Pharmacogenomics of Ventricular Conduction in Multi-Ethnic Populations

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LIST OF ABBREVIATIONS

AA	African American or African descent population
AC	Active comparitor
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

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. Pharmacogenomics studies 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.).[19-25] 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.[21] 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 begin by examining 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.

This work will then 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,[26] coronary heart disease,[27] congestive heart failure,[28] stroke,[29] cardiovascular mortality, and all-cause mortality.[30] Furthermore, QT is highly heritable (35-40%),[31-35] with early family studies identifying rare and highly penetrant mutations associated with long- and short-QT syndrome[36] and more recent GWAS identifying multiple

common single nucleotide polymorphisms (SNPs) associated with modest increases in QT.[37-42] Thiazide diuretics, an increasingly common antihypertensive therapy used by over a quarter of the hypertensive population in the U.S.,[43, 44] are one of many common pharmaceuticals that may cause QT prolongation.[45-47] However, the mechanisms underlying thiazide-induced QT prolongation is not well understood.[48-50] 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,[51] 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.

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: Examine the influence of informative missingness 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 under differing levels of informative missingness caused by prevalent user bias
b. Compare the results of aim 1a under different study designs (e.g. whole cohort, active comparator, new-user).

Specific Aim 2: 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 both fixed-effect and trans-ethnic meta-analytic techniques (N_{total}=94,479).
c. Characterize identified genetic variants using *in silico* functional characterization techniques including computer databases and pathway analysis.
d. Calculate the proportion of the population with clinically significant pharmacogenomics interactions as defined by federally mandated QT prolongation thresholds.[51]

3. Background and Significance

A. 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.[59] 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).[58] 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. Discover of the Structures of the Cardiac Conduction System

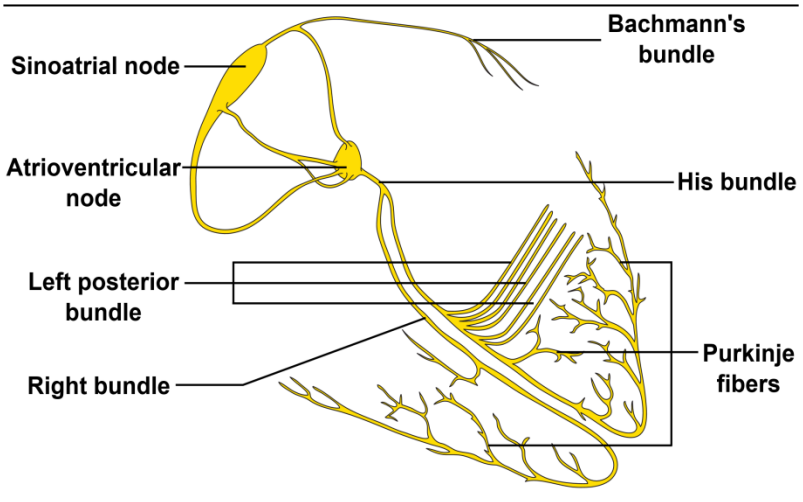
Year	Structure	Scientist
1845	Purkinje Fibers	J.E. Purkinje
1865/1893	Bundle of Kent	G. Paladino and A.F.S. Kent
1893	Bundle of His	W. His, Jr.
1906	AV Node	L. Aschoff and S. Tawara
1906/1907	Wenckebach Bundle	K.F. Wenckebach
1907	Sinus Node	A.G. Keith and M.W. Flack
1916	Bachmann Bundle	J.G. Bachmann

Adapted from Oto and Breithardt, 2001[58]

A.1. 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.

Figure 1. Electrical Conduction System of the Heart



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

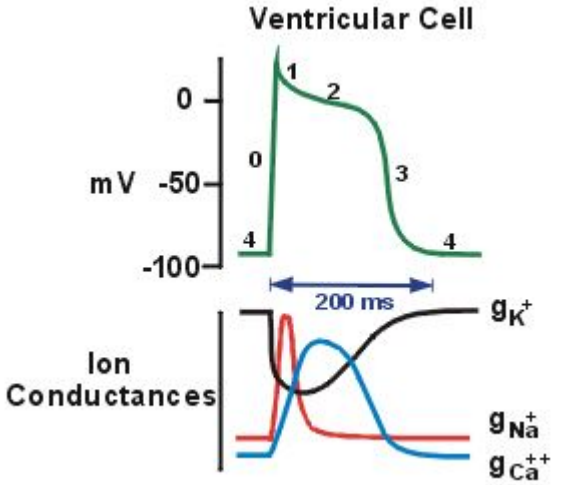
Pacemaker cells are capable of generating regular, spontaneous action potentials and are primarily found in the sinoatrial (SA) node and the atrioventricular (AV) node. These cells are responsible for generating the initial depolarizing current of the heartbeat. Myocytes make up the majority of cardiac 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.[59, 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

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

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 state, cardiac cells have a negative electrical potential relative to the outside of the cell.[59, 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.1.1. *Sodium Channels*

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

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]

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 C. QT Interval Genetics).

A.1.2. *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 $1/5^{th}$ 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.1.3. *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 replolarization.[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 C QT Interval Genetics).

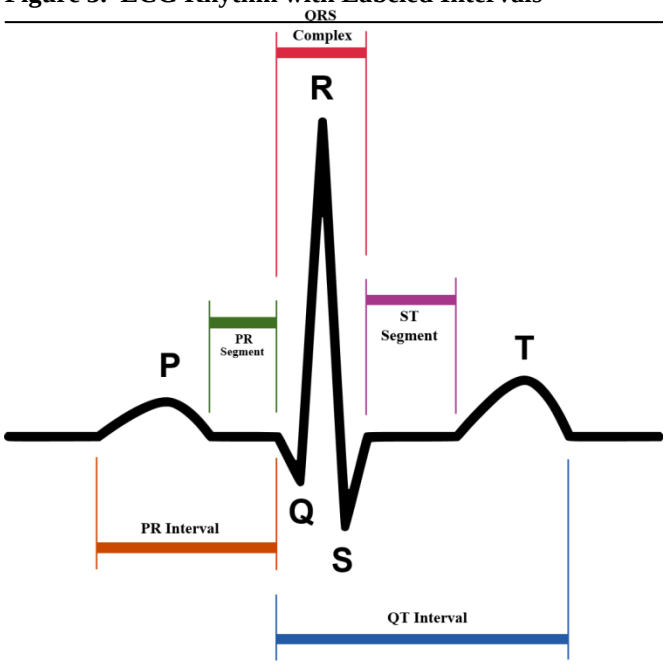
Table 3. Alpha Subunits of Cardiac Potassium Channels

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]

A.2. Ventricular Conduction on the Electrocardiogram

Figure 3. ECG Rhythm with Labeled Intervals



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

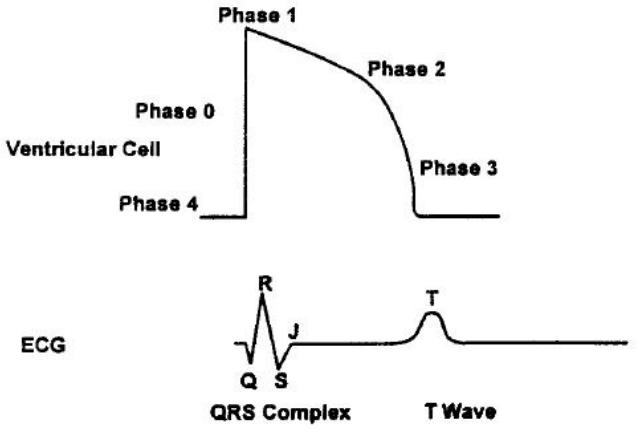
In 1887, a French scientist by the name of Gabriel Lippmann first demonstrated that the electrical impulses of the heart could be recorded from the body's surface.[58] Fifteen years later, Dutch physiologist Willem Einthoven published the first modern tracings from a surface electrocardiogram (ECG).[58] 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).[59, 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]

A.2.1. *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 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]

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

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 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]

A.2.1.1. *Heart Rate Correction Formulas for QT 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.[45, 75-77] 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.[45, 60] One of the most commonly used correction formula is Bazett's formula.[45, 77] However, Bazett's correction can be inaccurate at elevated heart rates.[45]

Table 4. Heart Rate Correction Formulae for QT Interval

Formula	Mathematical Form
Bazett[77]	$QT_c = \frac{QT}{\sqrt{RR}}$
Fridericia[79]	$QT_c = \frac{QT}{\sqrt[3]{RR}}$
Hodges[80]	$QT_c = QT + 1.75(Heart\ Rate - 60)$
Framingham[78]	$QT_c = QT + 0.154(1 - RR)$
Normogram[81]	$QT_c = QT + Correction\ Factor$

