence of a SNP main effect over a varied drug-SNP interaction effect. The false positive proportion or power in the drug effect and SNP effect estimated for a pharmacogenomics study of QT in the absence of a simulated drug-SNP interaction effect over a varied main effect or in the absence of a SNP main effect over a varied drug-SNP interaction effect. Left column panels represent the drug effect under the modest scenario (5 ms increase in QT among drug users). Left center column panels represent the drug effect under the extreme scenario (30 ms increase in QT among drug users). Right center column panels represent the SNP effect under the modest scenario. Right column panels represent the SNP effect under the extreme scenario. Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 29. Bias and False Positive Proportion in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a Drug-SNP Interaction Effect



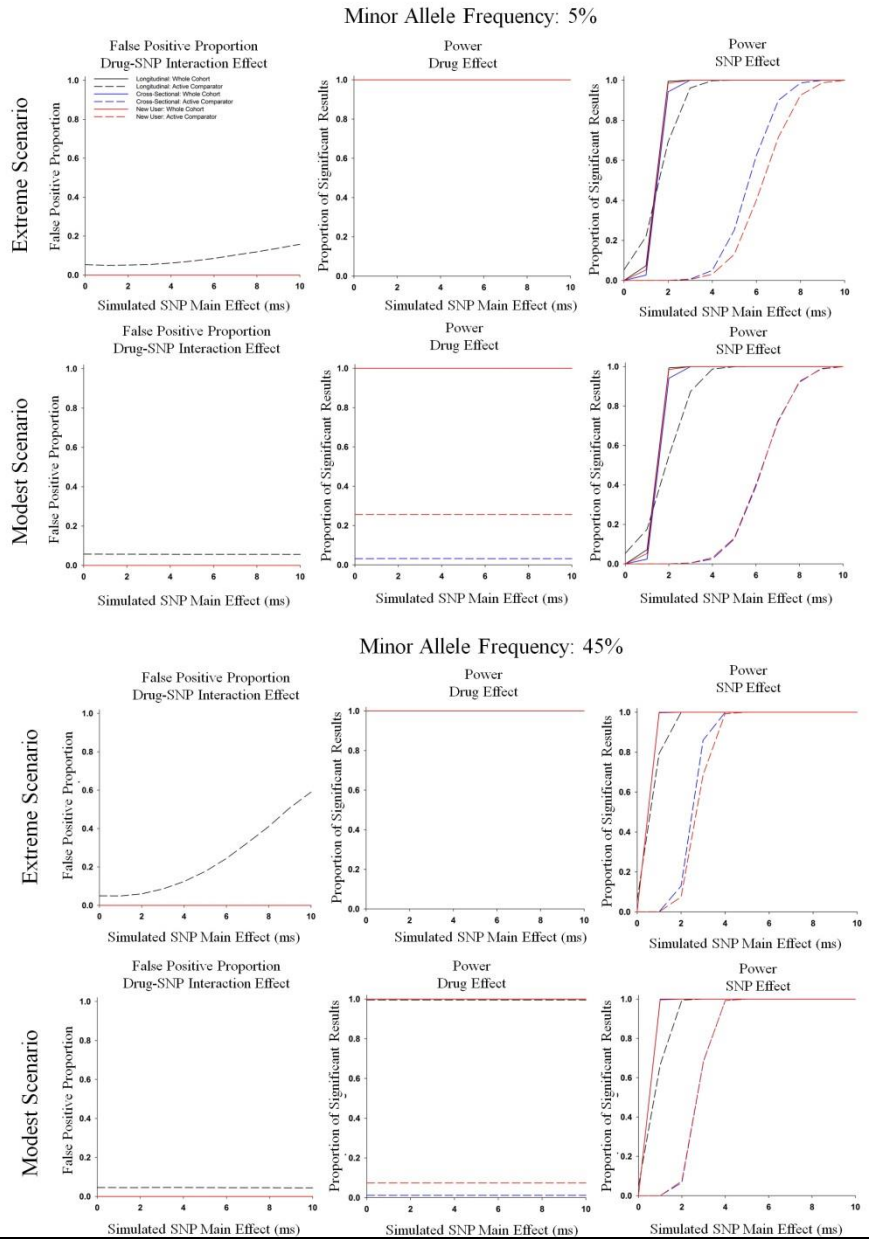
The bias (A, B) and false positive proportion (C, D) estimated for a pharmacogenomics study of QT in the absence of a simulated drug-SNP interaction effect over a varied main effect. Left column panels represent the Modest scenario (5 ms increase in QT among drug users). Right column panels represent the Extreme scenario (30 ms increase in QT among drug users). Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 30. Bias in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a Drug-SNP Interaction Effect Assuming a Minor Allele Frequency of 5% or 45%



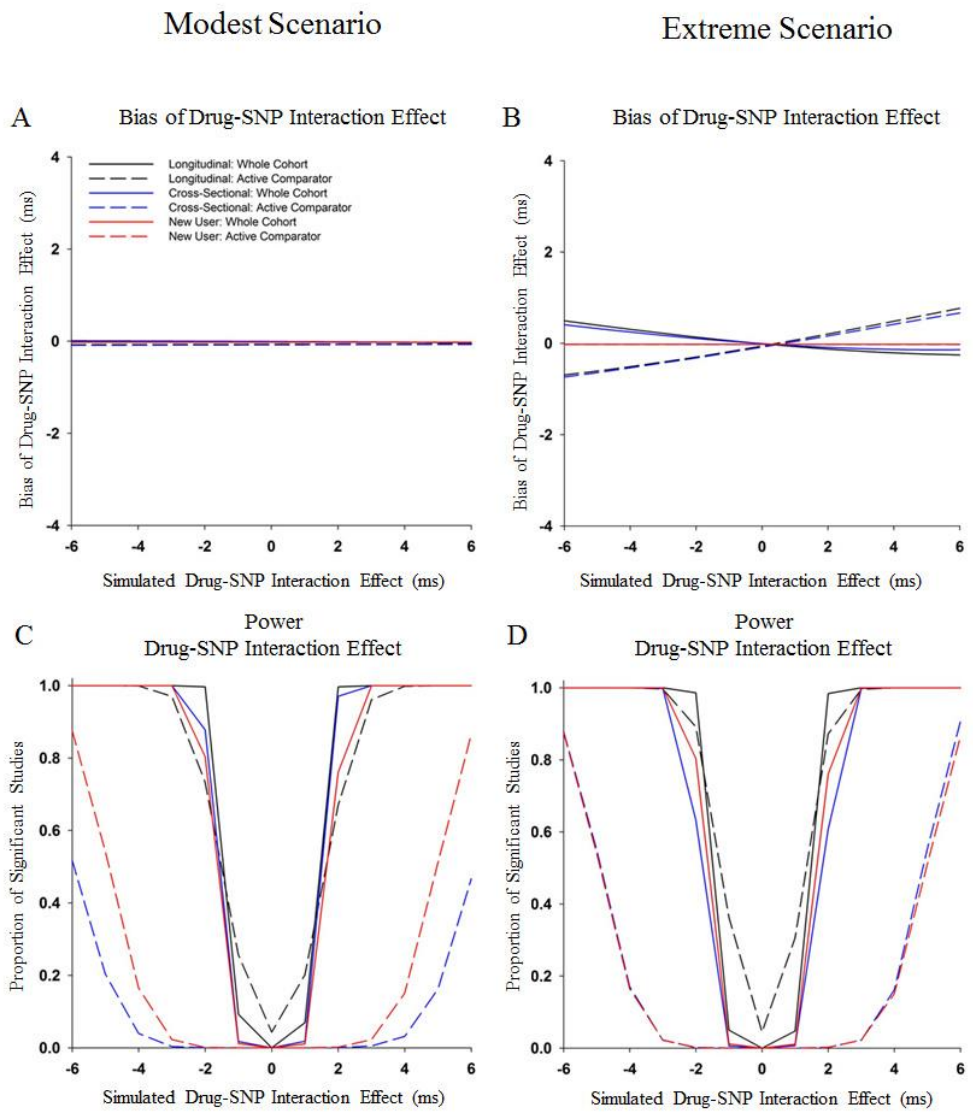
The bias estimated for a pharmacogenomics study of QT in the absence of a simulated drug-SNP interaction effect under an extreme drug effect on QT and a modest drug effect on QT. Left column panels represent the bias in the drug-SNP interaction effect. Middle column panels represent the bias in the drug main effect. Right column panels represent the bias in the SNP main effect. Top two rows represent simulations with a minor allele frequency (MAF) of 5%. Bottom two rows represent simulations with a MAF of 45%. Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (—) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 31. False Positive Proportion or Power in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a Drug Main Effect or the Absence of a Drug-SNP Interaction Effect



