1.a. **Full Title:** Association of biochemical and cellular markers with a single SNP in the 9p21 chromosomal region.

2. **Writing Group:**
   Writing group members:
   - Ariel Brautbar, MD
   - Christie Ballantyne, MD
   - Alanna Morrison PhD
   - Ron Hoogeveen PhD
   - Nena Matijevic PhD
   - Salim Virani, MD
   - Eric Boerwinkle, PhD

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ___AB___ [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:** Recently multiple GWAS studies have shown the association of certain single point polymorphisms (SNP’s) on a particular 9p21 chromosomal region and increased risk for coronary heart disease (CHD). The mechanism by which this 58KB area in the 9p21 chromosome is involved in the pathogenesis of CHD remains unclear. This region does not encompass any gene or coding region, open reading frame or MicroRNA. In addition the 9p21 SNP’s were not associated with the traditional risk factors for CHD such as age, gender, HDL, HTN or diabetes. However, SNP’s in the 9p21 region were recently reported to be associated with coronary calcification on MRI and Family history of CHD (Ridker, abstract at American Heart Association). The ARIC MRI study has examined numerous novel measurements including both biochemical and cellular markers in approximately 2000 individuals and some of these markers are associated with carotid atherosclerosis as measured by MRI. We propose to examine if any of the biochemical and cellular markers are associated with SNP’s in the 9p21 region. If we do find an association, this may help to identify pathway(s) which are influenced by 9p21 region.

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

**Hypothesis:** The rs10757274 SNP on the 9p21 chromosomal region (9p21 SNP) is associated with biochemical and cellular markers measured in the ARIC MRI study.

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

1. Are any of the biochemical and cellular biomarkers measured in ARIC MRI associated with any of the rs10757274 genotypes?
2. Is family history associated with the rs10757274 genotypes

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).**
In this study we will examine the allele frequency of the 9p21 SNP and certain biochemical and cellular markers in the entire ARIC MRI white population.

Study population will include all white participants in the ARIC MRI study and will be conducted with relation to age and gender.

Study participants will be analyzed for baseline epidemiologic data:

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
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<tbody>
<tr>
<td>Age, years</td>
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<td>Smoking, years</td>
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<td>BMI, m/kg^2</td>
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<td>Waist/hip ratio</td>
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<tr>
<td>HDL-C</td>
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<tr>
<td>Total cholesterol</td>
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<tr>
<td>Triglyceride</td>
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<td>IMT, mm</td>
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<tr>
<td>Family history</td>
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<tr>
<td>Systolic BP</td>
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<td>Diastolic BP</td>
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<tr>
<td>Diabetes</td>
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### Table 2

<table>
<thead>
<tr>
<th>Characteristics</th>
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<th>Characteristics</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>YY(SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Male</td>
<td>XX(SD)</td>
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<td>Family history</td>
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<tr>
<td>Biomarker 1</td>
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<tr>
<td>Biomarker 2</td>
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</table>

The list of biomarkers and indices to be included in table 2 are as follows:

(Sampling design will be considered for each analyte for each tested association)

Lactate, apo A-I, apo B, MMP-1,2,3,7,8,9, TIMP-1, RANTES, hs-CRP, homocysteine, HbA1c, glucose, blood creatinine, urine creatinine, microalbumin

**Monocytes markers** - TLR-4+, CD14+, TLR-2+, CD162+, COX-2+, CD45+, MPO+

**Granulocytes markers** - TLR-4+, CD162+, TLR-2+, COX-2+, MPO+

**Lymphocytes markers** - TLR-4+, CD162+, TLR-2+, COX-2+, CD45+

**Platelet markers** - Total platelets CD61+, Single platelets CD61+, Non-activated platelet CD61+, CD62P+, CD41+, CD154+

**Cell aggregates** - Platelet-platelet aggregates, Platelet-monocyte aggregates, Platelet-granulocyte aggregates, Platelet-lymphocyte aggregates
7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __x__ 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? __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 ICTDER02 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)?

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* _ARIC MRI_______)

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.