Adapted from Aytemir 1999[82]
QT: Uncorrected QT interval; QT_c: Corrected QT interval; RR: RR interval

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.[79] 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).[82] 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.[82, 86, 87]

A.2.2. *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.[59]

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]

A.2.3. *JT Interval*

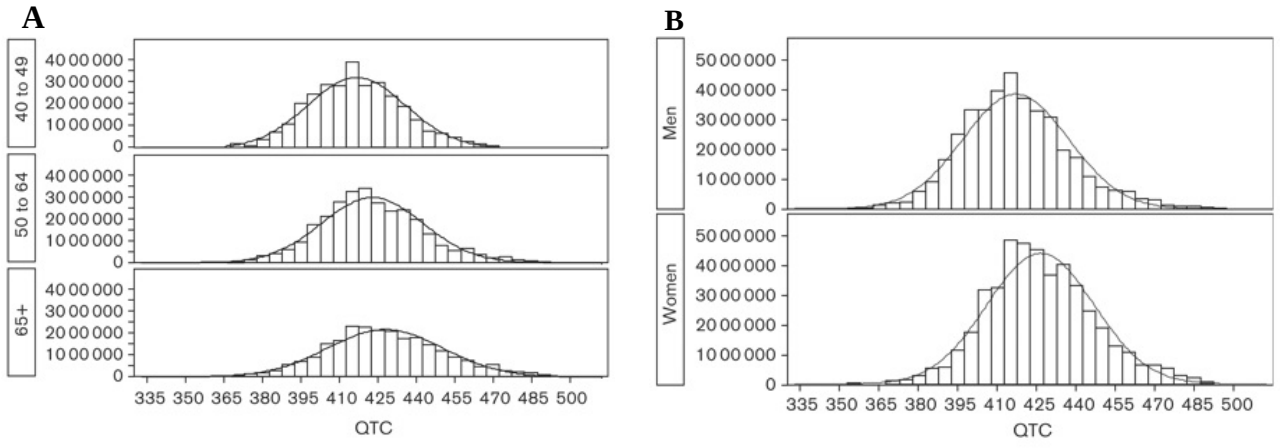
The JT interval is a measure of ventricular repolarization and is composed of the ST segment and the T wave. The ST segment represents the plateau phase of repolarization while the T wave represents phase 3 repolarization.[59] JT is generally calculated as $JT = QT - QRS$ rather than measured directly from the surface ECG. JT is highly correlated with QT but, unlike QT and QRS, JT has not been as commonly studied.[35] 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 B.3. 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 B.4.2. *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.

B. QT Interval Prolongation

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).[77, 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

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

innate and acquired risk factors.

B.1. 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.

B.1.1. *Acquired Clinical Conditions*

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]

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

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 B.3. Drug-Induced QT Prolongation).[46]

B.1.2. *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.[31, 32, 40, 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.[58] 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 C. QT Interval Genetics.

B.1.3. *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]

B.2. 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.[46, 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.[51] 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.

B.2.1. *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]

B.2.1.1 *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] 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

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]

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 D. Thiazide Diuretics.

B.2.1.2 *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 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 Iriibarren *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]

B.2.2. *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

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		Citalopram	Ketoconazole	Diphenhydramine
Gatifloxacin	trioxide	**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**	Crizotinib	Desipramine	Pimozide	Telaprevir
Sparfloxacin	**Relaxant**	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	Dexmedetomide
		Cocaine	Probucol	Quetiapine	Chloral hydrate
		Tetrabenazine	Lithium	Risperidone	
		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]

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.

B.2.3. *Clinical Considerations*

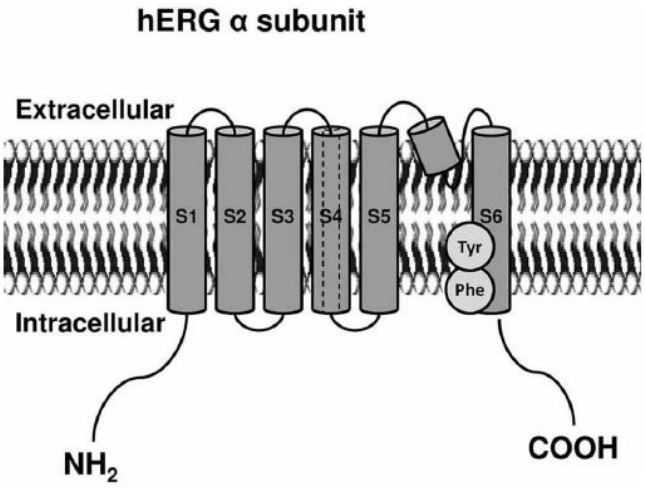
The continued use of QT prolonging medications despite the risk of severe negative outcomes has been widely acknowledged by the medical community.[151, 152] Physicians must weigh the risks and benefits of the use of such medications. In cases where effective alternative treatments are available, the risks of using a QT prolonging agent would outweigh the benefits.[46] Also, in cases where multiple risk factors for prolonged QT are present, adding a QT prolonging medication presents particular concern.[45] 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.[46] 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.[45] 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.

B.2.4. *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] 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]

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

B.3. 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,[27, 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.[46, 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 C. QT Interval Genetics). Thus, we have chosen to evaluate QT as a continuous outcomes in order to identify risk factors which have smaller although still important effects on QT.

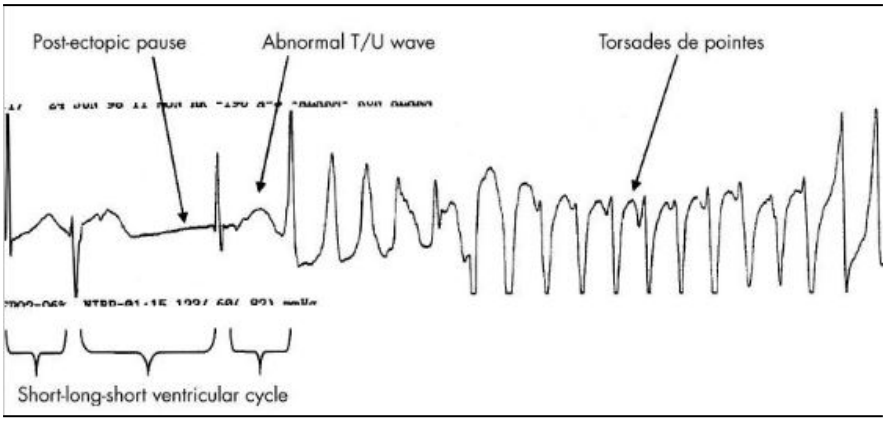
B.4. Potential Clinical Outcomes of Prolonged QT

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),[27] congestive heart failure (CHF),[164] stroke,[165] and both cardiovascular and all-cause mortality.[30]

B.4.1. *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

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



Adapted from Yap and Camm 2003[153]

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 fibrillation and cardiac arrest, often leading to SCD.[26, 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.

B.4.2. *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.[43] QT_c prolongation is an established risk factor for CHD and CHD mortality.[27, 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).[27] 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, 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

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

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]

B.4.3. *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.[43, 181] Both prolonged QT and QRS have been associated with CHF.[28, 164, 177, 182] Dhingra *et al.* found that, in an elderly population of 1,759 white men and women, extreme values of QRS (QRS $\geq$ 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.[28] 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.

B.4.4. *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.[43] 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).[29] 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 $\geq$ 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.

B.4.5. *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,[27, 158, 177, 185] Hispanics,[185] and American Indians,[157] suggesting that the association generalizes across race/ethnicities. Finally,

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[187]	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[188]	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[189]	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

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.