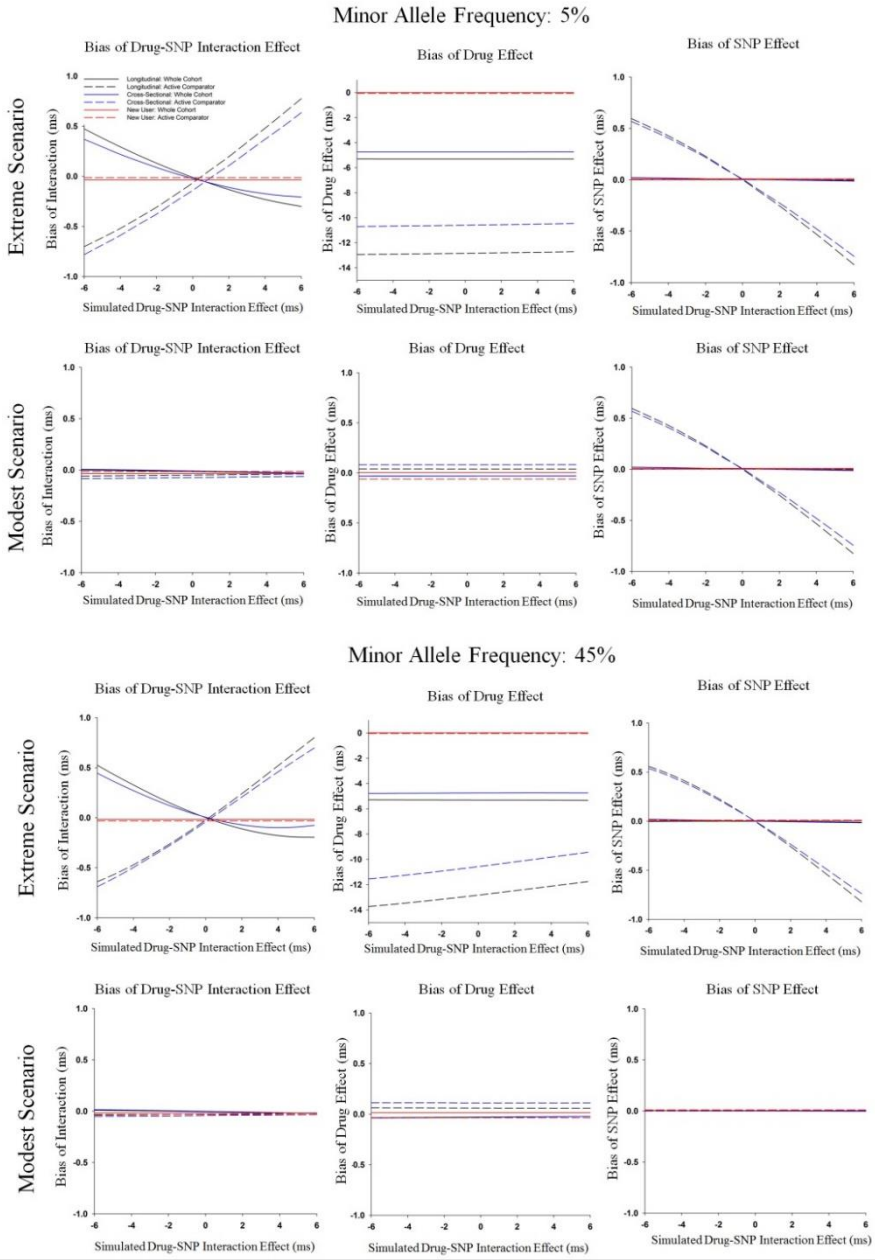
The false positive proportion (FPP) or power estimated for a pharmacogenomics study of QT in the absence of a simulated drug-SNP interaction effect over a varied main effect and the FPP or power estimated in the absence of a SNP main effect over a varied drug-SNP interaction effect under an extreme drug effect on QT and a modest drug effect on QT . Left column panels represent the FPP or power in the drug-SNP interaction effect. Middle column panels represent the power in the drug main effect. Right column panels represent the FPP or power in the SNP main effect. Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 32. Bias and Power in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a Drug Main Effect



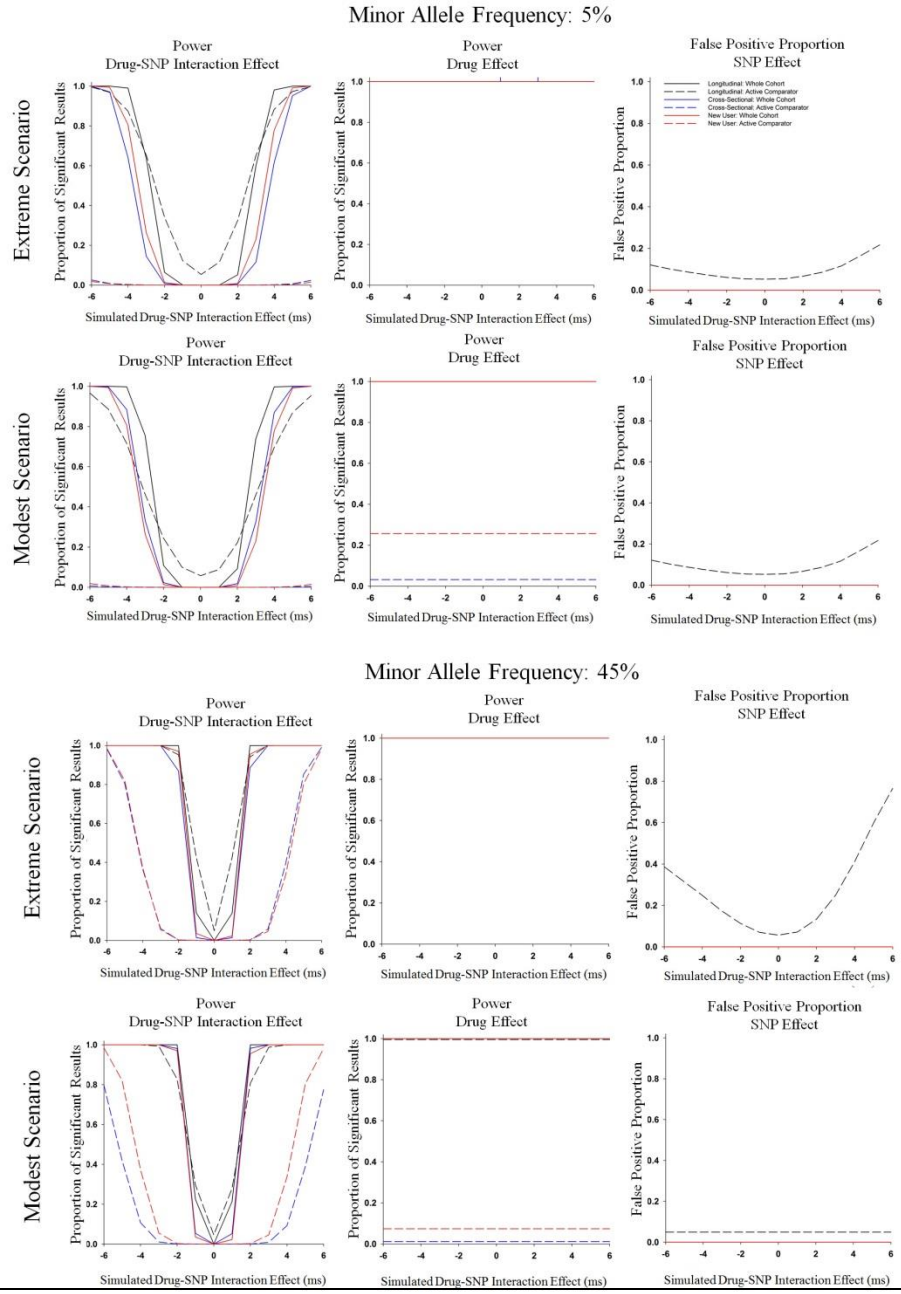
The bias (A, B) and power (C, D) estimated for a pharmacogenomics study of QT in the absence of a SNP main effect over a varied drug-SNP interaction effect. Left column panels represent the Modest scenario (5 ms increase in QT among drug users). Right column panels represent the Extreme scenario (30 ms increase in QT among drug users). Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 33. Bias in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a SNP Main Effect Assuming a Minor Allele Frequency of 5% or 45%.



