1.a. Full Title: Association Between Leukocyte Markers and Genotypes of Myeloperoxidase (MPO) (ARIC CAR MRI Study)

b. Abbreviated Title (Length 26 characters): MPO Leukocyte Markers and Genotypes

2. Writing Group: Matthew B. Schabath, Suzette Bielinski, Jack Folis, Eric Boerwinkle, and any other ARIC member(s) as deemed necessary by the coordinating center.

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:
Manuscript Prep: May 2008 to July 2008
Manuscript Revisions: Aug 2008
Manuscript Submission: Sept 2008
4. Rationale:
Myeloperoxidase (MPO) is an iron-containing heme protein localized in the azurophilic granules of neutrophil granulocytes and in the lysosomes of monocytes (1). MPO is the most abundant protein in neutrophils constituting approximately 5% of their dry weight (2). MPO catalyzes the reaction between hydrogen peroxide and the chloride ion and generates hypochlorous acid and other reactive oxygen species (3,4).

MPO plays an important role in human defense against microorganisms by catalyzing the formation of hypochlorous acid, a potent microbicidal agent (5). MPO has been involved in the pathogenesis of several diseases through excessive production of reactive oxygen species (ROS). The stimulation of leukocytes leads to the release of MPO into the extracellular medium and its plasma concentration may therefore be considered as a specific index of leukocyte activation. MPO is highly polymorphic and the implication of MPO and its genotypes have been reported in a variety of disease pathologies including atherosclerosis (6), stroke (7), Alzheimer's disease (8), as well as a variety of cancers (9,10).

Although MPO appears to be involved in wide variety of disease pathologies, there is limited evidence for functional relevance of MPO SNPs. The aims of this analysis is to identify the factors affecting MPO levels as measured by flow cytometry (Table 1) and to investigate the association between polymorphisms of the MPO gene and MPO levels in the ARIC Carotid MRI study. The MPO SNPs chosen for the proposed analyses have been genotyped on the entire ARIC Carotid MRI study (Table 2). Although previous association studies have explored the functional effects for some of the MPO SNPs, this will be the largest analysis to explore such associations.

<table>
<thead>
<tr>
<th>Table 1. MPO flow cytometry data</th>
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<tbody>
<tr>
<td>Cell Type</td>
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<tr>
<td>monocytes</td>
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<tr>
<td>granulocytes</td>
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</tbody>
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<table>
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<th>Table 2. List of available MPO SNPs</th>
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<tr>
<td>SNP ID</td>
</tr>
<tr>
<td>rs2071409</td>
</tr>
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<td>rs2759</td>
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<tr>
<td>rs28730837</td>
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<td>rs7208693</td>
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5. Main Hypothesis/Study Questions:

1. Assess the relationship between MPO levels, as measured by flow cytometry, and population characteristics including age, gender, smoking status, BMI, pack-years smoked, years quit smoking, existing medical conditions (e.g., prior history of lung disease, diabetes, pre-existing cardiovascular disease, hypertension, and carotid IMT thickness) and medication use (e.g. statin use). Furthermore, assess the relationship between the MPO genotypes and these same population characteristics. **This aim will test whether the MPO genotypes or levels are associated with population characteristics.**

2. Assess the association between the MPO genotypes and MPO levels and adjusting, where necessary, for potential confounders as identified in aim 1. **This aim will test whether the MPO genotypes modulate MPO levels.** Stratified analyses will be performed to determine if the associations are more evident in specific subgroups.

6. Design and analysis

**Study Design:** The proposed analysis will utilize all available data from the ARIC Carotid MRI study.
**Inclusion/exclusion:** The usual DNA and non-CVD restrictions, ethnic group and missing data exclusion criteria will be used.

**Outcome:** The primary outcome for this analysis will be MPO levels (Table 1), as measured by flow cytometry, from monocytes (P6MONOL2XD) and from granulocytes (P4GRCAXD). MPO from monocytes and granulocytes will be assessed separately and combined as an estimate of “total MPO”.

**Other variables of interest:** MPO genotypes (Table 2), smoking data, SES, occupation, BMI, prior medical history (e.g., prior history of lung disease, diabetes, pre-existing cardiovascular disease, hypertension, and carotid IMT thickness), medication use (e.g. statins), and covariate data will be required.

**Summary of data analysis:** All analysis is based on methods appropriate for stratified random sample methods. In particular, all analyses are weighted by the inverse of the sampling fractions in the 8 sampling strata (4 field centers X 2 IMT groups). Genotype and allele frequencies will be calculated and tests for Hardy-Weinberg equilibrium will be performed. Where appropriate, parametric and non-parametric analyses will be used to describe and characterize the study population, the MPO genotypes, MPO flow cytometry data, and covariates. MPO from monocytes and granulocytes will be assessed separately and combined as an estimate of “total MPO”. Genotypes will be coded, by a priori information, and analyzed using both the co-dominant model (i.e., wildtype = 0, heterozygote = 1 and variant = 2) and the mutation-dominant model (i.e., wildtype = 0, heterozygote & variant = 1). Also, an “additive model/score” will be considered by combining all MPO variants. Generalized linear models will be used to determine if the MPO flow cytometry data varies by MPO genotypes and if necessary, adjusted for potential confounders. Stratified analyses will be explored to determine if the associations are more evident in certain subgroups or associated with specific existing medical histories.

**Any anticipated methodological limitations or challenges:** At this point we do not anticipate any substantial methodological imitation or challenges.

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 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? **X** 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](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)?
   #1290: The effects of polymorphisms of *TCF7L2, CD14, MPO, TLR2,* and *TLR4* on monocyte activation: The Atherosclerosis Risk in Communities (ARIC) MRI Study.
   #1206 Association of risk factors with blood platelet and monocyte cell-markers and cell aggregates (ARIC MRI)
   #1219 Peripheral blood monocyte myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2) levels and carotid artery plaque presence/progression (ARIC CAR MRI Study)
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)* Novel Genes Influencing Cardiovascular Disease Risk in the Population-at-Large: The ARIC Study (CARMRI) (#2004.11)

*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.

References for rationale