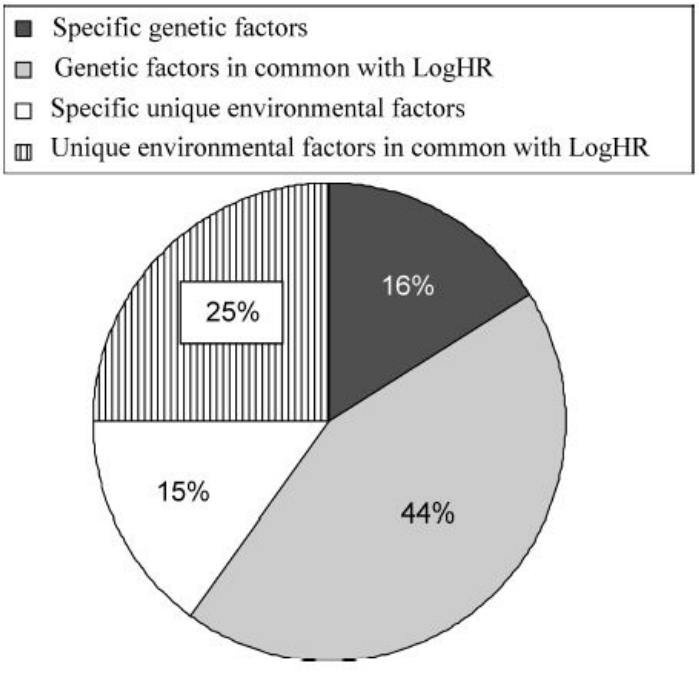
C. QT Interval Genetics

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.

C.1. 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.[31, 33, 35, 131, 190-193] 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%.

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).[190] 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.[192] 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.[192] 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%).[192] 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.[33, 193] A more recent study by Newton-Cheh *et al.* found the heritability of JT to be 25%.[35] 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.

C.2. 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.

C.2.1. *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

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

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.[36] 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.[36]

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.[36] 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*).[36, 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.

C.2.2. *Family/Twin Studies*

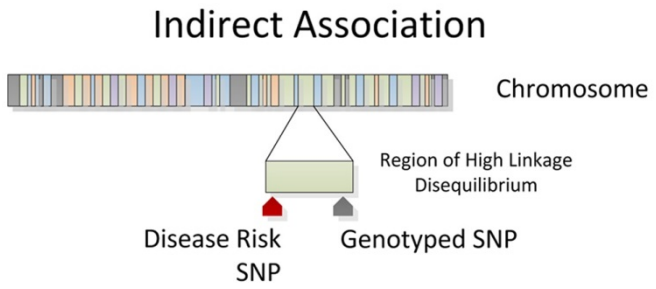
While research in families with LQTS or SQTS worked in identifying the genes associated with the rare congenital forms of the disorders, they did not establish if the same genes, or others, influenced QT variability on a population level. To determine if the same genes were involved, early research on 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*.[193] 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.[35]

C.2.3. *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 (*KCN Q1, 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.3. Genome-Wide Association Studies

Figure 9. Indirect Associations in GWAS Using Linkage Diseqiulibrium



Adapted from Bush 2012[201]

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, Table A1).[37] However, Arking studied only the extremes of the QT distribution. Later GWAS which

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[206]	QT	EU	1,345	FHS		*NOS1AP*
Marroni, 2009[39]	QT	EU	2,325	EUROSPAN		*NOS1AP*
Newton-Cheh, 2009[40]	QT	EU	13,685	CHS, FHS, RS	*KCNH2* *KCNQ1* *SCN5A*	*LIG3* *LITAF* *NDRG4* *NOS1AP* *PLN*
Nolte, 2009[41]	QT	EU	3,558	BRIGHT, DCCT/EDIC, TwinsUK		*NOS1AP* *PLN*
Pfeufer, 2009[42]	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[38]	QT QRS	AS	6,543	London Life Sciences on the Indian Subcontinent	*KCNH2* *KCNJ2* *SCN5A* *SCN10A*	*ATP1B1* *LITAF* *NOS1AP* *PLN*
Holm, 2010[208]	QT QRS	EU	9,860	Icelandic Cohort	*KCNE1* *KCNH2* *KCNQ1*	*ATP1B1* *LITAF* *NDRG4* *TBX5*
Sotoodehnia, 2010[209]	QRS	EU	40,407	CHARGE	*CACNA1D* *SCN10A* *SCN5A*	*PLN* *TBX5*
Kim, 2012[210]	QT	AS	6,805	KARE	*KCNH2* *KCNQ1* *SCN5A*	*NDRG4* *NOS1AP* *PLN* *SLC8A1*
Smith, 2012[211]	QT	AA	12,097	COGENT, CARe, WHI		*ATP1B1* *NOS1AP* *SLC8A1*
Ritchie, 2013[212]	QRS	EU	5,272	eMERGE	*SCN5A* *SCN10A*	
Arking, 2014[213]	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

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[38, 207, 210] and one in African descent populations.[211] 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.3.1. *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 (Table A1). 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,[42, 209, 213] *SCN10A* has only been associated with QRS,[209, 212] 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.[209] Furthermore, the single calcium channel gene identified is not the same as the calcium channel genes potentially associated with congenital LQTS.[198, 209]

C.3.2. *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 (Table A1). 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.[38, 40-42, 211, 213] 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.*[215-218] Furthermore, *PLN* has also been associated with left ventricular structure and is known to affect rates of cardiac contraction in mice.[41, 219] 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.3.3. *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).[220-222] Furthermore, SNPs from *NDRG4, KCNE1, SCN5A, SCN10A,* and *KCNH2* have been generalized to AA populations for QT and QRS.[221, 223] Additionally, the following loci have been generalized to American Indian populations: *ATP1B1*, *SCN5A, PLN, KCNH2, KCNQ1, LITAF*, and *NDRG4.*[221] Only four additional loci have been generalized to HLs: *ATP1B1, KCNH2, LITAF,* and *NDRG4.*[221] 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.[224] 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.[225] 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.[226] 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.[210, 216, 227] This illustrates why it is imperative to expand genetic studies beyond EU populations and include a diverse range of populations.

C.4. 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.[228, 229] Furthermore, a recent study in AAs identified a mutation in *SCN5A* for which hypokalemia can moderate its association.[230] 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.[213] One common hypothesis for this "missing heritability" is GxE interactions.[231] 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.

D. Thiazide Diuretics

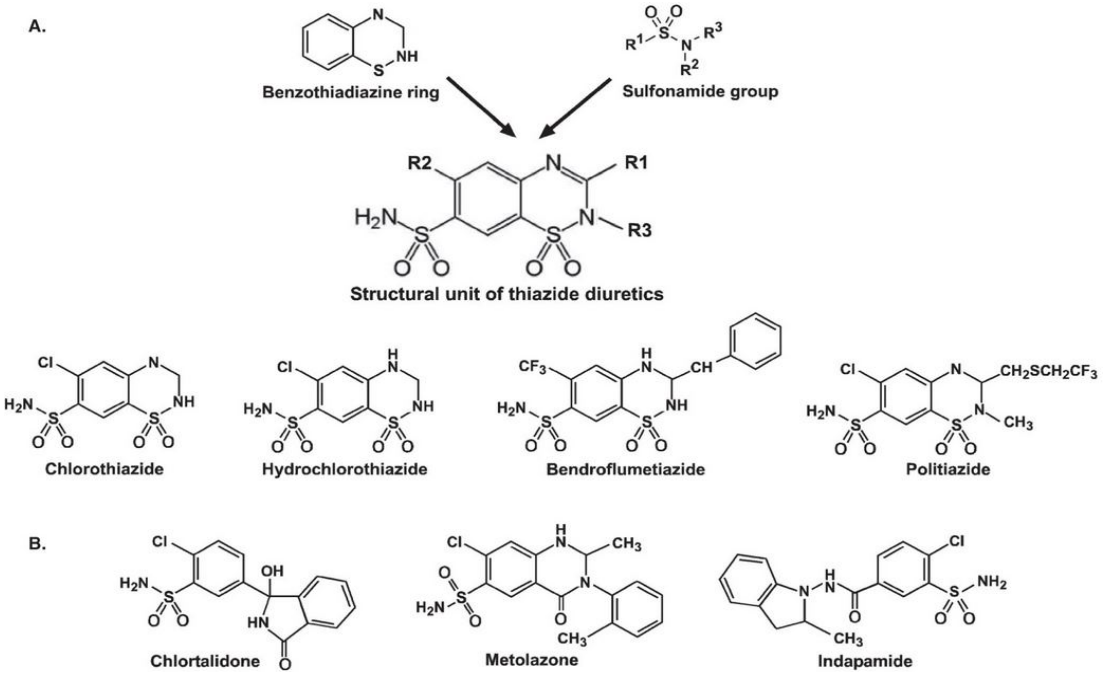
Thiazide diuretics are a promising candidate for GxE inquiry in QT, as this class of medications has been associated with QT prolongation (Section D.4. Thiazide-Induced QT Prolongation). Briefly, thiazide and thiazide-like diuretics are members of a class of pharmaceuticals which increase renal excretion of sodium and water. Thiazides were first

released in 1957 as an antihypertensive and have since become a critical drug in hypertension treatment.[47, 232] 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.[47] 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 pharmacology of thiazides and the association between thiazides and QT.