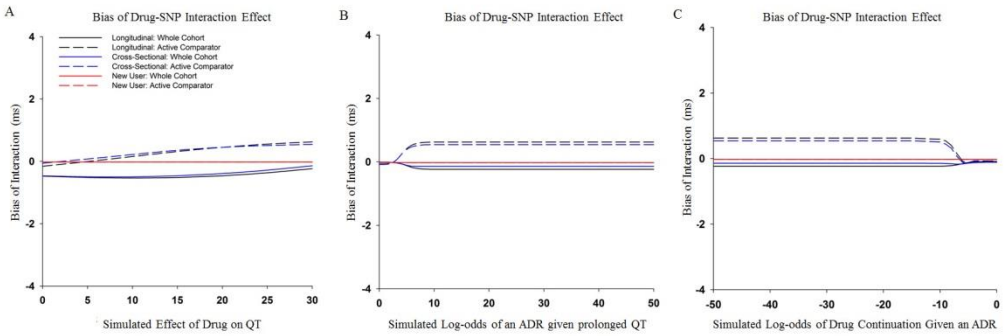
The bias estimated for a pharmacogenomics study of QT in the absence of a simulated SNP main effect under an extreme drug effect on QT and a modest drug effect on QT. Left column panels represent the bias in the drug-SNP interaction effect. Middle column panels represent the bias in the drug main effect. Right column panels represent the bias in the SNP main effect. Top two rows represent simulations with a minor allele frequency (MAF) of 5%. Bottom two rows represent simulations with a MAF of 45%. Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 34. False Positive Proportion or Power in a Pharmacogenomic Study of QT Under an Extreme and Modest Scenario in the Absence of a Drug-SNP Interaction Effect Assuming a Minor Allele Frequency of 5% or 45%



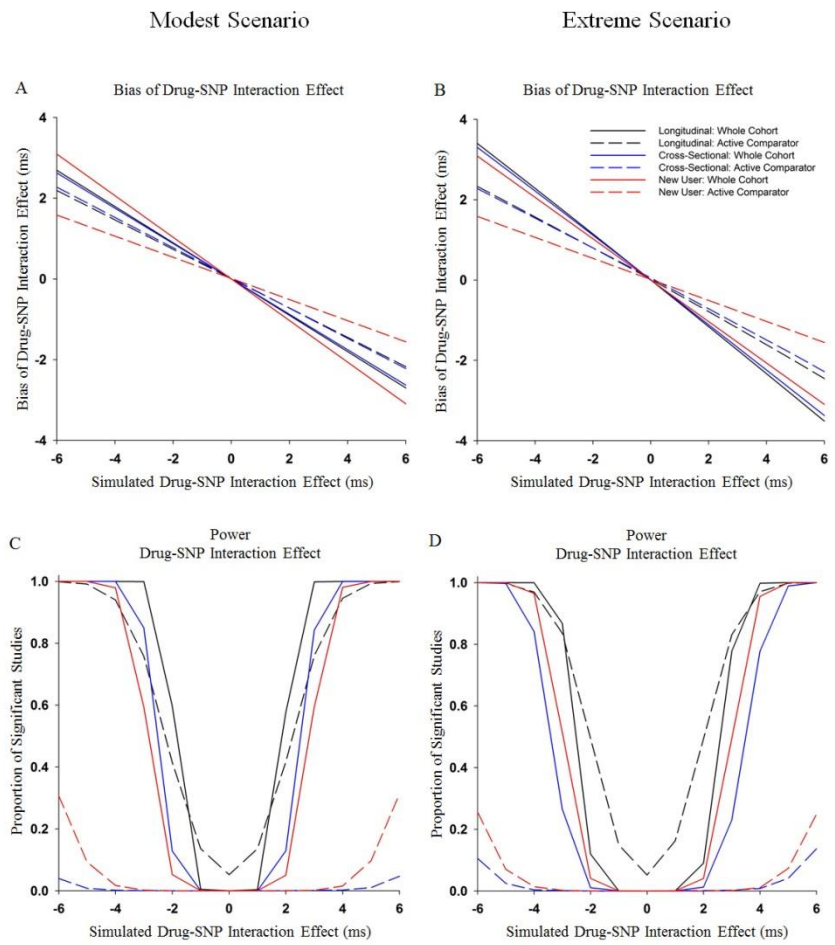
The false positive proportion (FPP) or power estimated for a pharmacogenomics study of QT in the absence of a simulated drug-SNP interaction effect under an extreme drug effect on QT and a modest drug effect on QT. Left column panels represent the FPP in the drug-SNP interaction effect. Middle column panels represent the power in the drug main effect. Right column panels represent the power in the SNP main effect. Top two rows represent simulations with a minor allele frequency (MAF) of 5%. Bottom two rows represent simulations with a MAF of 45%. Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 35. Bias in a Pharmacogenomics Study With Varying Levels of Drug Effect on QT Duration, Prolonged QT Effect on Adverse Drug Reactions and Adverse Drug Reactions on Drug Continuation



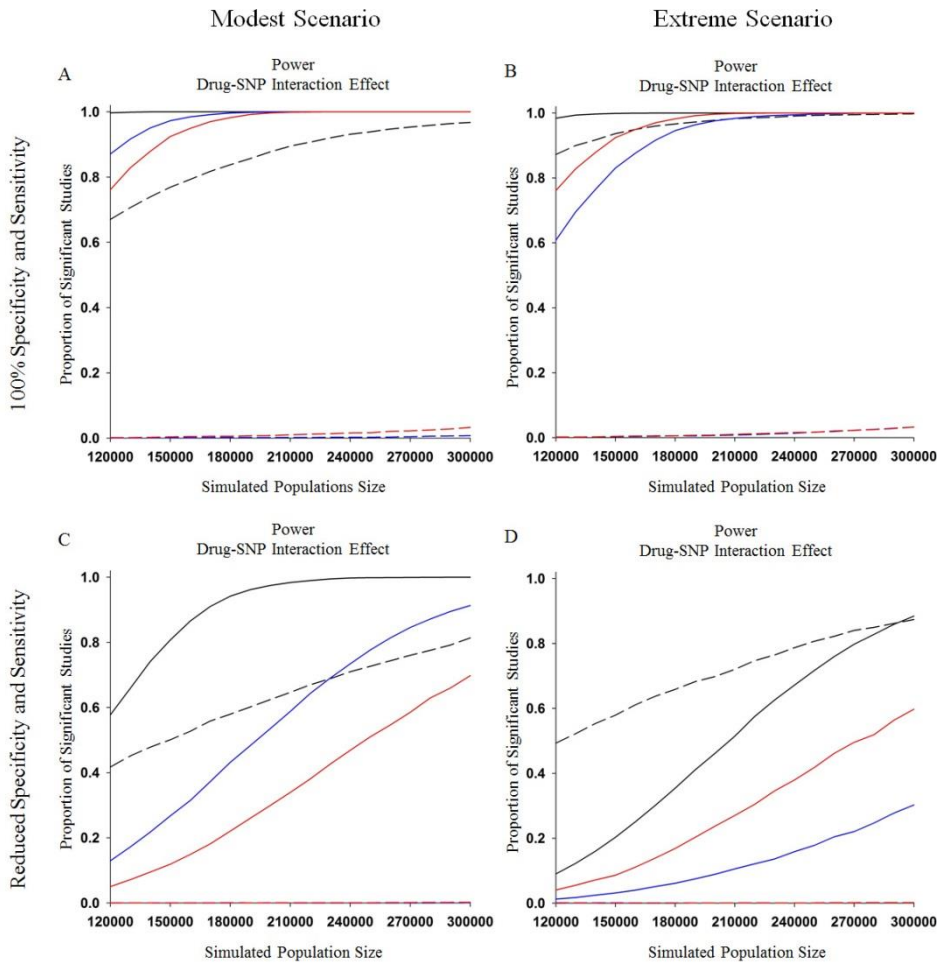
The bias estimated for a pharmacogenomics study of QT with bias estimated in the absence of a SNP main effect and with a drug-SNP interaction effect of 5 ms under varying effect sizes of drug use on QT interval (A), varying effects of QT > 450 ms (QT_{long}) on the occurrence of an adverse drug reaction (ADR, B), or varying effects of having an ADR on drug continuation (C). Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 36. Bias and Power in a Pharmacogenomics Study Under an Extreme or Modest Scenario with Reduced Specificity and Sensitivity of Medication Assessment



The bias and power to detect a drug-SNP interaction effect estimated for a pharmacogenomics study of QT in the absence of a simulated SNP main effect over a varied drug-SNP interaction effect under an extreme drug effect on QT (B, D) and a modest drug effect on QT (A, C). Left column panels represent the modest scenario (5 ms increase in QT among drug users). Right column panels represent the extreme scenario (30 ms increase in QT among drug users). Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

Figure 37. Power to Detect a Drug-SNP Interaction Effect of 2 ms in a Pharmacogenomics Study Under an Extreme or Modest Scenario with Perfect or Reduced Specificity and Sensitivity of Medication Assessment with Increasing Population Size



The power to detect a drug-SNP interaction effect estimated for a pharmacogenomics study of QT in the absence of a simulated SNP main effect with a 2 ms drug-SNP interaction effect over increasing population size under an extreme drug effect on QT (B, D) and a modest drug effect on QT (A, C). Left column panels represent the modest scenario (5 ms increase in QT among drug users). Right column panels represent the extreme scenario (30 ms increase in QT among drug users). Top row represents perfect medication assessment. Bottom row represents imperfect medication assessment measured with reduced specificity and sensitivity. Black lines represent the longitudinal study design, blue lines represent the cross-sectional study design, and red lines represent the new-user study design contrasting whole-cohort (─) and active comparator (- - -) control groups. Simulations were performed assuming a population of 120,000 to 300,000 participants with ~17% of participants receiving a QT-prolonging medication, with 10,000 simulations performed per scenario.

