ARIC Manuscript Proposal # 1556

1a. Full Title: QT-Prolonging Drug-Gene Interactions and Ventricular Repolarization: the CHARGE Drug-Gene GWAS Consortium

b. Abbreviated Title: CHARGE Drug-Gene GWAS of QT

2. Writing Group: Eric A. Whitsel, Christy L. Avery, Til Stürmer, Eric Boerwinkle, Dan Arking (and attempting to maintain symmetry across contributing cohorts), other members of the CHARGE Drug-Gene GWAS Consortium, as well as other interested members of the ARIC ECG Phenotype Working Group.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal.

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3. Timeline:
   Statistical analyses: September 2009 – October, 2009 (on availability of final Freeze 3 data)
   Manuscript revision: January, 2010 – March, 2010
   Manuscript submission: March, 2010

4. Rationale:
In December 1990, Monahan and colleagues reported a case of torsades de pointes, an abnormal heart rhythm that can cause syncope and sudden death, in a patient taking both terfenadine, a non-sedating anti-histamine, and ketoconazole, an antifungal agent (1). A major risk factor for torsades de pointes is prolongation of the heart-rate-corrected QT interval (QTc), an electrocardiographic measure of myocardial repolarization. Soon, basic-science studies identified the mechanism of the toxicity: terfenadine blocks the cardiac potassium channels (2), specifically the rapid component of delayed rectifier current (IKr) (3). For the first time, a non-cardiac drug was associated with potentially lethal cardiac toxicity. In this instance, ketoconazole inhibited the metabolism of
terfenadine and increased the QTc prolongation seen with normal doses (6 msec). Withdrawn from the market in 1998, terfenadine was an effective drug with a signature toxicity—QTc prolongation and torsades de pointes. The same cardiotoxicity occasioned the withdrawal of cisapride, a prokinetic agent used to treat heartburn (4). Due to its strong association with fatal arrhythmias, prolongation of QTc has become a major obstacle in drug-development (5-7). In the general population, QTc is related to sudden cardiac death (8-12). The main drug classes that exert a QT-prolonging effect are anti-arrhythmic, anti-psychotics, anti-infectants, anti-depressants and anti-histamines (5-6,13-14). A list is available from the University of Arizona Center for Education and Research on Therapeutics (UAZ CERT), a U.S. AHRQ-funded program of the Critical Path Institute (15). Many of the QT prolonging drugs exert their effects through binding to the human ether-a-Go-Go related (hERG) potassium channel, encoded by the $KCNH2$ gene (6), but this not the sole mechanism. The determinants of individual susceptibility to drug-induced QT prolongation remain largely unknown (6,16). Research on the genetic background of drug-induced QT-prolongation has largely focused on the candidate genes responsible for the QTc-related Mendelian disorders. However only a small proportion of persons with drug-induced torsade carry a mutation in one of these channels (17,18). Recently a drug- and patient group-specific GWAS was performed, by its license holder, to identify common variation associated with QT interval change in schizophrenics treated with the atypical anti-psychotic iloperidone with the goal of directing therapy to those with the best benefit-risk profile (19). The search for new genetic variants that predispose for QT-prolongation during drug exposure through GWAS may provide new insights that can improve drug development and lead to preventive measures and tailored pharmacotherapy.

In 2006, Arking and colleagues reported the results of a GWAS study of 200 subjects at the extremes of the QTc distribution (20). They identified $NOS1AP$, a regulator of neuronal nitric oxide synthetase, as a new locus associated with cardiac repolarization. This genetic variant was common, present in about 60% of subjects of European ancestry. The findings were replicated in two other populations. The same association was demonstrated in African Americans (83), and fine-mapping efforts identified a strong sex-interaction and a second independent $NOS1AP$ site associated prolonged QTc (21). $NOS1AP$ variants were strongly associated not only with QT prolongation but also with the incidence of sudden cardiac death in a joint study from ARIC and CHS (22). Additional studies have extended the findings to new drug-gene interactions. Stricker and colleagues reported a drug-gene interaction between $NOS1AP$ and glibenclamide. In patients with the TG or GG genotype at rs10494366, glibenclamide was less effective in glucose lowering and associated with an increased risk of mortality (23). In a second study (24), genetic variation in $NOS1AP$ significantly potentiated the QT prolonging effect of verapamil but not other calcium channel blockers.