D.1. 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).[47] The NCC is encoded by *SLC12A3* from the SLC family of genes.[47, 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

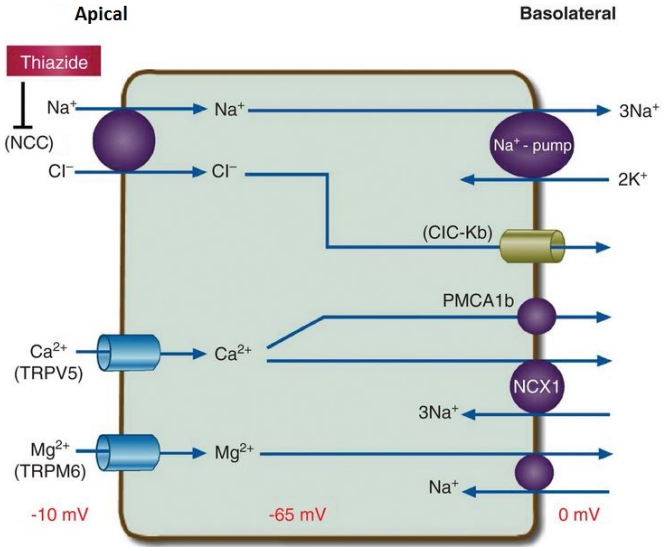
Figure 10. Molecular Structure of Thiazide and Thiazide-Like Diuretics



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 benzothiadiazine group

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*.[47] Conversely, thiazide use increases 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).[47]

Figure 11. Transport Mechanisms of the Distal Convoluted Tubule



Adapted from Tamargo 2014[47]

D.2.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.[47] 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.[233] 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.[234] Similarly, thiazides are used to treat edema associated with liver cirrhosis and renal therapy.[235] Thiazides have also been found effective in treating osteoporosis, likely due to its effects on calcium reasbsorption.[236, 237] The same mechanism leads to the usage of thiazides to treat calcium-based kidney stones.[238]

D.2.1. *Contraindications*

Thiazides are contraindicated in patients with anuria, renal failure, an allergy to thiazides or other sulfonamide drugs, or hepatic coma.[235] 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.[47]

D.3. 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.[43, 44, 239] 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).[44] The majority of those using a thiazide (>90%) were using it in conjunction with other drugs in polytherapy.[44] 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).[44, 47, 240] 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%

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]

adherence to angiotensin receptor blockers, the drug class with the highest levels of adherence.[241, 242] 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]

D.4. 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.[45-47] 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.4.1. *Pharmacoepidemiology*

Associations between thiazide diuretics, ECG abnormalities, and arrhythmogenic death was first reported in the 1980s,[243-246] when Hollifield and Slaton found that patients taking hydrochlorothiazide were more likely to suffer SCD than patients not on a thiazide diuretic.[243] 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.[247, 248] It was estimated that, in 1994, thiazide use was responsible for more than 10% of all SCD in the Netherlands, totaling 120 deaths.[249] 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).[250] 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.[243, 251] 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 (hydroclorothiazide or chlorthalidone).[244] 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.[252] 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.[252]

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.[246] 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.[245] 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).[253]

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.4.2. *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,[254-257] 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.[49] Most cases of GS are caused by mutations in the *SLC12A3* gene, which encodes the thiazide-sensitive NCC (Figure 11).[49] Studies of patients with GS have found that QT is significantly prolonged in these patients and they are at higher risk for cardiac arrhythmias.[48, 258]

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.[50] 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.[259] 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.

E. 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.[260] Genetic variants identified in the *CYP2C9* and *VKORC1* explain up to 50% of the variability in dose response to warfarin.[260] 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, 261] 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.[260, 262] These examples illustrate the potential clinical significance of pharmacogenomics research.

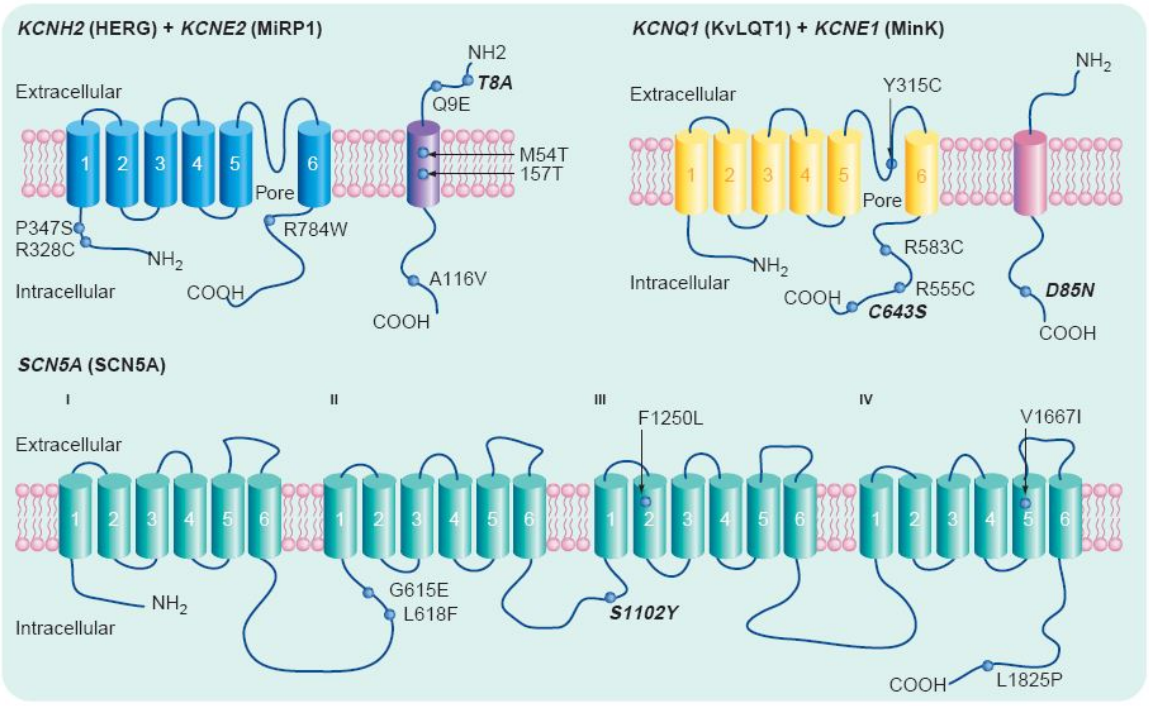
E.1. Pharmacogenomics of QT-Prolonging Drugs

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.[263-268] 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).[267, 269] 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).[265]

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).[270] A candidate gene analysis of antipsychotics and QT also identified *NOS1AP* as a modifier of diLQTS.[271] In addition, two genes encoding cytochrome P450 enzymes, *CYP2D6* and *CYP3A4*, enzymes involved in drug metabolism, have

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

been associated with diLQTS.[264, 272] 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.[264] 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.

E.2. 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.[273-275] 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*.[275-277] 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.[278, 279]

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.[280] 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.[281] 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.[282] 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. Additioanlly, 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.

E.2.1. *Thiazide Diuretics and QT Prolongation*

To date, one study of the pharmacogenomics of thiazides and QT in EU populations has been conducted.[283] 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.

F. 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.[284] 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.[19, 22, 285] Observational settings often provide larger sample sizes, greater statistical power, and better generalization to a broader population than RCTs.[285] Unfortunately, observational studies also are subject to many biases, such as selection bias.[285] 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.[285, 286] 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.[287-289] 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.[21] 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.[21, 22] 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.[21] 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.

G. Multi-Ethnic Populations

Minority populations are historically underrepresented in clinical and health research.[290-292] 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.[293] Minority populations hold a disproportionate burden of negative health outcomes compared to the EU populations which represent the majority of healthcare research to date.[294, 295] In particular, AAs have a higher risk of diLQTS,[296] a higher risk of mortality due to QT prolongation,[297] and a higher prevalence of thiazide use compared to other race/ethnic groups.[44]

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.[298] 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.[299] As of 2014, there are over 130 pharmaceuticals with FDA-approved genetic information on their labels.[300] 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.[301] 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.

H. 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, 302-314] As of 2002, the FDA's Adverse Event Reporting System, begun in 1969, had recorded 2.3 million cases of adverse events.[315] 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, 316] 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.[44] 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[317] or clopidogrel prescribing.[318] Furthermore, pharmacogenomics findings can be used to alter medication labels, or, in extreme cases, remove drugs from the market.[319, 320] 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.