CHAPTER 7: DISCUSSION AND CONCLUSION

QT interval (QT) prolongation is a long-known, potentially fatal side effect of many common pharmaceuticals, including thiazide diuretics, a common antihypertensive treatment.[36, 37, 39] Pharmacogenomics research represents a promising step forward in understanding and preventing adverse drug reactions like QT prolongation, as QT is highly heritable (35-40%) and influenced by many common SNPs.[24-28, 209] Furthermore, pharmacogenomics studies of thiazides have identified numerous loci influencing patient response and ADRs.[274, 276, 279, 280] However, underlying mechanisms governing thiazide-QT associations are poorly understood. Theories range from a direct effect of thiazides on cardiac conduction mechanisms[43, 258] to an indirect effect through electrolyte levels.[41, 42, 257] Together, these lines of evidence suggest that the thiazide-QT relationship is a promising candidate for pharmacogenomics study.

We therefore conducted the first large, multi-ethnic pharmacogenomics study of thiazide diuretics and QT. Using fourteen large, observational cohort studies (N=78,199), we performed a genome-wide analysis of the thiazide-SNP interaction on QT and its component parts (QRS interval, JT interval). Although we used a comprehensive approach that considered multi-ethnic populations, leveraged pleiotropy, and accommodated population heterogeneity, we did not identify any genome-wide significant SNPs modifying the association between thiazides and these ECG intervals. However, we identified 74 loci with suggestive evidence of association through both univariate and cross-phenotype analyses as well as enrichment in pathways involved in transcription and translation.

Interestingly, our suggestive results included multiple loci involved in ion transport and handling, the disruption of which is believed to be an underlying mechanism in drug-induced QT prolongation,[111] supporting the hypothesis that common SNPs modify the thiazide-QT relationship. For example, our suggestive results include the *PITX2* and *RYR3* QRS loci identified in Hispanic/Latinos, which may directly regulate ion channel genes and genes involved in calcium handling.[377] Moreover, we found suggestive evidence of thiazide-SNP interactions on QT, QRS, or JT in other genes involved in ion transport and handling, including *NELL1,*[279] *STC2,*[378] *EDN1,*[379] *TRPC7,*[380] *PKP2,*[381] and *DISC1,*[382] as well as a voltage-gated potassium channel gene (*KCNQ3*). Additionally, our power simulations suggested there was limited power to detect interaction effects of 2 ms, sizes consistent with QT main effects analyses.[209]

Our pharmacogenomics study of thiazides and QT, particularly the lack of genome-wide significant results despite biological plausibility and a sample size surpassing that of most published gene-environment studies to date suggested to us that further efforts are needed to better characterize pharmacogenomics studies conducted in observational settings and potential causes of reduced power. For example, pharmacogenomics studies are increasingly being conducted in observational cohort settings, which provide large, diverse sample, deep phenotype characterization, numerous medication exposures, and improved external validity compared to clinical trials. [284] However, a form of selection bias called prevalent user bias affects pharmaco*epidemiologic* studies conducted in observational settings, as does exposure misclassification. Yet, the effects of prevalent user bias and exposure misclassification on the interaction effects under evaluation in pharmaco*genomics* studies are unclear.[284, 287] Therefore, we conducted a simulation analysis to examine the

influence of prevalent user bias, exposure misclassification, study design, and referent group on bias, power, and type I error in pharmacogenomics studies conducted in observational cohort settings.

Specifically, we examined the influence of prevalent user bias and exposure misclassification in pharmacogenomics studies conducted in observational settings by contrasting three designs (new user, cross-sectional, and longitudinal) and two control groups (whole cohort and active comparator) under two scenarios (modest and extreme drug effects on QT), totaling 12 different settings. Our simulations identified settings where prevalent user bias caused moderate bias on the drug-SNP interaction effect. Yet, the greatest bias, as well as the largest power reductions, were detected when simulations were extended to examine exposure misclassification. For example, our simulations indicated that, even in a longitudinal setting which provided the greatest power to detect interaction effects, pharmacogenomics studies require at least 150,000 participants to achieve 80% power to detect a 2 ms interaction effect, an effect consistent with, or slightly larger than, published main effects GWAS,[209] providing further evidence that our sample size of 78,199 participants in our thiazides-QT pharmacogenomics GWAS was insufficient.

Given that the amount of bias and potential for reduced power, which varied by the design, control group, and strength of the drug effect on the outcome, these results have broad implications for pharmacogenomics studies conducted in observational cohort settings, beyond the work presented here. For example, recommendations for the optimal pharmacogenomics design and control group must balance bias and power and be tailored to the research question of interest. In the context of thiazide-SNP interactions on QT, even if increasing the sample size by50% was possible, the influence of exposure misclassification

and other yet evaluated sources of measurement error makes it difficult to rule-out clinically significant results, generally defined as interactions with effects of 5 ms or greater. [44] However, such increases in sample size are unlikely to occur through incorporating data from yet represented observational cohort studies, as the remaining large population-based cohort studies (e.g. The National Longitudinal Study of Adolescent to Adult Health [AddHealth][417] or the Reasons for Geographic and Racial Differences in Stroke Study [REGARDS][418]) do not have ECGs or have inadequate ECG characterization (EZ Soliman personal communication). Additionally, while other phenotypes of interest (i.e. blood pressure or glycemic traits) are measured in more cohorts than ECG traits, many of these traits show greater variability in measurement, [407-409] which may require even larger sample sizes than required for QT. Future efforts to evaluate the effect of differing levels of outcome measurement error is therefore warranted. Finally, funding institutes, particularly the National Institutes of Health, are moving away from funding new large, population-based cohorts, making the de novo collection of requisite data in the future unlikely.[419, 420] Yet, our results underscore the massive sample sizes required in gene-environment interaction studies to both identify loci as well as rule out pharmacogenomics efforts of large magnitude,[282, 402-404] as false negatives can negatively impact dissemination of results, innovation in drug design to prevent future ADRs, and future pharmacogenomics efforts.

Given that we have identified the majority of cohort studies to date with the requisite drug, ECG, and GWAS data, additional avenues are likely needed to grow the analytic sample for future efforts. One potentially attractive option is offered by studies of electronic medical records (EMRs). Strengths of EMRs include the potential to provide a more complete medication history, which could enable sensitivity analyses examining variables

such as medication dose and duration of use. Consortia such as the Electronic Medical Records and Genomics (eMERGE) Network have demonstrated the feasibility of linking EMRs to genetic data for use in genetic research, [386] and have successfully identified genetic variants modifying drug response. [387] However, investigators using EMR data cannot control participant recruitment, timing and accuracy of data collection, or population representativeness. [388] Considering ECG research specifically, cohort studies administer ECGs to all participants at study visits using strict quality control procedures, whereas EMRs may capture ECGs for patients with medical indications, providing an inherently different population. The selection bias caused through the inclusion of only participants with medical indications for specific test would also be of concern to pharmacogenomics studies of numerous other phenotypes that are not part of standard medical examinations (e.g. cognitive decline, hearing, biomarker assays, etc.).

Thus, before resources like EMRs can be combined with results from observational cohort studies to illuminate pharmacogenomics studies, efforts to understand the potential bias and threats to precision are needed. Unfortunately, prior efforts suggest that most pharmacogenomics studies have been performed (and published) before extensive efforts validating study designs have been conducted. [47] In the context of EMRs, future studies should therefore investigate the impact of non-routine testing (e.g. ECGs) on a population with medical indications on bias in a pharmacogenomics analysis. Furthermore, EMRs provide the opportunity to include more precise medication data (e.g. dose and duration of use), but the impacts of including this information, particularly when it is only available in a subset of populations, warrants investigation. Finally, researchers also must consider the limitations of combining different sources of data (e.g. population based cohorts, EMRs,

biobanks, etc.) and better characterize how combining such heterogeneous data sources impacts bias and power.

