1.a. **Full Title**: Genome wide association (GWAS) and candidate gene approach to identify relationship between SNP’s and aortic and carotid diameter measurements in the ARIC study.

b. **Abbreviated Title (Length 26 characters)**: SNP’s and aortic/carotid diameter

2. **Writing Group**:
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   and others

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

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3. **Timeline**: Analysis to start as soon as approval is obtained. Manuscript is to be prepared as soon as analysis is available. We hope that the analysis and manuscript preparation will take place within 1 year from approval of the proposal.

4. **Rationale**: Thoracic aortic aneurysm and dissection (TAAD) is a severe disorder that occurs with a lifetime risk of approximately 1 per 200. TAAD results in about 18,000 deaths per year in the U.S. There is very limited understanding of the pathogenesis of TAAD and it is often not detected until there is catastrophic dissection. Current hypotheses about the major factors include disordered transforming growth factor beta (TGF-b) signaling within the medial layer of the aortic wall and abnormal proliferation and survival of vascular smooth muscle cells. These two major pathways have been uncovered by analysis of rare families in which TAAD segregates as a dominant trait. To date six genes have been identified to play a role in TAAD – FBN1, TGFBR1, TGFBR2, ACTA2, MYH11, and MLCK. A seventh gene, FLNA, is responsible for a group of rare X linked conditions that also predispose to aortic aneurysm with dissection. Identification of the genes involved in these families has pointed to specific molecular mechanisms that may also have a role in sporadic TAAD.

Although TAAD itself was not directly included in ARIC, we hypothesize that aortic root diameter could serve surrogate quantitative trait for susceptibility to TAAD. If this hypothesis has merit, then we predict that polymorphisms in the seven genes that cause Mendelian forms of aortic aneurysm may have an effect on aortic root diameter as a quantitative trait. If association of SNPs with aortic root size is found, this information could be used in several important ways. First, the results could be compared to TAAD genome wide association study results from U.S. and European groups that will be available in the next several years. Second, it would validate aortic root diameter as a useful surrogate for TAAD in a genome wide analysis using the full SNP data available on the ARIC samples. Third, the results could be used to inform clinical risk assessment when taken together with other known risk factors (hypertension, smoking, age, sex, and atherosclerosis) which could then be used to develop targeted monitoring programs for high risk individuals. The measurements of aortic root diameter are available only for a subset of the ARIC population and thus candidate gene approach will be used and not GWAS due to statistical power issues.

Carotid dissection and tortuosity are less frequent than events of aortic aneurysm but has also been shown more common in some mendelian connective tissue diseases such as Ehlers-Danlos Syndrome (EDS) type 4 and Loyes-Diets syndrome. Carotid root diameter may play a role as an intermediate phenotype and associated SNP’s may have a role in identifying at risk individuals.

5. **Main Hypothesis/Study Questions:  
   **Hypothesis:**
   1. Common genetic variants (marked by SNPs and SNP haplotypes) in seven candidate genes (FBN1, TGFBR1, TGFBR2, ACTA2, MYH11, MLCK and FLNA) have an effect on aortic root diameter taken as a quantitative trait. The candidate genes are selected because it is known that rare deleterious variants in those genes are causally involved in thoracic aortic aneurysm.
2. Common genetic variants (marked by SNPs and SNP haplotypes) have an effect on carotid root diameter taken as a quantitative trait.

**Questions to be addressed in a stepwise manner:**

1. a. Are there alleles in the candidate SNPs associated with either increased or decreased aortic root diameter corrected for sex and age?
b. Are SNP haplotypes in candidate genes better predictors of aortic diameter than single SNPs?
c. Are there interactions of SNPs in the selected candidate loci with known risk covariates including hypertension, smoking.

2. Are there alleles associated with either increased or decreased aortic root diameter corrected for sex and age?

**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).**

After excluding patients with who have not provided consent for use of genetic information, patients in the ARIC study on whom aortic root diameter data are available (N=2041???) will be eligible for the analysis.

We would:

1. Absolute measures of aortic root diameter will be normalized (mean 0, unit variance) using sex and age in a linear model. The resulting transformed trait will be used for the remaining analyses.

2. Carry out basic tests for association using asymptotic (likelihood ratio test and Wald test) and empirical significance values (via permutation). For the additive effects of SNPs, the sign of the regression coefficient represents the effect of each extra minor allele (i.e. a positive regression coefficient means that the minor allele increases risk/phenotype mean). We will also compute the means and standard deviations stratified by genotype.

3. We will carry out tests for a difference in association with aortic root diameter between groups based on presence or absence of several known risk factors including hypertension, and smoking. This test will be based on comparison the difference between the regression coefficients.

4. We will use logistic regression models (compared to allele counting as in goal #2 above) to allow for known covariates including blood pressure, BMI/height, smoking. We will compute separate tests of the dominance component or a 2 df joint test of both additive and dominance (i.e. corresponding the general, genotypic). Unlike the dominance model which will be tested in goal #2 this test evaluates a variable coded 0,1,0 for the three genotypes AA, Aa, aa, i.e. representing the dominance deviation from
additivity, rather specifying that a particular allele is dominant or recessive. Under this model dominance deviation is fitted jointly with the additive term in a single model. All covariates will be included in the regression model so that a single model is fit to the data with a form similar to:

\[ Y = b_0 + b_1.AD + b_2.DOMDEV + b_3.COV_1 + b_4.COV_2 \ldots + e \]

This represents coefficients from the terms in a multiple regression of aortic diameter on ADD, DOMDEV, covariates jointly. The final test is a 2df test that tests the coefficients for ADD and DOMDEV together. The p-values reflect the 2df test of the SNP whilst controlling for the covariates. Potential SNP interactions will also be tested using the allele count as a covariate.

5. We will repeat analyses described above using imputed haplotypes. Haplotypes will be based on the standard E-M algorithm. We will perform tests based on the distribution of probabilistically-inferred set of haplotypes for each individual. We will use either short SNP windows (e.g. 3 marker haplotypes) or LD-structure based haplotypes based on block boundaries to carry out these tests.

6. A GWAS analysis will be preformed to for carotid root diameter (systolic, diastolic and mean) diameter using the set of genotyped and imputed SNPs. The primary analysis will be age and sex adjusted. A multivariable adjustment will also be considered as well.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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
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? ____ Yes x No

11.b. If yes, is the proposal

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

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/

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.