Two GWAS meta-analyses, published together in *Nature Genetics*, have identified 10 new loci associated with QT duration. The paper from the “Cohorts for Heart and Aging Research in Genomic Epidemiology” (CHARGE) consortium (25) detected nine common variants at five known candidate genes and an additional five common variants at loci not previously recognized to modulate myocardial repolarization. One new loci is in $PLN$, which encodes phospholambam, an inhibitor of cardiac sarcoplasmic reticulum Ca+2-ATPase. A unifying hypothesis for this finding together with $NOS1AP$ and rare variants in $CACNA1C$ suggests that genetic variation influencing calcium cycling in cardiac myocytes may affect cardiac repolarization and QTc. Altogether, these variants explained a substantial proportion of the QT variation (5.4% in FHS, 6.5% in RS, and 2.3% in CHS). In a companion paper, the QTSCD study confirmed loci at four
genes associated with monogenic long QT syndrome, and identified 5 others, including *PLN* (26).

5. **Main Hypotheses/Study Questions:**
To examine gene-drug interactions as they relate to time-domain ECG measures of ventricular repolarization of greatest clinical, pharmaceutical and regulatory interest.

6. **Design and Analysis:**

**Overview**
The goal of the proposed analysis is to systematically examine within a common working group resting, standard twelve-lead ECG measures of ventricular repolarization as they relate to interactions between known, QT-prolonging drugs and genes in the CHARGE consortium. The consortium was formed to facilitate GWAS meta-analyses and replication opportunities among multiple large population-based prospective cohort studies, including the Age, Gene/Environment Susceptibility (AGES) -- Reykjavik Study, the Atherosclerosis Risk in Communities Study (ARIC), the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Rotterdam Study (RS). HealthABC (HABC), which has had GWAS data since June 2009, recently joined the effort. With genome-wide data on more than 40,000 participants (>5000 of them African Americans), this collaboration represents a unique resource for evaluating drug-gene interactions and ventricular repolarization in the “real world” of community-based studies.

The general approach is first to conduct within-study analyses of the association between phenotype and genotype for each of the 2.5 imputed autosomal CEPH HapMap SNPs and then to combine the findings from the within-study analyses by the method of meta-analysis. Imputation for the African-American populations requires data becoming available through the extended HapMap project. Analyses will be conducted separately for the major ethnic groups (European and African-Americans). Use of GWAS data in African-Americans will follow CARE procedures.

To maximize power in the face of infrequent prescription, intermittent use, and/or interim withdrawal of QT-prolonging drugs from the marketplace due to adverse affects, several initial strategies are proposed. The first involves working with all QT-prolonging drugs, systematically grouped according to the risk of torsades de pointes with which each is associated. In this scenario, grouping would be accomplished using a current classification of risk (definite; possible; conditional; no/unknown) assigned by the UAZ CERT. If sample sizes are insufficient to support new user analysis of change in phenotype between serial ECGs (as described below), drug-gene interactions would be examined in longitudinal models leveraging the availability of repeated ECG and drug exposure measures over time. Sample size permitting, mechanistic (or therapeutic) classes and subclasses would then be investigated in separate manuscript proposals under the rotating leadership of lead authors with relatively focused substantive interests within the broader topical area.

**Phenotypes**
It is well-known that QT interval duration varies inversely with heart rate. Dual analysis of both measured and rate-corrected QT is therefore proposed. Model- and formula-based correction strategies recommended by e.g. the U.S. Food and Drug Administration for evaluation of QT-prolonging and proarrhythmic potential of drugs (27-29) will be adopted because the best method of rate correction is controversial (30-31).

