1.a. Full Title: The Genetics of Left Ventricular Hypertrophy and associated “independent” phenotypes in the community

b. Abbreviated Title (Length 26 characters): The Genetics of LVH

2. Writing Group:
   Writing group members: C. Cristina Quarta, Calum A. MacRae, Susan Cheng, Amil M. Shah, Gabriel Musso, OTHERS WELCOME, Eric Boerwinkle, Scott D. Solomon

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

First author: Candida Cristina Quarta
Address: 75 Francis Street, 02115 Boston, MA

Phone: 617-732-2733 Fax: 617-582-6027
E-mail: cquarta@partners.org

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Name: Scott D. Solomon
Address: Brigham and Women's Hospital
Cardiovascular Division
75 Francis Street
Boston, MA 02115

Phone: 857-307-1960 Fax: 857-307-1944
E-mail: ssolomon@rics.bwh.harvard.edu

3. Timeline: Analysis: 4-6 months. Anticipate one or possibly two manuscripts will be completed within 6 months after completion of the analyses: one regarding the genetics of left ventricular hypertrophy (LVH), the other concerning the genetics of “independent” phenotypes associated with LVH.
4. **Rationale:**

LVH is an abnormal increase in the mass of the left ventricular (LV) myocardium (1). Echocardiographically detected LVH identifies a population at high risk for cardiovascular disease (2). LVH is a common condition that profoundly affects morbidity and mortality from cardiovascular diseases, including myocardial infarction, congestive heart failure, and stroke: the Framingham Heart Study showed that LVH was present in 15–20% of adults, and that, for each 50 g/m² increment of left ventricular mass, the relative risk of cardiovascular death increased by 1.73 in men and 2.12 in women (3). Increased LV mass is also associated with an increased risk for sudden cardiac death (4).

LVH occurs in a wide range of disorders. The most common causes of LVH or associations with LVH include hypertension, diabetes, obesity and valvular heart diseases. Less common causes include heart muscle disorders presenting with LVH due to inherited mutations in genes encoding cardiac sarcomeric contractile proteins (5). Other rarer conditions also can manifest with increased LV mass and thickness, mimicking a hypertrophic phenotype. These “phenocopies” include metabolic diseases, such as Anderson-Fabry disease, and infiltrative disorders, such as amyloidosis (6,7).

The development of LVH in the context of predisposing conditions shows considerable inter-individual variability (8,9). This is partly because LVH (or more precisely LV myocardial volume) as a quantitative phenotype behaves as a complex genetic disease, likely representing an interaction of several genes with the environment. In addition, the common traits associated with LVH are likely aggregates of many different disorders each with etiologies that are dependent on genetic, acquired or environmental contributions to varying degrees. Thus, the apparent complex genetic architecture of LVH may represent a series of discrete traits which the granularity of traditional phenotyping may not resolve. We hypothesize that in combination with traditional measures of LVH, other distinct phenotypic elements (including extreme degrees of LVH, upper septal thickening –DUST– or other peculiar regional distributions of LVH, bicuspid aortic valve, lipomatous hypertrophy of the interatrial septum, interatrial septum aneurysm, myxomatous mitral valve, LV non-compaction) may reflect some of these underlying discrete traits or alternatively the interaction between discrete sets of genetic variants contributing to LVH. Although the recognition of such “independent phenotypes” would help in characterizing the inter-individual variability, much remains unknown about their genetic basis.

In recent years there has been growing recognition of a widespread heritability of cardiac structural traits (10-12). Genome-wide association studies (GWAS) interrogate whether variation across the human genome in the form of single nucleotide polymorphisms (SNPs) is associated with given phenotypes. GWAS are now widely recognized as powerful data-driven tools for identifying genetic variants related to complex diseases (10,12). Early studies seeking association between candidate genes and LVH have looked at exclusively hypertensive patients (13,14). Two more recent population-based GWAS have sought SNPs associated with LVH in the general population: Vasan et al found 2 SNPs to be associated with increased LV wall thickness but they could not replicate this finding in an independent cohort (11); Shah and colleagues found 4 SNPs associated with LVH defined by ECG criteria with genome wide significance and replicated in an independent cohort (12); in a GWAS of a large
population of patients with type 2 diabetes, Parry et al were able to replicate two SNPs previously identified as predictors of LVH (11,12,15). Regarding rarer causes of LVH, Bick and colleagues found the prevalence of likely pathogenic sarcomere variants to be 0.6% in individuals from the Framingham Heart Study and Jackson Heart Study cohorts (16).

However, all these studies took into consideration only the main morphological changes related to LVH (increased LV wall thickness and mass), and did not incorporate orthogonal data such as indices of cardiac function.

