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

b. Abbreviated Title: Sulfonylurea-Gene GWAS of QT (CHARGE)

2. Writing Group: Eric A. Whitsel, Christy L. Avery, Til Stürmer (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: February 2010 – March, 2010
Manuscript preparation: April, 2010 – May, 2010
Manuscript revision: May, 2010 – Jun, 2010
Manuscript submission: Jun, 2010

4. Rationale:
The University Group Diabetes Program (UGDP) was an early randomized trial that compared placebo, insulin and the sulfonylurea, tolbutamide (1). In 1969, the tolbutamide arm was stopped early because of a pronounced increase in the risk of cardiovascular mortality (2). The sulfonylurea receptor 1 (SUR1) forms part of the ATP-dependent potassium channel that regulates beta-cell insulin secretion, and a second cardiac member of the SUR family appears to be critical in the cardiac protection provided by ischemic pre-conditioning (3). Schwartz and Meinert have tried to explain the cardiotoxicity seen in the UGDP trial on the basis of ischemic pre-conditioning, which relies on SUR2 and is blocked by tolbutamide and other sulfonylureas such as glyburide (glibenclamide) (2). Although sulfonylureas are not identified by the University of Arizona Center
for Education and Research on Therapeutics (UAZ CERT) as QT-prolonging drugs per se (4), they also prolong the QT interval. In a randomized trial, for instance, glyburide (glibenclamide) was associated with a significant increase in QTc (34 msec increase in QTc) compared with metformin (12 msec decrease in QTc) (5). In 2006, Arking and colleagues used GWAS methods to identify NOS1AP, a regulator of neuronal nitric oxide synthetase, as a new locus associated with cardiac repolarization (6), and in a recent report (7), Stricker and colleagues from Rotterdam reported a drug-gene interaction between NOS1AP variants and glyburide (glibenclamide) that increased the risk of mortality. Additional research on sulfonylurea-gene interactions with QT is therefore warranted.

5. Main Hypotheses/Study Questions:
To examine sulfonylurea-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 sulfonylureas 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 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, several initial strategies are proposed. The first involves working with all sulfonylureas at visit 1, tabulated below:

SULFONYLUREA USE @ ARIC V1 BY YEAR, THERAPEUTIC SUBCLASS & MEDICATION

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>FIRST GENERATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; CHLORPROPAMIDE</td>
<td>3</td>
<td>21</td>
<td>18</td>
<td>22</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>&gt; TOLAZAMIDE</td>
<td>0</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>&gt; TOLBUTAMIDE</td>
<td>0</td>
<td>11</td>
<td>6</td>
<td>12</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>&gt; ACETOHEXAMIDE</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>SECOND GENERATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; GLIBENCLAMIDE or GLYBURIDE</td>
<td>1</td>
<td>60</td>
<td>88</td>
<td>102</td>
<td>7</td>
<td>258</td>
</tr>
<tr>
<td>&gt; GLIPIZIDE</td>
<td>0</td>
<td>23</td>
<td>29</td>
<td>53</td>
<td>3</td>
<td>108</td>
</tr>
<tr>
<td>&gt; GLICLAZIDE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; GLIMEPIRIDE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALL SULFONYLUREAS</td>
<td>4</td>
<td>129</td>
<td>154</td>
<td>201</td>
<td>15</td>
<td>503</td>
</tr>
</tbody>
</table>
If sample sizes remain insufficient to support the cross-sectional analyses described below, sulfonylurea-gene interactions will be examined by leveraging e.g. (i) more stringent minor allele frequency (MAF) filters, (ii) the availability of repeated ECG and drug exposure measures over time in longitudinal models, or (iii) a better asymptotic approximator being finalized by our collaborators on the CHARGE Analysis Committee.

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

**Drug exposure definition**  
Sulfonylurea use (yes/no)

**Inclusions**  
Consenting participants, GWAS data, data QT data

**Exclusions**  
Poor quality ECG, pacemaker, QRS > 120 ms, atrial fibrillation, 2° or 3° AV block or heart failure

**Exposure**  
Additive genetic model of inheritance

**Model**  
We propose selecting from two candidate analysis strategies on the basis of available sample size. 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 the phenotype. The second involves using generalized estimation equations (GEE) among all participants with genotype data and at least one measurement of the phenotype. The OLS and GEE models are given by

\[ Y_{ij} = \beta_0 + \beta_1 I_{ij} + \beta_2 \text{SNP}_i + \beta_3 I_{ij} \times \text{SNP}_i + \beta_4 C_{ij} , \]

where \( Y_{ij} \) 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), \( \text{SNP}_i \) is the genetic variant of interest, and \( C_{ij} \) is a vector of covariates. The parameter of interest is \( \beta_3 \), the multiplicative interaction term.

**Adjustments**  
Cross-sectional framework: age (yr), gender, where RR = median RR interval duration (s) across all twelve leads (ms), 3-level UAZ-CERT classification of non-sulfonylurea QT-prolonging medications (as defined in ARIC MS# 1556), and center

Longitudinal framework: + controls for confounding by indication

**Meta-Analysis**  
Fixed effects

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

7.a. Will the data be used for non-CVD analysis in this manuscript?  
___ Yes  
___ No

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?
_ x_ 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”?
_ x_ 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
_ x_ 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”), #1152 (Post, “Genomic predictors of sudden cardiac death”), and MS# 1556 (Whitsel, “QT-Prolonging Drug-Gene Interactions and Ventricular Repolarization: the CHARGE Drug-Gene GWAS Consortium”) are related to this proposal. 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. #1556 focuses on UAZ-CERT-classified QT-prolonging medications, which exclude sulfonylureas, the focus of this proposal. Although this proposal is distinct from #1434, #1152 and #1556 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?
_ x_ Yes
____ No

11.b. If yes, is the proposal
_ X_ 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