Given the massive statistical testing penalty of genome-wide association studies ($\alpha=5x10^{-8}$), the potential for bias in study designs that optimize power (i.e. longitudinal), and uncertainty surrounding the use of EMRs, a third tempting option may be to consider hypothesis-based genomic analyses such as a candidate gene approach. There has been some success in identifying genetic variants in genes associated with congenital long QT syndrome that modify drug-induced QT-prolongation.[410] However, our evaluation of loci from main effects QT GWAS as candidate genes in pharmacogenomics studies of QT-prolonging drugs did not yielded any positive results. The observation of loci from main effects GWAS having no interactive effect is not unique to pharmacogenomics studies of drug-induced QT-prolongation. Efforts by Bis *et al*. that evaluated whether SNPs with previously reported main effects on coronary heart disease also modified the association between anti-hypertensives and cardiovascular disease were similarly unsuccessful.[403] It is not surprising that SNPs identified in studies of main effects do not demonstrate an interactive effect in gene-environment such as pharmacogenomics, as SNPs selected on the basis of an extreme *P*-value for a single main effect may be less likely to harbor heterogeneity across population subgroups (i.e. drug users vs. non-users).[282]

In conclusion, pharmacogenomics research is currently one of the few areas of public health genomics for which interventions are not only possible, but are underway, as genetics are used to guide drug selection.[7, 13, 259] In addition to informing drug selection, pharmacogenomics research also has the potential to illuminate novel pathways in drug response, inform drug development, alter policy and drug labelling, modify dosing regimens,

and prevent ADRs, thereby influencing public health at many points of intervention.[10, 11, 13] However, the limitations currently posed by pharmacogenomics research have the potential to minimize the role of genetics in variable drug response and to naively constrain the advancement of pharmacogenomics inquiry. Therefore, future analyses must seek innovative solutions to overcome the inherent challenges in pharmacogenomics work so that this contemporary field can reach its full public health potential.

APPENDIX 1: SUMMARY RESULTS FROM FIVE LARGEST GENOME-WIDE ASSOCIATION STUDIES OF QT (POPULATIONS~10,000 OR GREATER)

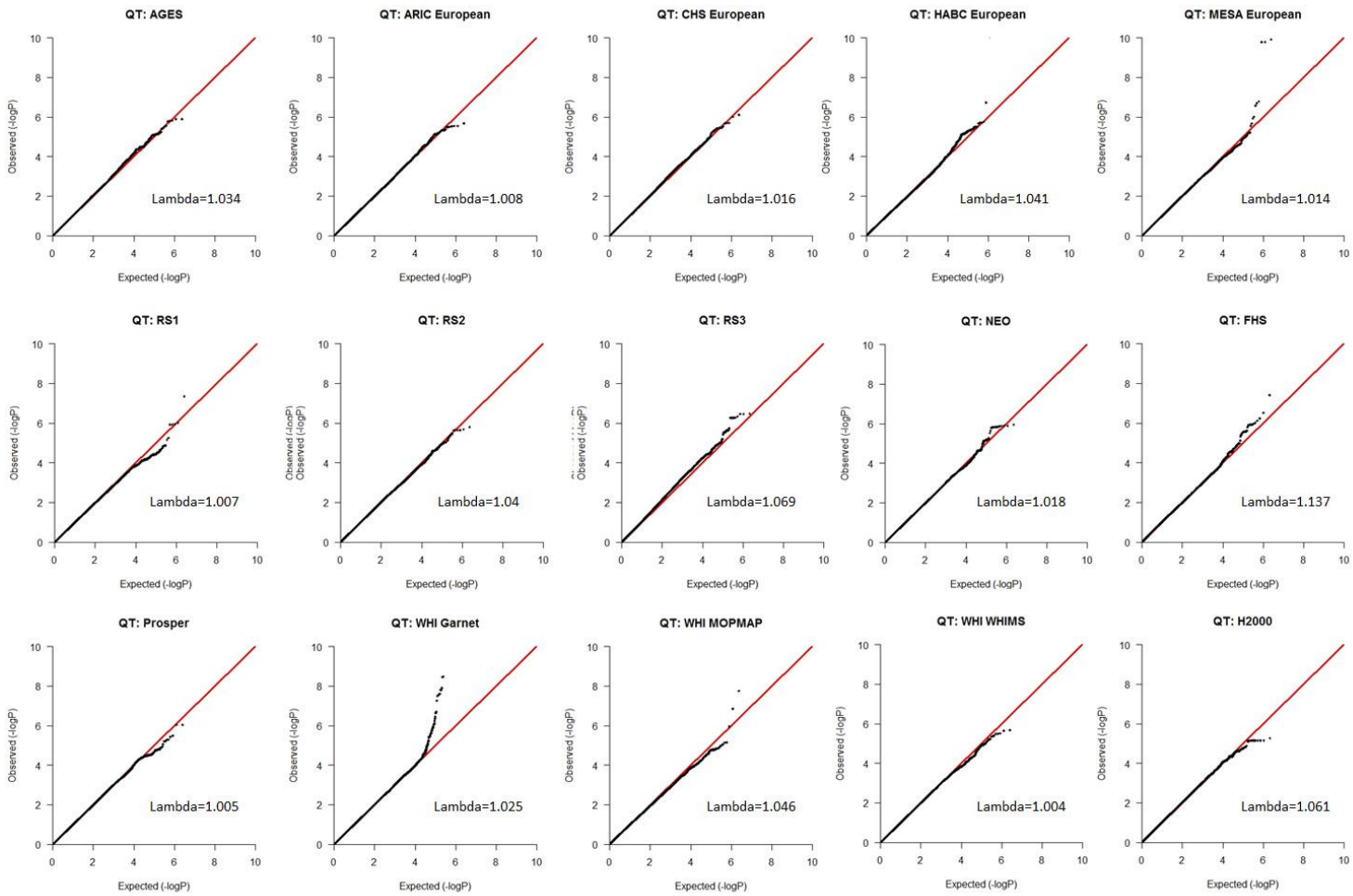
		Author and Year		Newton-Cheh 2009			Pfeufer 2009			Holm 2010			Smith 2012			Arking 2014		
		Ancestry		European			European			European			African			European		
		N		13,685			15,842			9,860			12,097			76,061		
Gene	Chr	SNP	CA	MAF	β (SE)	*P*	MAF	β (SE)	*P*	MAF	β (SE)	*P*	MAF	β (SE)	*P*	MAF	β (SE)	*P*
RNF207	1	rs846111	C	0.28	1.75 (0.18)	1E-16	0.29	1.49 (0.25)	4E-9							0.28	1.73 (0.13)	7E-40
TCEA3	1	rs2298632	T													0.50	0.70 (0.09)	1E-14
NOS1AP	1	rs12143842	T	0.26	3.15 (0.18)	2E-78	0.24	2.88 (0.23)	2E-35				0.20	3.14 (0.39)	2E-15	0.24	3.50 (0.11)	1E-213
		rs16847548	C										0.22	2.17 (0.33)	2E-10			
		rs16857031	G	0.14	2.63 (0.18)	1E-34												
		rs12029454	A	0.15	2.98 (0.18)	3E-45							0.31	1.73 (0.29)	4E-9			
		rs7534004	A										0.31	1.73 (0.29)	3E-9			
		rs10127719	C										0.32	1.64 (0.29)	2E-8			
		rs12567315	A										0.33	1.69 (0.28)	2E-9			
		rs6692381	T										0.34	-1.71 (0.28)	1E-10			
		rs6667431	A										0.33	1.69 (0.28)	2E-9			
		rs4306106	A										0.33	1.66 (0.28)	5E-9			
		rs10800352	G										0.33	-1.66 (0.28)	5E-9			
		rs4480335	C										0.33	-1.67 (0.28)	4E-9			
		rs12116744	A										0.33	1.67 (0.28)	4E-9			
		rs12027785	A										0.33	1.67 (0.28)	3E-9			
		rs3934467	T										0.33	-1.69 (0.28)	3E-9			
		rs4391647	G										0.33	-1.74 (0.28)	8E-10			
		rs4657175	G										0.33	1.74 (0.28)	7E-10			
		rs12123267	T										0.34	-1.70	2E-9			

