1.a. Full Title: Meta-analysis of gene-drug interaction in relation to PR interval: the CHARGE consortium

b. Abbreviated Title (Length 26 characters):
Gene-drug interactions and PR interval

2. Writing Group:
Writing group members:
Alvaro Alonso, Dan Arking, Eric Boerwinkle, Elsayed Z. Soliman, others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _AA_ [please confirm with your initials electronically or in writing]

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3. Timeline:
We anticipate that the primary analyses will be finished within 4-6 weeks.

4. Rationale:
The PR interval in the standard electrocardiogram (ECG) reflects atrial and atroventricular nodal conduction, disturbances in which may lead to atrial fibrillation (AF) and other cardiac conditions. To identify common variants associated with PR interval, a
A meta-analysis of genome-wide association (GWA) was conducted in seven community-based cohorts of European ancestry individuals: AGES, ARIC, CHS, FHS, KORA, the Rotterdam Study and SardiNIA. Genome-wide significant associations at 9 loci ($P < 5 \times 10^{-8}$) were identified. The strongest was at chromosome 3p22.2 where independent association signals were in the sodium channel genes $SCN10A$ and $SCN5A$. In addition, six associations were identified near genes known to be involved in cardiac pathology ($CAV1/CAV2$, $NKX2-5$, $WNT11$, $MEIS1$, $SOX5/C12orf67$ and $TBX3/TBX5$) while one was near a gene not known to be relevant to the heart ($ARHGAP24$).

Case reports and experiments suggest that the calcium-channel blockers (CCBs) verapamil and diltiazem may aggravate or induce AF in susceptible patients.[1-4] The mechanism is unknown. Since CCBs have been suggested to have an increased risk on developing AF and thereby affecting the PR interval, we want to investigate whether CCBs have a different effect on the PR interval in persons with the wildtype alleles than in persons with the variant alleles.

5. Main Hypothesis/Study Questions:
To conduct a meta-analysis of gene-drug interactions in relation to PR interval, in adults of European ancestry.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).
We will conduct a cross-sectional analysis using information from ARIC visit 1. As a secondary analysis, we will use ECG information from visits 2, 3 and 4.

Subjects:
European/European-American subjects with PR interval measurements.

Exclusions – conditions at baseline:
Prevalent AF, pacemaker, WPW syndrome, third degree AV-block, CHF, myocardial infarction and extreme PR trait values (<80 ms, >320 ms) and use of digoxin and type 1 & III antiarrhythmic drugs.

Exposure:
9 top hits from GWA PR interval and use of CCBs.
Drug exposure: pharmacy records or interview data at the time of the ECG.

Outcome:
Length of PR interval on first eligible ECG. A secondary analysis will consider PR interval from follow-up visits.

Primary statistical approach:
We will use a two-step procedure. First, we will examine the association between use of CCBs, and the length of the PR-interval with linear regression analyses, adjusting for age, sex, RR interval (to account for heart rate), BMI, height, systolic BP, use of beta-blockers and diuretics. Second, we will examine the association between the interaction of CCBs and the 9 top hits and the PR interval with linear regression analyses, with complete adjustment.
Furthermore, we will stratify by use of CCBs and assess the association between the 9 top hits and the PR interval in users and non-users, complete adjustment.

In the secondary analysis, we will use a general linear model with repeated measures to study PR interval change over time according to CCBs and the 9 SNPs associated with PR interval.

Meta-analysis:
Our primary analysis will use a meta-analysis of all contributing cohorts' results. Meta-analysis of results will be performed using inverse-variance weighting. Analyses will be conducted using R.

Statistical significance:
To correct for multiple testing, Bonferroni correction will be performed. Significance thresholds will be if a P-value of <0.0055 (0.05/9) is reached.

Cohorts Included in Analysis
We anticipate including AGES, ARIC, CHS, FHS, MONICA/ KORA and the Rotterdam Study.

Limitations
Two potential limitations threaten the study:
1. The number of CCB users is limited, approximately 5%, reducing the statistical power to detect interactions. Still, we expect that pooled results from different cohorts will provide adequate sample size, particularly considering that the outcome is a continuous variable.
2. The main analysis has a cross-sectional approach. Therefore, an association between CCBs and PR interval could be due to reverse causality (PR interval leading to CCB use). This is unlikely, however, because PR interval is not usually taken into account by physicians when deciding to prescribe CCBs. Other factors, such as presence of CVD or systolic blood pressure and use of other antihypertensives will be controlled through restricting the analysis to individuals without CVD or adjusting for systolic BP and use of betablockers and diuretics.

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

b. If Yes, is the author aware that the file ICTDER03 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 ICTDER03 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 ICTDER03 must be used to
exclude those with value RES_DNA = “No use/storage DNA”?

_X_ Yes _____ No

8.c. If yes, is the author aware that the participants with RES_DNA = ‘not for profit’ restriction must be excluded if the data are used by a for profit group?

_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.cscn.unc.edu/ARIC/search.php

_X__ Yes _______ No No overlap with other proposals

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)?

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

___  A. primarily the result of an ancillary study (list number* _________)

_X_  B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 2006.03, 2007.02)

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

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.

Agree