4. Research Plan

A. Overview

This work will be conducted in two parts. In specific aim 1, 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. Results from specific aim 1 will then be used to inform the interpretation of results from specific aim 2, which will be a pharmacogenomics study of the thiazide-QT relationship. Specific aim 2 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.

B. Specific Aim 1

Examine the influence of informative missingness caused by prevalent user bias on a pharmacogenomics study conducted in an observational setting.

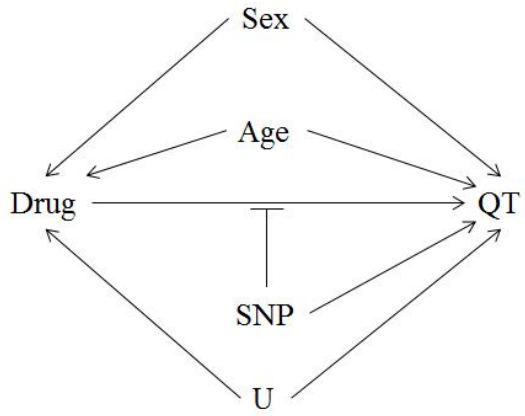
> *Using simulations, evaluate bias, power, and type I error in the drug-SNP interaction associated with specific reference groups (e.g. whole cohort, active comparator, new-user) alone and under differing levels of informative missingness.*

B.1. 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 13). 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 current conceptual model includes three covariates besides the SNP: age, sex, U, which represents unknown/unmeasured confounders of the drug-QT relationship. The conceptual model represented in Figure 13 is based on a single visit. However, for our simulation analysis, we will

simulate two visits. The correlation between variables at Visit 1 and Visit 2 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).

Figure 13. 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
U represents unknown/unmeasured confounders of the thiazide-QT relationship

B.2. Simulation Parameters and Values

For a summary of all variables to be simulated in this analysis, see Table 13.

B.2.1. *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.

B.2.2. *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.2.3. *Drug Use*

QT-prolonging drug use at visit 1 will be predicted conditional on age and sex using a logit function:

$$Logit\big(pr(Drug = 1)\big) = \alpha_0 + \alpha_1 Age_{visit\ 1} + \alpha_2 Sex$$

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. QT-prolonging drug use at visit 2 will be predicted using the same prediction function as visit one along with a term for QT-

prolonging drug use at visit 1, for which the correlation will be determined using ARIC visit 1 and 2.

QT-prolonging drug use at visit 2 will be predicted conditional on age, sex, use of a QT-prolonging drug at visit 1, and the probability of an adverse drug event (ADR) occurring between visits 1 and 2 using a logit function:

$$Logit\big(pr(Drug = 1)\big) = \alpha_0 + \alpha_1 Age_{V2} + \alpha_2 Sex + \alpha_3 Drug_{V1} + \alpha_4 \Pr(ADR) \times \Pr(Drug_{V1-2})$$

The probability of an adverse drug reaction between visits will be varied between models. The probability that an observation was using a QT-prolonging medication during the periods between visits 1 and 2 ($Drug_{V1-2)}$ will be 1 if the observation was on a QT-prolonger at visit 1. For all remaining observations, this probability will be determined by the expected rate of QT initiation over a 2 year period, according to rates reported in the literature.

For the AC study design, an alternative drug variable is needed. The alternative drug for visit *i* will be simulated using a logit function, conditional on age and sex, excluding all individuals on a QT-prolonger at visit *i*:

$$Logit\big(pr(Drug_{Ai} = 1)\big) = \alpha_0 + \alpha_1 Age_i + \alpha_2 Sex$$

For the NU study design, the duration of use for QT-prolongers at visit 2 is needed.

Table 13. Simulation Parameters and Scenarios

Parameter	Simulation Scenario	Static or Variable
Age	Normal distribution, mean determined using ARIC data	Static
Sex	Uniform random variable with defined probability of being male	Static
Unmeasured/Unknown Confounders (U)	Normal distribution, mean determined by the correlation between QT in visits 1 and 2 in ARIC study	Static
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	Variable
QT Prolonging Drug Use at Visit 1 ($Drug_1$)	Logit function conditional on age and sex	Static
QT Prolonging Drug Use at Visit 2 ($Drug_2$)	Logit function conditional on age, sex, $drug_1$, and the product of the probability of QT prolonging drug use between visits and probability of an adverse event among those on a QT prolonger	Variable
QT at Visit i (QT_i)	Linear function conditional on $drug_i$, SNP, age, sex, U, and the product of $drug_i$ and SNP, where i indicates visit 1 or 2	Variable
Alternative Drug at Visits i ($Drug_{Ai}$)	Logit function conditional on age and sex, and excluding those on QT prolonger at visit *i*	Variable
Time on QT Prolonging Drug at Visit 2 (Time)	Normal distribution, mean determined using WHI data, and excluding those on QT prolonger at visit 1	Variable

Duration of use will be simulated using a normal distribution with a mean value determined using WHI data. Observations with drug use at visit 1 will be excluded.

B.2.4. *QT Interval*

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

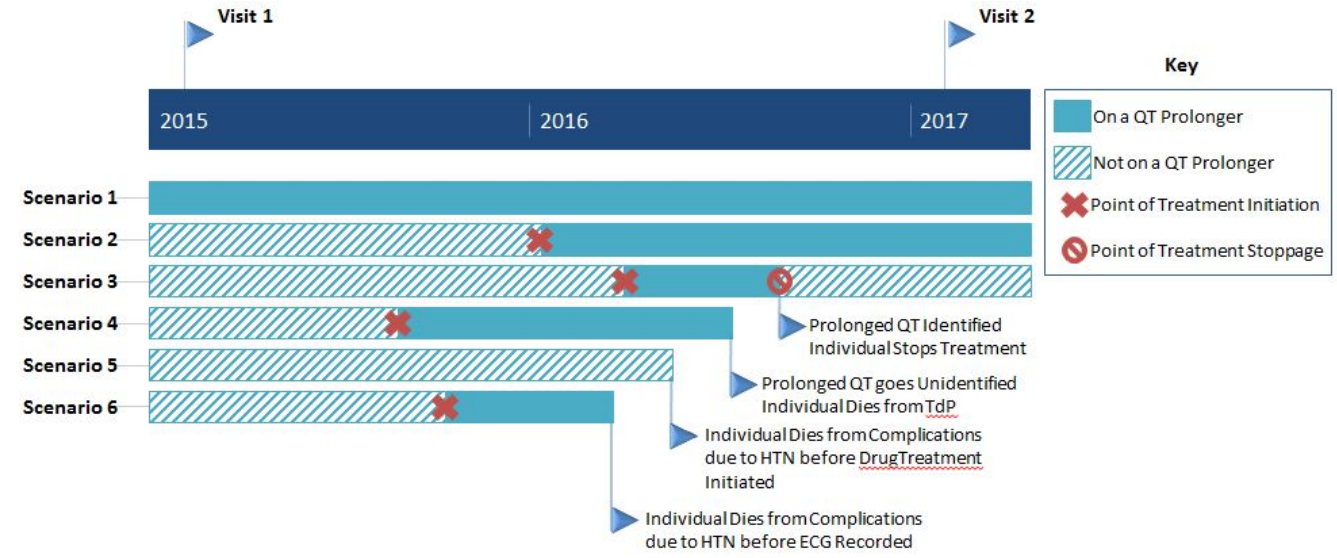
$$QT_i = \beta_0 + \beta_1 Drug_i + \beta_2 SNP + \beta_3 Drug_i \times SNP + \beta_4 Age_i + \beta_5 Sex + \beta_6 U + \varepsilon$$