The Atherosclerosis Risk in Communities (ARIC) study began in 1987 and enrolled over 15000 individuals aged 45-64 years in four heterogeneous communities in the U.S. (30% African-Americans). Data for ~6500 exomes sequenced both at UW and at the Broad Institute have been collected. From 2011 to 2013, as part of the currently funded ARIC study, the cohort survivors have undergone a new full clinical and instrumental assessment, which included serum, urine collection, ECG and echocardiographic evaluation (visit 5). In particular the echocardiographic assessment included a comprehensive acquisition of both conventional and novel parameters of cardiac structure and function.

ARIC therefore presents a unique opportunity for exome level assessment in a large cohort of the genetics of a wide range of conditions associated with LVH, including the distinctive subsets of LVH which we have outlined.

5. **Main Hypothesis/Study Questions:**

The primary objective of this study is to define a series of distinctive subsets of LVH and to investigate the genetic background (either known or unknown, common or rare variants, including Mendelian or non-Mendelian genetic variants) of these subsets as well as the interaction between genetic and non-genetic factors in determining the left ventricular mass and thickness across a wide range of structural and functional changes occurring along with LVH.

To meet this goal, we have the following specific aims:

1. *To analyze the genetic background (already known and novel genes, including single nucleotide polymorphisms) of patients displaying echocardiographic LVH.* This approach is clinically oriented, and is based on a morphological and functional phenotype rather than putative pathophysiological mechanisms. This strategy will include the replication in the ARIC cohort of previously identified SNPs as well as the search for novel unknown variants.
2. *To assess the frequency and to study the genetic basis of associated echocardiographic structural and functional abnormalities (“independent” phenotypes), including extreme degrees of LVH dust, bicuspid aortic valve, lipomatous hypertrophy of the interatrial septum, interatrial septum aneurysm, myxomatous mitral valve, LV non-compaction, different degrees of systolic or diastolic dysfunction.* This approach offers the potential identification of variation in genes that may cause genocopies or overlapping phenotypes which might modify the prognosis of LVH (associated diseases). This approach will allow a
better understanding of more complex presentations of LVH and aid in the
dissection of the phenotypic and genetic architecture of LVH.

3. To analyze and correlate electrocardiographic features (including low or high
QRS voltages, pre-excitation, pseudo-infarct patterns, short or long QT, short or
long PR) to LVH and/or “independent” phenotypes in order to direct towards the
correct underlying causes of LVH in order to define distinctive subsets of LVH.

4. Based on the genetic variants identified through the previous specific aims, to
analyze the patients’ outcome according to the genetic variants themselves and
their interaction with non-genetic factors. Specifically, to analyze how the genetic
factors interact with non-genetic factors (blood pressure, BMI, serum glucose,
renal function) in determining LVH phenotype and outcome.

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).

1) The study will start with the identification, among the ~6000 patients undergone
echocardiographic visit 5, of those displaying with LVH. Those manifesting
“independent” phenotypes associated with LVH (extreme LVH, upper septal
thickening –dust, bicuspid aortic valve, lipomatous hypertrophy of the interatrial
septum, interatrial septum aneurysm, myxomatous mitral valve, and LV non-
 compaction) will also be identified. As aortic stenosis promotes LVH through
increased hemodynamic load on the LV, patients with aortic stenosis greater than
mild severity will be excluded from the primary analyses to prevent confounding.
These individuals will be analyzed separately in secondary analyses. As aortic
stenosis promotes LVH through increased hemodynamic load on the LV, patients
with aortic stenosis greater than mild severity will be excluded from the primary
analyses to prevent confounding. These individuals will be analyzed separately in
secondary analyses.

2) Genetic data from individuals meeting the definition of LVH (see below) will be
assembled for further analysis.

3) Electrocardiographic and clinical characteristics of the study population at the
time of echocardiography (visit 5) will be analyzed.

4) Clinical and laboratory parameters, including blood pressure, serum glucose,
BMI, collected at previous time points (from visit 1) will be analyzed for any
interaction with genetic variants in determining LVH.

5) Data for the ~6500 sequenced exomes will be used to identify novel variants
associated with LVH and “independent” phenotypes. Already known genetic
variants for sarcomeric, mitochondrial, metabolic, and infiltrative diseases will be
searched for. Already studied SNPs will be replicated, while unknown variants
associated with LVH will be searched for.

6) Genetic abnormalities (known, unknown, Mendelian, non Mendelian, frequent or
rare) identified from patients who underwent ARIC visit 5, will be searched for in
the entire population of enrolled patients with available DNA information in order
to assess the effect of genetic variants on survival and incident cardiovascular diseases.