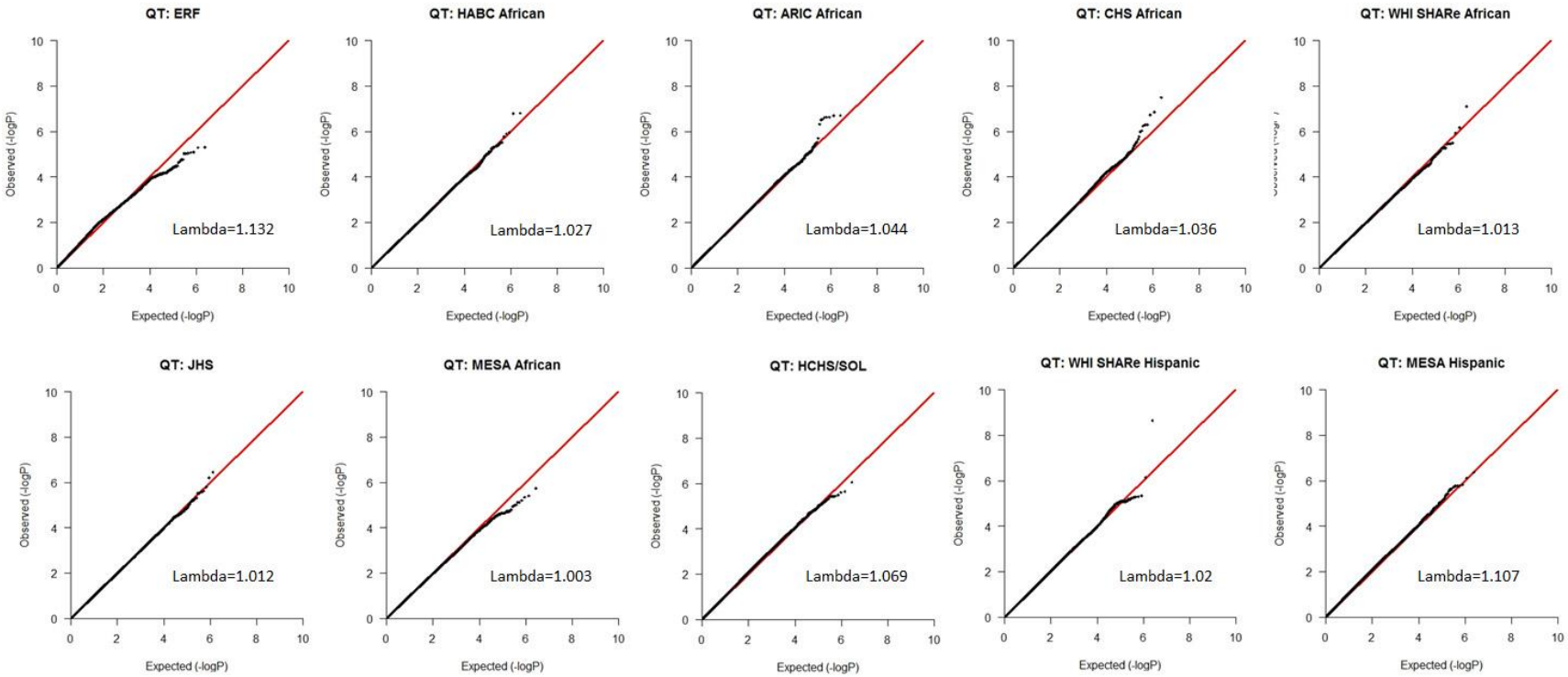
														(0.28)				
ATP1B1	1	rs12061601	C									0.29	-1.89 (0.30)	2E-10				
		rs1320976	A									0.25	-2.06 (0.32)	2E-10				
		rs10919071	A				0.87	2.05 (0.29)	2E-12	0.88	1.52 (0.71)	0.03				0.87	1.68 (0.14)	1E-31
SLC8A1	2	rs12997023	C													0.05	-1.69 (0.22)	5E-14
SP3	2	rs938291	G													0.39	0.53 (0.09)	6E-10
TTN-CCDC141	2	rs7561149	C													0.42	-0.52 (0.09)	7E-9
SPATS2L	2	rs295140	T													0.42	0.57 (0.09)	2E-11
SCN5A	3	rs11129795	A				0.23	-1.27 (0.23)	4E-8									
		rs12053903	C	0.34	-1.23 (0.18)	1E-14												
		rs6793245	A													0.32	-1.12 (0.10)	4E-27
C3ORF75	3	rs17784882	A													0.40	-0.54 (0.10)	3E-8
SLC4A4	4	rs2363719	A													0.11	0.97 (0.16)	8E-10
SMARCAD1	4	rs3857067	A													0.46	-0.51 (0.08)	1E-9
GFRA3	5	rs10040989	A													0.13	-0.85 (0.13)	5E-11
GMPR	6	rs7765828	G													0.40	0.55 (0.09)	3E-10
PLN	6	rs11153730	T													0.50	-1.65 (0.10)	2E-67
		rs11970286	T				0.44	1.64 (0.20)	2E-16									
		rs11756438	A	0.47	1.40 (0.18)	5E-22												
CAV1	7	rs9920	C													0.09	0.79 (0.14)	3E-8
KCNH2	7	rs2968864	T	0.25	1.40 (0.18)	8E-16				0.22	2.33 (0.01)	2E-5						
		rs2968863	T				0.29	-1.35 (0.23)	4E-9	0.22	-2.30 (0.55)	3E-5						
		rs4725982	T	0.22	1.58 (0.18)	5E-16				0.23	1.64 (0.55)	0.003						
		rs2072413	T										0.27	-1.68 (0.11)	1E-49			

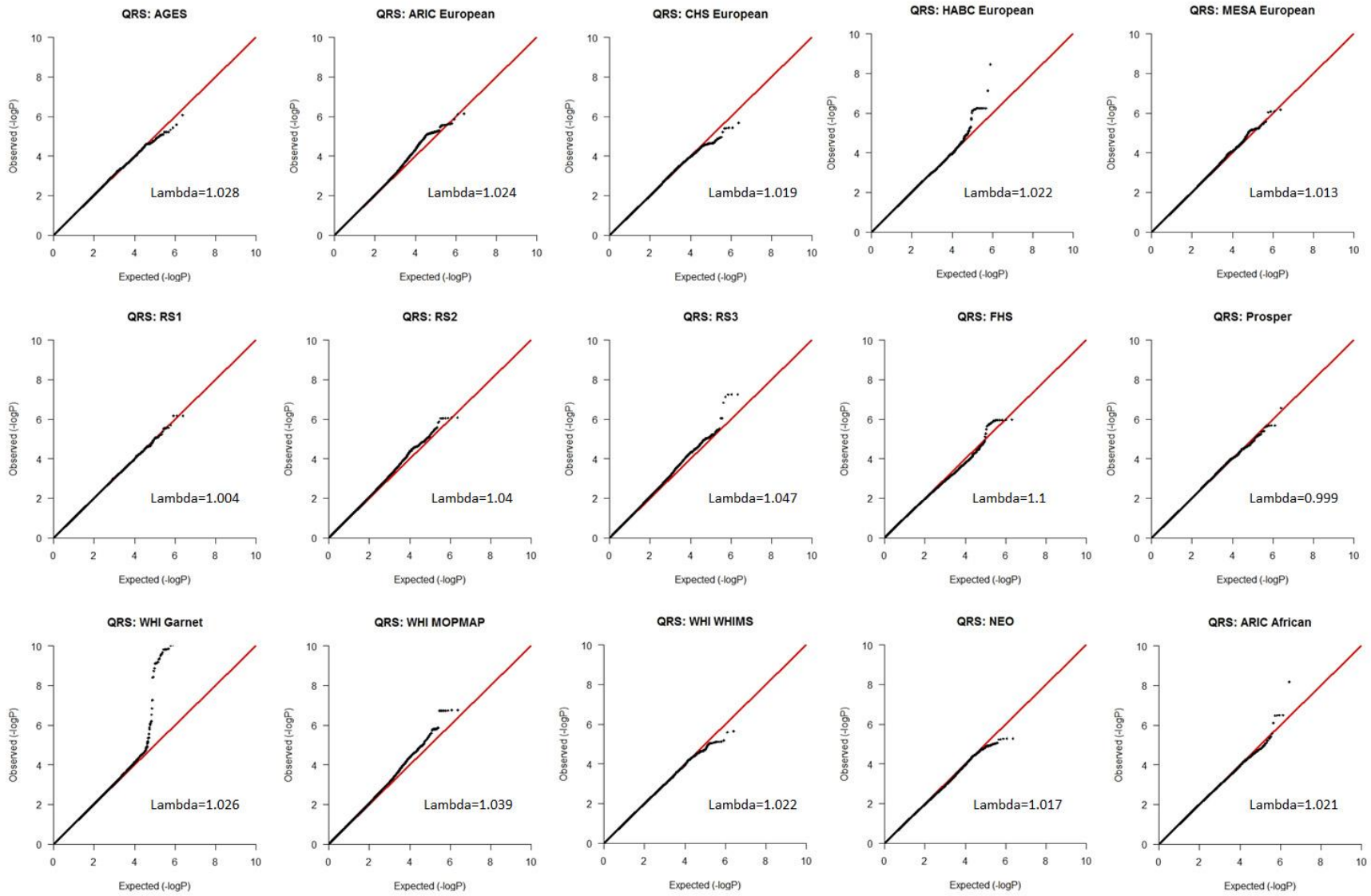
		rs3807375	T							0.35	4.42 (0.67)	5E-11					
NCOA2	8	rs16936870	A												0.10	0.99 (0.16)	1E-9
LAPTM4B	8	rs11779860	C												0.47	-0.61 (0.10)	2E-10
AZIN1	8	rs1961102	T												0.33	0.57 (0.10)	3E-9
GBF1	10	rs2485376	A												0.39	-0.56 (0.09)	3E-8
KCNQ1	11	rs2074238	T	0.06	-7.88 (0.88)	3E-17				0.04	-2.13 (0.49)	1E-5					
		rs7122937	T												0.19	1.93 (0.12)	1E-54
		rs12296050	T				0.20	1.44 (0.25)	9E-9	0.15	4.87 (0.75)	8E-11					
		rs757092	G							0.34	1.14 (0.48)	2E-2					
		rs12576239	T	0.13	1.75 (0.18)	1E-15				0.13	2.31 (0.52)	8E-6					
FEN1-GADS2	11	rs174583	T												0.34	-0.57 (0.09)	8E-12
ATP2A2	12	rs3026445	C												0.36	0.62 (0.09)	3E-12
TBX5	12	rs3825214	G							0.22	2.18 (0.41)	1E-7					
KLF12	13	rs728926	T												0.36	0.57 (0.10)	2E-8
ANKRD9	14	rs2273905	T												0.35	0.61 (0.09)	4E-11
USP50-TRPM7	15	rs3105593	T												0.45	0.66 (0.10)	3E-12
CREBBP	16	rs1296720	C												0.20	0.83 (0.13)	4E-10
LITAF	16	rs8049607	T	0.49	1.23 (0.18)	5E-15	0.49	1.25 (0.22)	3E-8	0.52	2.30 (0.52)	1E-5					
		rs735951	A										0.46	-1.15 (0.10)	2E-28		
MKL2	16	rs246185	C										0.34	0.72 (0.10)	3E-13		
NDRG4-CNOT1	16	rs37062	G	0.24	1.75 (0.18)	3E-25				0.28	2.25 (0.47)	1E-6					
		rs246196	C												0.26	-1.73 (0.11)	2E-15
		rs7188697	A				0.74	1.66 (0.23)	1E-12	0.71	1.75 (0.50)	5E-4					
LIG3	17	rs2074518	T	0.46	1.05 (0.18)	6E-12											