**Primary Phenotype**
QT, maximum QT interval duration (ms) across all twelve leads

**Secondary Phenotypes**

\[ \text{QT}_{\text{C}}, \text{ Bazett’s rate-corrected QT (ms)} = \frac{\text{QT}}{\sqrt{\text{RR}}} \]
QT_F, Fredericia’s rate-corrected QT (ms) = QT ÷ RR^{0.33}
QT_{RR}, Rautaharju’s linearly adjusted QT (ms) = QT – 185 × (RR – 1) + [6 in men]
where RR = median RR interval duration (s) across all twelve leads

**Inclusions**
New-user framework: GWAS data, QT-prolonging drug use, and QT pre- & post-treatment
Longitudinal framework: GWAS data, QT-prolonging drug use, and concurrently measured QT

**Exclusions**
Poor quality ECG, electronic pacemaker, QRS > 120 ms, or atrial fibrillation

**Exposure**
Additive genetic model of inheritance

**Model**
We propose selecting from two candidate analysis strategies on the basis of available sample size, which may be modest. Adopting longitudinal methods, using repeated measures, and establishing additional collaborations with other studies or consortia that have comparable data may therefore be helpful. The first strategy involves ordinary least squares (OLS) regression of post- minus pre-treatment differences in the phenotype restricted to new users of a QT-prolonging medication. The second involves using generalized estimation equations (GEE) among all participants with genotype data and at least one measurement of the phenotype. The OLS model is given by

\[ Y_{ij+1} - Y_{ij} = \beta_0 + \beta_1 SNP_i + \beta_2 C_i, \]

where \( Y_{ij+1} - Y_{ij} \) is the post- minus pre-treatment difference in the phenotype for the \( i \)-th new user across the \( j \)-th and subsequent visit, \( \beta_0 \) is the intercept, \( SNP_i \) is the genetic variant of interest, and \( C_i \) is a vector of covariables (see adjustments, below). The parameter of interest is \( \beta_1 \), which represents the effect of a one-unit increase in the genetic variant on post- minus pre-treatment difference in the phenotype among the newly treated. The GEE model is given by

\[ Y_j = \beta_0 + \beta_1 I_{ij} + \beta_2 SNP_i + \beta_3 I_{ij} \times SNP_i + \beta_4 C_{ij}, \]

where \( Y_j \) is the phenotype for the \( i \)-th participant at the \( j \)-th visit, \( \beta_0 \) is the intercept, \( I_{ij} \) is an indicator of medication use (1,0), \( SNP_i \) is the genetic variant of interest, and \( C_{ij} \) is a vector of covariables. The parameter of interest is \( \beta_3 \), the multiplicative interaction term.

**Adjustments**
All analyses: age (yr) & gender
New user framework: time between exams
In analyses of the primary phenotype: + RR
In multi-center studies: + k-1 indicators for center
In family studies: + control for known relationships
When necessary: + principal components to account for population substructure
Longitudinal framework: + controls for confounding by indication

**Sensitivity**
To dichotomizing the primary phenotype at the threshold of regulatory interest (+5 ms) (27).
To substituting secondary phenotypes for the primary phenotype
To adding k-1 indicators for UAZ CERT classification
To restricting to specific UAZ CERT classifications, e.g. definite only
To restricting to women (32-33)

**Meta-Analysis**
Fixed effects

**Genome-Wide Significance Level**
\[ 1 ÷ \text{number of tests} \]

7.a. Will the data be used for non-CVD analysis in this manuscript?
b. If Yes, is the author aware that the file ICTDER04 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used?

___ Yes
___ No

(This file ICTDER04 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?

___ Yes
___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER04 must be used to exclude those with value RES_DNA = “No use/storage DNA”?

___ Yes
___ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:
http://www.cscc.unc.edu/ARIC/search.php

___ Yes
___ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?
Manuscript proposals #1434 (Arking, “GWA and candidate gene studies for sudden cardiac death”) and #1152 (Post, “Genomic predictors of sudden cardiac death”) are related to the proposal presented herein. However, #1434 and #1152 focus on sudden cardiac death and main effects of selected SNPs. Moreover, neither focuses on drug-gene interactions as they relate to the QT interval or proposes collaboration within the CHARGE consortium. Although this proposal is distinct from #1434 and #1152 for these reasons, we are already collaborating with investigators named in these manuscript proposals.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?

___ Yes
___ No

11.b. If yes, is the proposal
___ A. primarily the result of an ancillary study (AS #2009.10; #2007.02; #2006.03)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

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12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

References