**Definition of LVH**
LV measurements will be used to identify individuals with LVH according to ASE criteria (17,18). LV measurements will be used to calculate LV mass according to the formula derived by Devereux et al. (18,19):

\[
LV_{mass} = 0.8*(1.04*((LVIDD+LVPW+IVS)^3-(LVIDD)^3))+0.6
\]

where LVIDD represents left ventricular internal diameter in diastole, LVPW refers to left ventricular posterior wall thickness and IVS is LV septal thickness. LV mass will be separately indexed to height in metres to the power 2.7 and to body surface area (BSA).

Individuals will be classed as having LVH if their LV mass will be outside the normal range when indexed to either height or BSA using ASE cut-off values (17). Participants with LV wall thickness exceeding the normal range according to direct 2D measures will also be classed as LVH. Additionally, individuals will be regarded as having LVH if relative wall thickness (RWT) will be increased using the formula (20):

\[
RWT = (2xLVPW)/LVIDD
\]

**Clinical and laboratory variables** (collected at different time points from visit 1 to visit 5) to be evaluated include:
age, gender, hypertension, diabetes mellitus, dyslipidemia, smoking, obesity, coronary heart disease, stroke/TIA, peripheral arterial disease, atrial fibrillation/flutter, chronic kidney disease, anemia, COPD, and alcohol use; heart rate, blood pressure (systolic, diastolic, mean arterial, and pulse pressure), height, weight, body mass index, body surface area, creatinine, hemoglobin, red cell distribution width, glucose, lipids, brain natriuretic peptide, high sensitivity troponin T.

**Electrocardiographic variables** (collected at visit 5) to be evaluated include:
heart rate, rhythm, left ventricular hypertrophy, low QRS voltage pattern, PR duration, QRS duration, QT duration, QRS axis deviation, peripheral and total QRS amplitude, Sokolow index, atrioventricular and ventricular conduction defects, pseudo-infarction pattern.

**Echocardiographic variables** (collected at visit 5) to be evaluated include:
Cardiac structure: LV size, LV wall thickness, LV mass, LV geometry, left atrial size and volumes, aortic root dimension, valvular disease, and right ventricular size. Search for “unique” features (extreme LVH, upper septal thickening –dust, bicuspid aortic valve, lipomatous hypertrophy of the interatrial septum, interatrial septum aneurysm, myxomatous mitral valve, and LV non-compaction) will be performed.
Cardiac function: LV ejection fraction, right ventricular fractional area change, Doppler mitral inflow E and A wave peak velocities, E/A ratio, deceleration time, tissue Doppler
systolic and diastolic indices at both the mitral and tricuspid annulus, as well as LV myocardial mechanics from speckle tracking imaging.

**Noninvasive hemodynamics**: stroke volume, cardiac output, LV filling pressures, pulmonary vascular resistance, and pulmonary artery pressures.

**Genetic Analysis.** Simple association for binary phenotypes and linear regression for continuous traits will be conducted. All analyses will be performed under a dominant model, but we will also consider a true additive model. Case control association will be performed across variants using Fisher’s exact test with permutation used to derive locus-wide significance thresholds and p values. We will undertake association analyses for quantitative traits using linear regression of the trait residuals on the variant burden. Again, we will empirically estimate significance using permutation. We will pre-specify a series of ordered subsets for association analyses on the initial frequency data for the “independent” phenotypes identified in the proposal.

Mendelian models: For potentially Mendelian disorders we will use both dominant and recessive models and will exploit variant burden measures with and without pathway filtering. We will use both the Combined Multivariate and Collapsing (CMC) method and SKAT. Where necessary, in secondary analyses we will also restrict burden testing to subsets of genes defined by pathway analyses conditioned on prior studies of LVH. We have developed a machine learning system to rank genes across the genome for their effects on LV mass and other cardiac phenotypes and will implement this system in conjunction with the typical burden testing approaches outlined above. In addition, we are able to empirically assay of the biologic effects of coding alleles on LV mass and LV function in the zebrafish to stratify variants for these analyses.

7.a. **Will the data be used for non-CVD analysis in this manuscript?**

- Yes  _X_ 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”?**

- Yes  _X_ 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.csc.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)?
ARIC Manuscript Proposal #1990: Christopher J. O’Donnell et al. Identification and analysis of known and novel variants in clinically relevant genes underlying cardiac arrhythmias and cardiomyopathies in the NHLBI Exome Sequencing Project

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 (list number* 1995.05)
____ 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.csc.unc.edu/aric/forms/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.csc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

References

17. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group. Developed in Conjunction with the