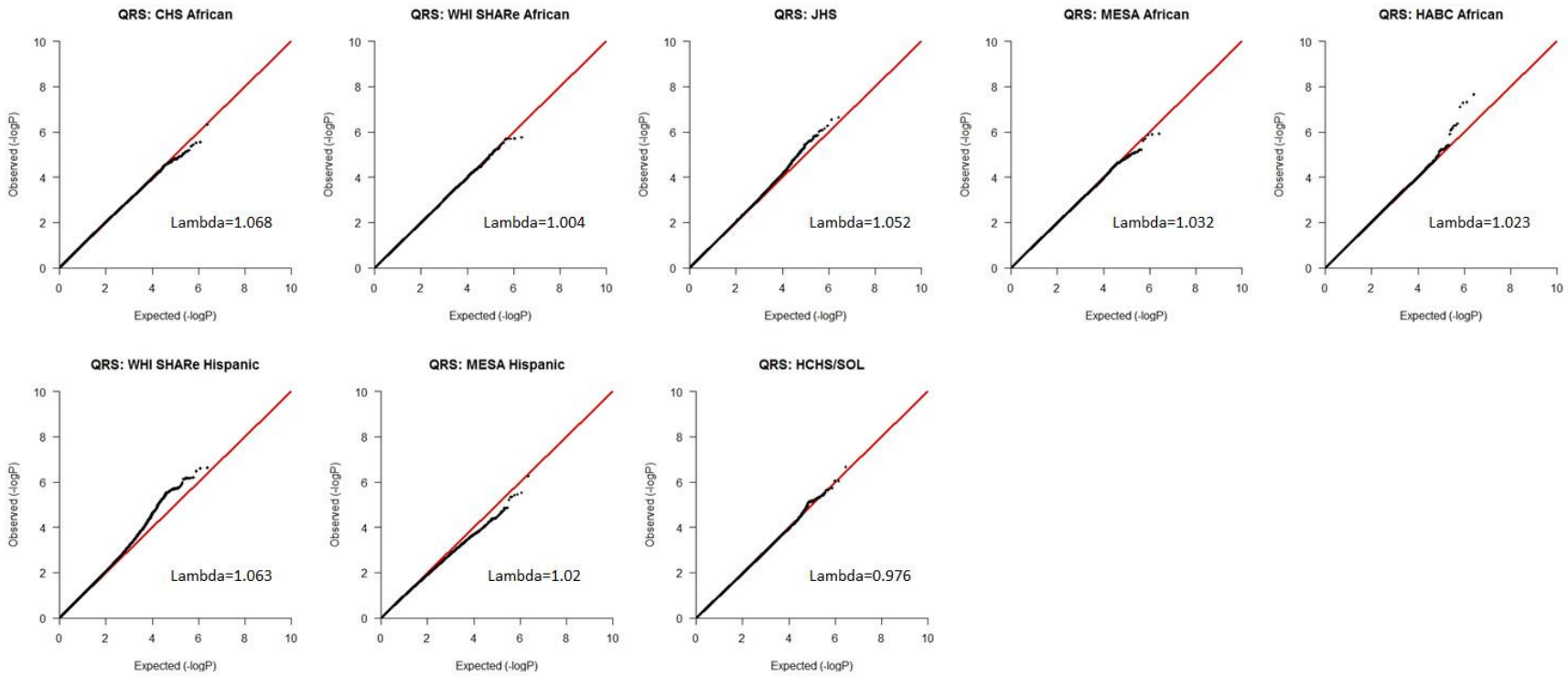
		rs1052536	C									0.53	0.98 (0.10)	6E-25
PRKCA	17	rs9892651	C									0.43	-0.74 (0.10)	3E-14
KCNJ2	17	rs1396515	C									0.52	-0.98 (0.09)	2E-25
		rs17779747	T		0.35	-1.16 (0.21)	3E-8							
KCNE1	21	rs1805128	T									0.01	7.42 (0.85)	2E-18
		rs1805127	T					0.39	3.09 (0.72)	2E-5				
		rs727957	T					0.19	4.33 (1.20)	2E-12				