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

B.3. Simulation Models and Analyses

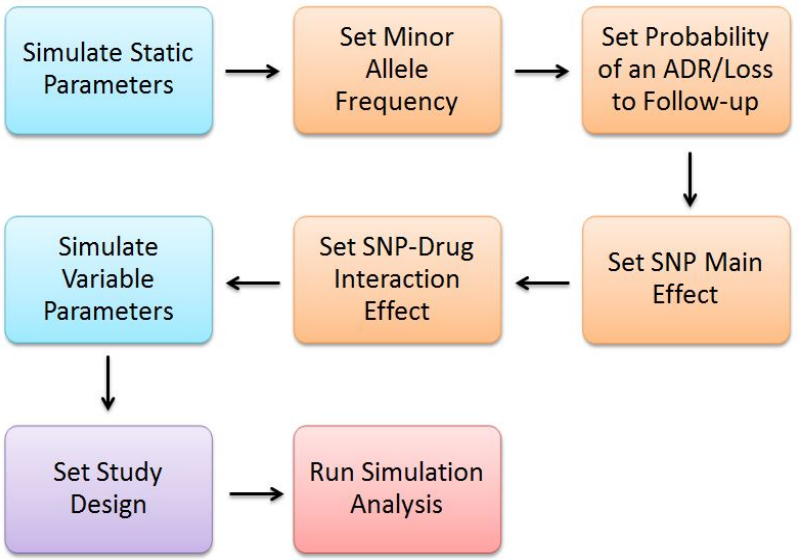
Multiple simulation models will be run to compare different conditions. The effect of age, sex, heart rate, and U will not vary across models. The SNP main effect, drug-SNP interaction, and the MAF will be varied across models. Furthermore, to test the effect of prevalent user bias on the interaction effect, different levels of informative missingness will be tested. Figure 14 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 user bias through differential misclassification (scenarios 3-4) or depletion of susceptibles (scenarios 5-6). The simulation of $Drug_2$ accounts for scenario 3 by accounting for both the probability that a QT-prolonger was initiated between visits 1 and 2 and the probability that an ADR occurred. Scenarios 4-6 will be accounted for by randomly assigning missing data at visit 2. The rate of

Figure 14. Scenarios Leading to Prevalent User Bias in Pharmacoepidemiology Studies



missing data will vary across studies and to simplify models, it will be assumed that the rate of informative missingness is equivalent to the rate of an ADR.

Figure 15. Flowchart of Simulation Analysis Process



The following parameters will be varied across models: MAF, probability of an ADR/loss to follow-up (Pr(ADR)), SNP main effect, and SNP-drug interaction effect. Variable MAF will cause the SNP parameter to vary across models. The variable Pr(ADR) will cause the $Drug_2$ parameter to vary across models. Variation in the SNP and $Drug_2$ parameters will subsequently cause the QT_1, QT_2, $Drug_{A2}$, and duration of use (Time) parameters 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 15. All models will estimate the QT-prolonging drug-SNP interaction using generalized estimating equations with an independence working correlation matrix.

C. Specific Aim 2

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.

> *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]*

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 both fixed-effect and trans-ethnic meta-analytic techniques (N_{total}=94,479).

Characterize identified genetic variants using in silico functional characterization techniques including computer databases and pathway analysis.

Calculate the proportion of the population with clinically significant pharmacogenomic interactions as defined by federally mandated QT prolongation thresholds.[51]

C.1. Study Populations

This study will make use of fifteen separate population-based cohort studies (Table 14). 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 14.

C.1.1. *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.

C.1.2. *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

Table 14. Study Population Characteristics

			CHARGE												
Characteristic	WHI	SOL	AGES	ARIC	CHS	FHS	RS	ERF	Health 2000	Health ABC	CARDIA	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	5,155	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	3,756	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	85-86	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	25	10	0	0	9
Mean Age (yrs)	64	46	77	54	72	55	65	48	50	74	25	62	75	56	55
% Female	100	46	58	55	61	53	58	59	52	52	56	54	54	57	55
Mean QT	401	415	406	398	414	414	399	398	389	413	387	412	414	NA	413
% Thiazides	18	NA	24	12	21	3	3	2	7	11	NA	13	26	NA	25
% Race/Eth															
European Am	65	0	100	77	85	100	94	100	100	60	53	32	100	100	0
African Am	24	0	0	23	15	0	0	0	0	40	47	33	0	0	100
Hispanic	10	100	0	0	0	0	0	0	0	0	0	26	0	0	0
Other	1	0	0	0	0	0	6	0	0	0	0	9	0	0	0

AGES, Age, Gene/Environment Susceptibility – Reykjavik Study; ARIC, Atherosclerosis Risk in Communities; CARDIA, Coronary Artery Risk Development in Young Adults; 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)

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.

C.1.3. *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).

C.1.3.1. *Age, Gene/Environment Susceptibility – Reykjavik Study*

The Age, Gene/Environment Susceptibility – Reykajavik 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.[321] 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.

C.1.3.2. *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.[322] 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.

C.1.3.3. *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.[323] 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.

C.1.3.4. *Coronary Artery Risk Development in Young Adults Study*

The Coronary Artery Risk Development in Young Adults (CARDIA) study collected data on 5,115 EU or AA individuals, aged 18-30 years, from four communities: Birmingham, Alabama; Minneapolis, Minnesota; Chicago, Illinois; and Oakland, California.[324] Participants with long-term disease or disability or who were pregnant or less than three months post-partum were ineligible.

C.1.3.5. *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.[325, 326] 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.

C.1.3.6. *Framingham Heart Study*

The Framingham Heart Study (FHS) collected data on 5,209 participants aged 28 – 62 from residents of Framingham, Massachusetts.[327-329] 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.

C.1.3.7. *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.[330] 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.

C.1.3.8. *Health 2000*

The Health 2000 study is a population-based health examination survey carried out in Finland from 2000 to 2001.[331, 332] 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.

C.1.3.9. *Jackson Heart Study*

The Jackson Heart Study (JHS) collected data on 5,301 non-institutionalized AA individuals from Jackson, Mississippi, aged 35 – 84 years.[333] Participants who were physically or mentally incompetent were excluded.

C.1.3.10. *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.[334] 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.

C.1.3.11. *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.[335] 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.

C.1.3.12. *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.[336, 337] 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.

C.1.3.13. *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.[338, 339] 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.

C.1.4. *Exclusion Criteria*

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 15, 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.

Table 15. 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

C.2. 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 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.3. Exposure Assessment

C.3.1. *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 14 shows the prevalence of individuals taking a thiazide diuretic at a minimum of one study visit.

Table 16. 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
CARDIA	Affymetrix Genome-wide Human SNP Array 6.0	934,940
MESA	Affymetrix Genome-wide Human SNP Array 6.0	934,940
PROSPER	Illumina 660K	557,192
NEO	TBD	TBD
JHS	Affymetrix Genome-wide Human SNP Array 6.0	934,940

Genotyping information for the NEO study was not available as of this proposal, as this study was a late addition to the list of participating cohorts. This information will be available for the final dissertation project.

C.3.2. *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 16 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[340-343] or 1000 Genomes[344, 345] data.

C.4. Data Analysis

C.4.1. *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 study specific measures of principal components of genetic ancestry, study site or region, and relatedness when appropriate.

C.4.1.1. *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 i^{th} 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.

C.4.1.2. *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 i^{th} participant at the j^{th} 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.

C.4.2. *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.[346] 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.[347]

C.4.2.1. *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 $P < 5x10^{-8}$.[346] 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.[348] 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,[349] 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.[350] 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 $N_{exposed}$.

C.4.2.2. *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.[347] 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.[347] 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. Because of this, we will rely on race/ethnic-specific effect sizes as determined using fixed-effect meta-analysis (see above) when extrapolating results to the U.S. population (see Section C.4.5. *Extrapolation to U.S. Population*)

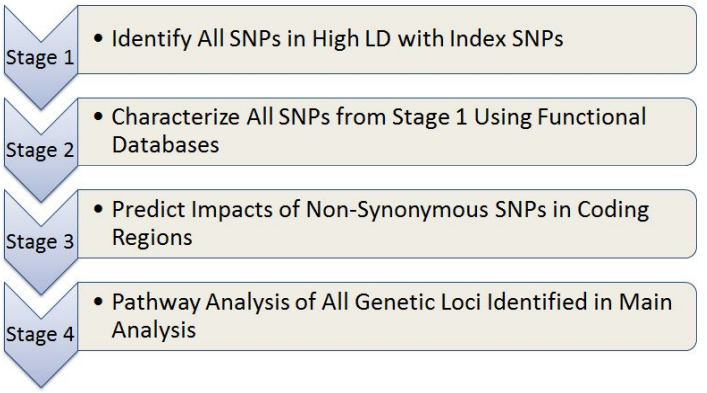
C.4.3. *Sensitivity Analyses*

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.[230] 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 C.2. 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.

C.4.4. *In-Silico Functional Characterization*

Figure 16. Flowchart of *In-Silico* Functional Characterization



For all SNPs identified in the above analyses, including the sensitivity analyses, I will conduct *in-silico* functional characterization. The *in-silico* characterization will be carried out in four stages (Figure 16). 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[344, 345] data and linked SNPs will be determined using two online databases: SNAP[351] and HaploReg.[352] 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[353] and the UCSC GenomeBrowser,[354] which makes use of previous functional characterization, as well as the recent ENCODE project,[355] 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[356-358] and PolyPhen 2[359] 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)[360] to identify potential linkages within the genome between the genetic loci associated with drug response.