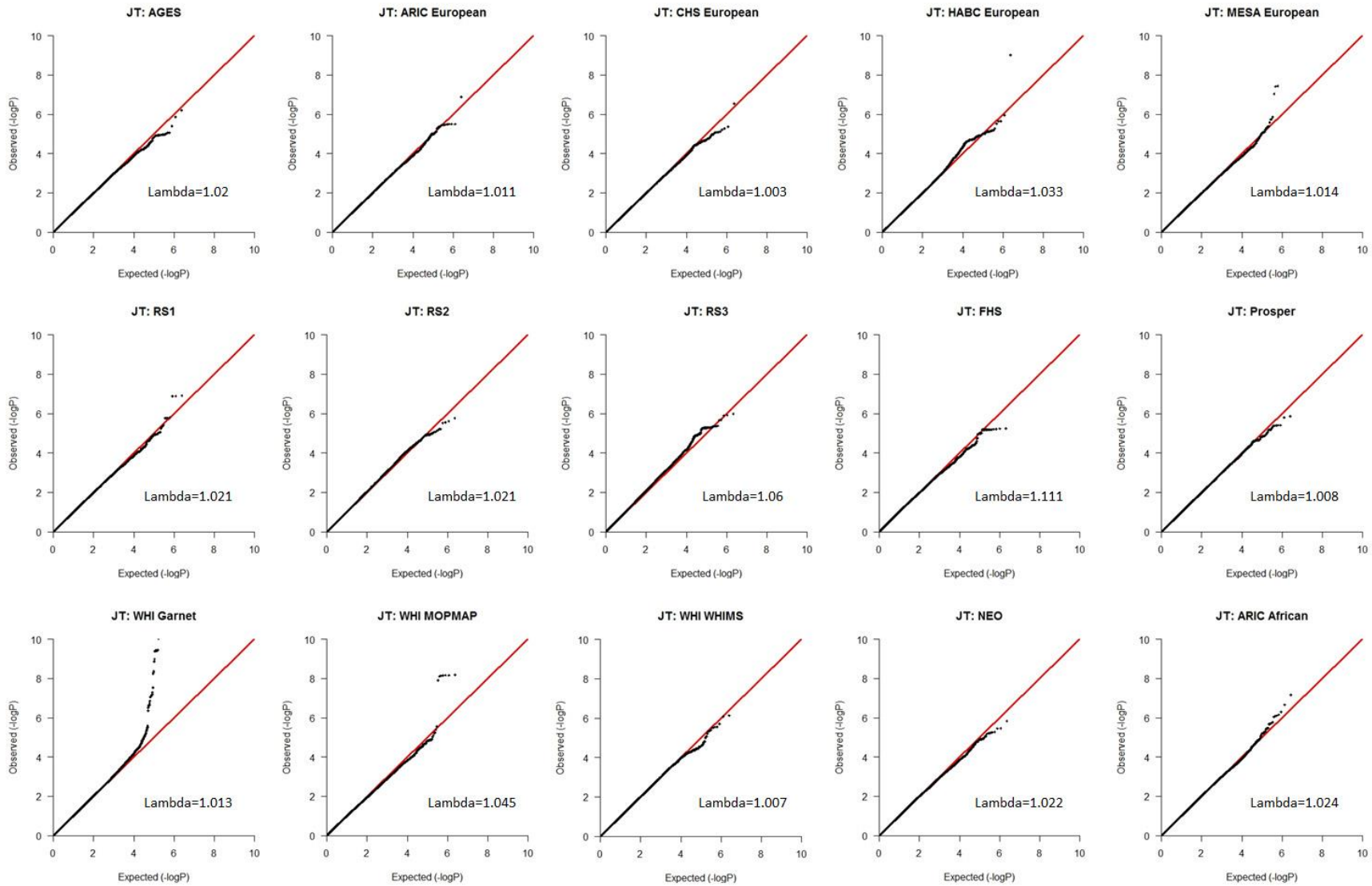
APPENDIX 2: STUDY- AND RACE/ETHNIC-SPECIFIC QUANTILE-QUANTILE PLOTS OF P-VALUES FOR THIAZIDE-SNP INTERACTION ESTIMATES IN ALL PARTICIPATING STUDIES FOR QT, QRS, AND JT INTERVAL ANALYSES

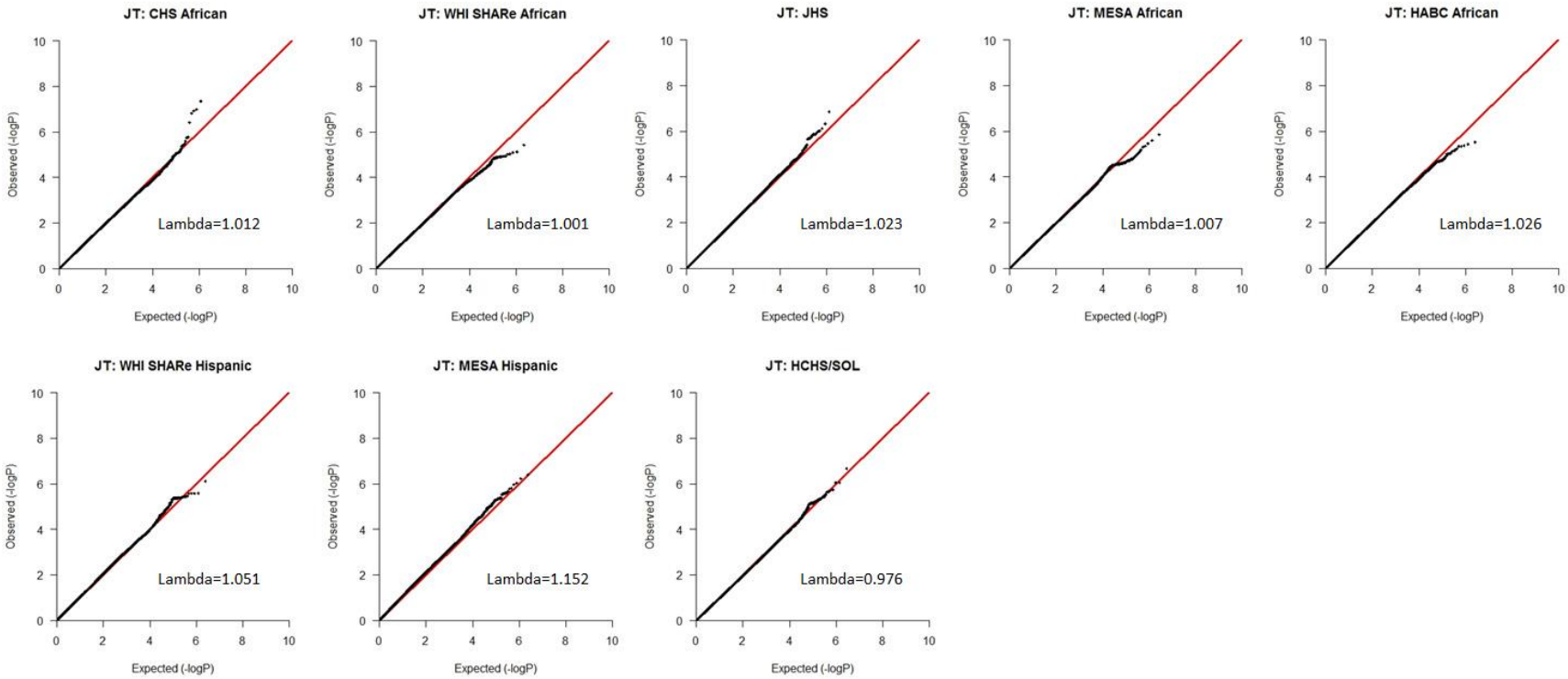



QT: ERF
Lambda=1.132
QT: HABC African
Lambda=1.027
QT: ARIC African
Lambda=1.044
QT: CHS African
Lambda=1.036
QT: WHI SHARe African
Lambda=1.013
QT: JHS
Lambda=1.012
QT: MESA African
Lambda=1.003
QT: HCHS/SOL
Lambda=1.069
QT: WHI SHARe Hispanic
Lambda=1.02
QT: MESA Hispanic
Lambda=1.107
Observed (-logP)
Expected (-logP)


QRS: AGES
Lambda=1.028
QRS: ARIC European
Lambda=1.024
QRS: CHS European
Lambda=1.019
QRS: HABC European
Lambda=1.022
QRS: MESA European
Lambda=1.013
QRS: RS1
Lambda=1.004
QRS: RS2
Lambda=1.04
QRS: RS3
Lambda=1.047
QRS: FHS
Lambda=1.1
QRS: Prosper
Lambda=0.999
QRS: WHI Garnet
Lambda=1.026
QRS: WHI MOPMAP
Lambda=1.039
QRS: WHI WHIMS
Lambda=1.022
QRS: NEO
Lambda=1.017
QRS: ARIC African
Lambda=1.021
Observed (-logP)
Expected (-logP)


QRS: CHS African
Lambda=1.068
QRS: WHI SHARe African
Lambda=1.004
QRS: JHS
Lambda=1.052
QRS: MESA African
Lambda=1.032
QRS: HABC African
Lambda=1.023
QRS: WHI SHARe Hispanic
Lambda=1.063
QRS: MESA Hispanic
Lambda=1.02
QRS: HCHS/SOL
Lambda=0.976
Observed (-logP)
Expected (-logP)


JT: AGES
Lambda=1.02
JT: ARIC European
Lambda=1.011
JT: CHS European
Lambda=1.003
JT: HABC European
Lambda=1.033
JT: MESA European
Lambda=1.014
JT: RS1
Lambda=1.021
JT: RS2
Lambda=1.021
JT: RS3
Lambda=1.06
JT: FHS
Lambda=1.111
JT: Prosper
Lambda=1.008
JT: WHI Garnet
Lambda=1.013
JT: WHI MOPMAP
Lambda=1.045
JT: WHI WHIMS
Lambda=1.007
JT: NEO
Lambda=1.022
JT: ARIC African
Lambda=1.024
Observed (-logP)
Expected (-logP)



AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; H2000, Health 2000; 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; MOPMAP, Modification of Particulate Matter-Mediated Arrhythmogenesis in Populations; NEO, the Netherlands Epidemiology of Obesity; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; QT, QT interval; RS, Rotterdam Study; SHARe, The SNP Health Association Resource; WHI CT, Women's Health Initiative Clinical Trial; WHIMS, the WHI Memory Study

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