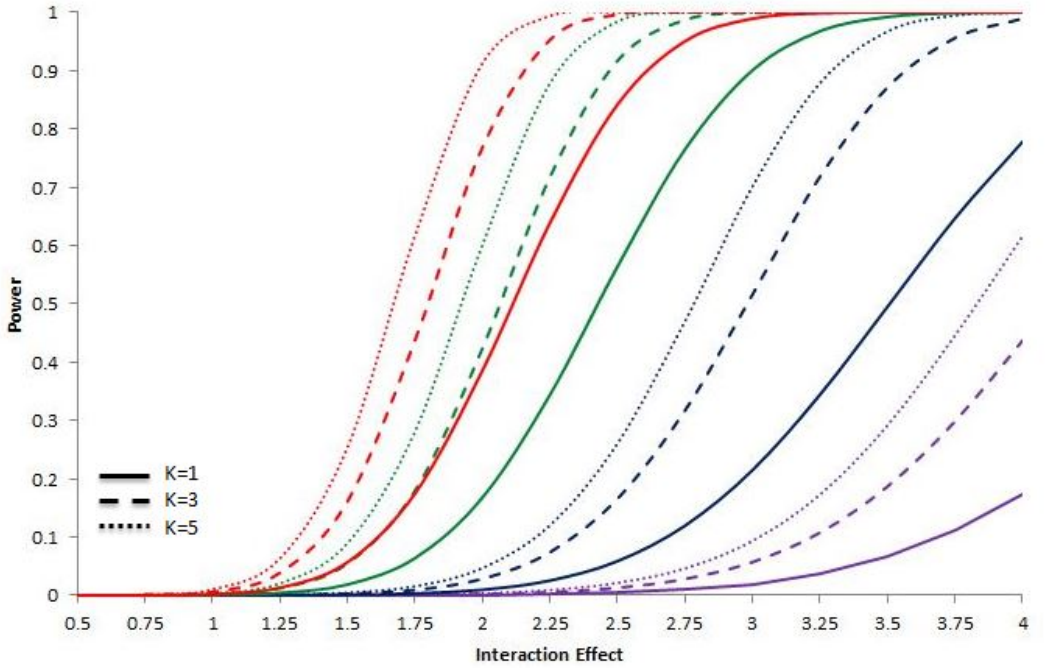
C.4.5. *Extrapolation to U.S. Population*

To determine the potential impact of my findings, I will calculate the proportion of the U.S. population with clinical significant pharmacogenomic interactions. Clinical significance will be defined using the Food and Drug Administration's guidelines for QT prolongation. These guidelines define QT prolongation as an increase of 5 ms or more.[51] For the most significant associations at each locus, I will determine the expected minor allele frequency by race/ethnicity using the following populations: the ARIC study (EU and AA) and SOL (HL). Genotypes will be assigned assuming Hardy-Weinberg equilibrium and Mendelian laws of segregation. The population size for each race/ethnicity will be determined using the 2010 U.S. census. The NHANES III population will be used to determine the proportion of the U.S. population using a thiazide diuretic. All calculations will be made under the assumption that the genotype does not affect thiazide usage. Proportions will be calculated in EU, AA, and HL populations separately to account for differing allele frequencies and thiazide use by race/ethnicity.

C.5. Sample Size and Statistical Power

Power was calculated using Quanto[361] 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 14), 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 17). 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

Figure 17. 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)

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.

D. Integration of Specific Aims 1 and 2

I will use the results of Specific Aim 1 to inform the interpretation of results from Specific Aim 2. 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 1 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 use the results from Specific Aim 1 to inform interpretation of the results of Specific Aim 2.

5. 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 (94,479), 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.

6. Conclusions

QT interval prolongation is a known and potentially fatal side effect of many common pharmaceuticals, including thiazide diuretics, a common antihypertensive treatment.[43, 44, 46] Pharmacogenomics research represents a promising step forward in understanding and preventing adverse drug reactions like QT prolongation. QT is highly heritable (35-40%), with many known genetic loci influencing its duration.[31-35, 213] Furthermore, there have been multiple pharmacogenomic studies of thiazides, which have identified numerous loci influencing patient response and side effects to thiazide use.[275, 277, 280, 281] However, the underlying mechanism of the relationship between thiazides and QT is poorly understood. Theories range from a direct effect of thiazides on cardiac conduction mechanisms[50, 259] to an indirect effect through electrolyte levels.[48, 49, 258] Together, these facts indicate the thiazide-QT relationship is a promising candidate for pharmacogenomics study.

I therefore have proposed the first large, multi-ethnic pharmacogenomics study of thiazide diuretics and QT. Using fourteen large, observational cohort studies, I will conduct a genome-wide analysis of the thiazide-gene interaction and its effects on QT. However, because a form of selection bias called prevalent user bias is a known concern of pharmacoepidemiologic studies conducted in observational settings but its effects are unclear on the interaction effects under study in pharmacogenomics studies,[285, 288] I will first conduct simulation analyses to examine the influence of prevalent user bias and the choice of referent group on pharmacogenomics studies conducted in observational settings. I will use the results of these analyses to inform the design of my subsequent work.

After determining the best study design, I will seek to identify genetic variants that modify the association between thiazides and QT, including its component parts (QRS interval and JT interval). QT represents both the depolarization and repolarization phases of ventricular conduction there is evidence that genetic factors can impact the two components inversely, thus cancelling out possible effects when studying QT alone.[230] For this reason, I will include all three outcomes in my analyses. Furthermore, I will incorporate both race-stratified and trans-ethnic meta-analytic conversions to take advantage of the large multi-ethnic populations available in the participating cohorts. Additionally, I will incorporate functional characterization data to prioritize potentially causal genetic variants and determine the potential burden of these

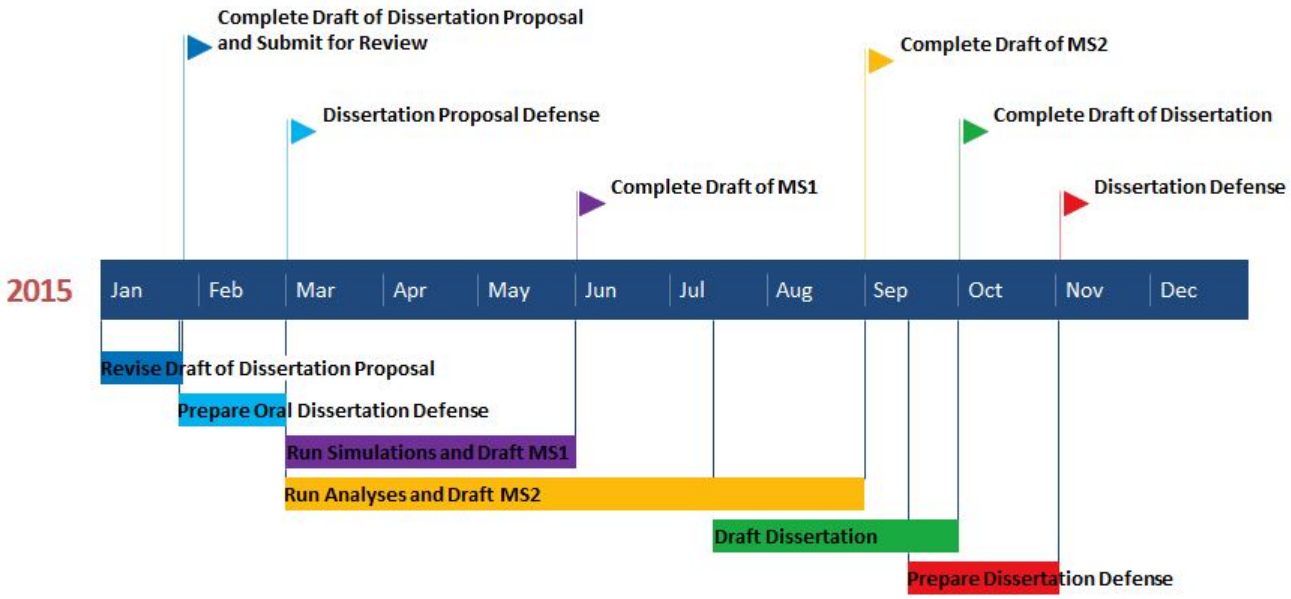
findings by calculating the proportion of the U.S. population with clinically significant pharmacogenomic interactions.

This work has broad potential public health impacts. Variable drug response and adverse drug reactions pose significant problems in the effective treatment of disease, with ADRs accounting for over two million serious health effects annually.[2-5] Pharmacogenomics work has the potential to illuminate novel pathways in drug response, inform drug development, alter policy and drug labelling, influence drug selection, modify dosing regimens, and prevent ADRs.[10, 11, 13] Furthermore, it can help to understand the etiology of drug response. In studying thiazides, which are one of the most common treatments of hypertension, and QT prolongation, a highly regulated physiologic marker of adverse drug reactions, the work proposed in this study impact a broad segment of the population directly and, through the potential to better understand the genetic underpinnings of human health, benefit the entire population.

7. Manuscript Preparation and Timeline

The first manuscript for this project (MS1) will be a methods paper developed from Specific Aim 1. The second manuscript (MS2) will be a pharmacogenomics paper developed from Specific Aim 2. Potential target journals include *Pharmacogenomics* and the *American Journal of Epidemiology*. Figure 18 shows the timeline for the completion of this project. Timeline goals include the completion of MS1 by the end of May 2015, the completion of MS2 by the end of August 2015, the completion of the dissertation document by the end of September 2015, and the defense of my dissertation in early November 2015.

Figure 18. Dissertation Manuscript Timeline



APPENDIX

Table A1. 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	BP	CA	MAF	β (SE)	*P*	MAF	β (SE)	*P*	MAF	β (SE)	*P*	MAF	β (SE)	*P*	MAF	β (SE)	*P*
RNF207	1	rs846111	6219310	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	23383982	T													0.50	0.70 (0.09)	1E-14
NOS1AP	1	rs12143842	162064100	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	162065484	C										0.22	2.17 (0.33)	2E-10			
		rs16857031	162143120	G	0.14	2.63 (0.18)	1E-34												
		rs12029454	162163327	A	0.15	2.98 (0.18)	3E-45							0.31	1.73 (0.29)	4E-9			
		rs7534004	162176919	A										0.31	1.73 (0.29)	3E-9			
		rs10127719	162186380	C										0.32	1.64 (0.29)	2E-8			
		rs12567315	162196856	A										0.33	1.69 (0.28)	2E-9			
		rs6692381	162198094	T										0.34	-1.71 (0.28)	1E-10			
		rs6667431	162198131	A										0.33	1.69 (0.28)	2E-9			
		rs4306106	162202204	A										0.33	1.66 (0.28)	5E-9			
		rs10800352	162202899	G										0.33	-1.66 (0.28)	5E-9			
		rs4480335	162203587	C										0.33	-1.67 (0.28)	4E-9			
		rs12116744	162210666	A										0.33	1.67 (0.28)	4E-9			
		rs12027785	162211355	A										0.33	1.67 (0.28)	3E-9			
		rs3934467	162212887	T										0.33	-1.69 (0.28)	3E-9			

		rs4391647	162217141	G									0.33	-1.74 (0.28)	8E-10			
		rs4657175	162225948	G									0.33	1.74 (0.28)	7E-10			
		rs12123267	162229561	T									0.34	-1.70 (0.28)	2E-9			
ATP1B1	1	rs12061601	169101212	C									0.29	-1.89 (0.30)	2E-10			
		rs1320976	169104108	A									0.25	-2.06 (0.32)	2E-10			
		rs10919071	169130245	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	40525842	C												0.05	-1.69 (0.22)	5E-14
SP3	2	rs938291	173877880	G												0.39	0.53 (0.09)	6E-10
TTN-CCDC141	2	rs7561149	178825129	C												0.42	-0.52 (0.09)	7E-9
SPATS2L	2	rs295140	200295976	T												0.42	0.57 (0.09)	2E-11
SCN5A	3	rs11129795	38547672	A				0.23	-1.27 (0.23)	4E-8								
		rs12053903	38551902	C	0.34	-1.23 (0.18)	1E-14											
		rs6793245	38557546	A												0.32	-1.12 (0.10)	4E-27
C3ORF75	3	rs17784882	47502513	A												0.40	-0.54 (0.10)	3E-8
SLC4A4	4	rs2363719	71272499	A												0.11	0.97 (0.16)	8E-10
SMARCAD1	4	rs3857067	94105283	A												0.46	-0.51 (0.08)	1E-9
GFRA3	5	rs10040989	138238036	A												0.13	-0.85 (0.13)	5E-11
GMPR	6	rs7765828	16294491	G												0.40	0.55 (0.09)	3E-10
PLN	6	rs11153730	118346359	T												0.50	-1.65 (0.10)	2E-67
		rs11970286	118359211	T				0.44	1.64 (0.20)	2E-16								
		rs11756438	118672469	A	0.47	1.40 (0.18)	5E-22											
CAV1	7	rs9920	116560038	C												0.09	0.79 (0.14)	3E-8
KCNH2	7	rs2968864	150925074	T	0.25	1.40	8E-16				0.22	2.33	2E-5					

						(0.18)						(0.01)						
		rs2968863	150926049	T				0.29	-1.35 (0.23)	4E-9	0.22	-2.30 (0.55)	3E-5					
		rs4725982	150940775	T	0.22	1.58 (0.18)	5E-16				0.23	1.64 (0.55)	0.003					
		rs2072413	150950881	T										0.27	-1.68 (0.11)	1E-49		
		rs3807375	150970122	T							0.35	4.42 (0.67)	5E-11					
NCOA2	8	rs16936870	70277107	A													0.10	0.99 (0.16)	1E-9
LAPTM4B	8	rs11779860	97838102	C													0.47	-0.61 (0.10)	2E-10
AZIN1	8	rs1961102	102920617	T													0.33	0.57 (0.10)	3E-9
GBF1	10	rs2485376	102290249	A													0.39	-0.56 (0.09)	3E-8
KCNQ1	11	rs2074238	2463573	T	0.06	-7.88 (0.88)	3E-17				0.04	-2.13 (0.49)	1E-5						
		rs7122937	2465320	T													0.19	1.93 (0.12)	1E-54
		rs12296050	2468112	T				0.20	1.44 (0.25)	9E-9	0.15	4.87 (0.75)	8E-11						
		rs757092	2477948	G							0.34	1.14 (0.48)	2E-2						
		rs12576239	2481089	T	0.13	1.75 (0.18)	1E-15				0.13	2.31 (0.52)	8E-6						
FEN1-GADS2	11	rs174583	61842278	T													0.34	-0.57 (0.09)	8E-12
ATP2A2	12	rs3026445	110285398	C													0.36	0.62 (0.09)	3E-12
TBX5	12	rs3825214	114357638	G							0.22	2.18 (0.41)	1E-7						
KLF12	13	rs728926	73938985	T													0.36	0.57 (0.10)	2E-8
ANKRD9	14	rs2273905	102508662	T													0.35	0.61 (0.09)	4E-11
USP50-TRPM7	15	rs3105593	50552821	T													0.45	0.66 (0.10)	3E-12
CREBBP	16	rs1296720	3823641	C													0.20	0.83 (0.13)	4E-10
LITAF	16	rs8049607	11597897	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	11599680	A										0.46	-1.15 (0.10)	2E-28			

MKL2	16	rs246185	14301575	C										0.34	0.72 (0.10)	3E-13			
NDRG4-CNOT1	16	rs37062	58533334	G	0.24	1.75 (0.18)	3E-25				0.28	2.25 (0.47)	1E-6						
		rs246196	58540349	C													0.26	-1.73 (0.11)	2E-15
		rs7188697	58588274	A				0.74	1.66 (0.23)	1E-12	0.71	1.75 (0.50)	5E-4						
LIG3	17	rs2074518	34997363	T	0.46	1.05 (0.18)	6E-12												
		rs1052536	35004556	C													0.53	0.98 (0.10)	6E-25
PRKCA	17	rs9892651	66307675	C													0.43	-0.74 (0.10)	3E-14
KCNJ2	17	rs1396515	70434852	C													0.52	-0.98 (0.09)	2E-25
		rs17779747	70498851	T				0.35	-1.16 (0.21)	3E-8									
KCNE1	21	rs1805128	34449382	T													0.01	7.42 (0.85)	2E-18
		rs1805127	34449523	T							0.39	3.09 (0.72)	2E-5						
		rs727957	34507774	T							0.19	4.33 (1.20)	2E-12						

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