I. INVESTIGATOR INFORMATION:

Name of Lead Author: Jonathan Kocarnik  
Email Address: kocarnik@fhcrc.org  
Telephone Number: 206-667-5257  
Fax Number: 206-667-7850  
Mailing Address: 1100 Fairview Ave. N / M4-B402  
P.O. Box 19024, Seattle, WA 98109

Name of Corresponding Author (if different): Ulrike Peters  
Email Address: upeters@fhcrc.org  
Telephone Number: 206-667-2450  
Fax Number: 206-667-7850  
Mailing Address: 1100 Fairview Ave. N / M4-B402  
P.O. Box 19024, Seattle, WA 98109

Names, affiliations and email address of PAGE Investigators proposed as co-authors:

<table>
<thead>
<tr>
<th>N, N</th>
<th>Affiliation in PAGE</th>
<th>EMAIL</th>
<th>Junior Investigator?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peters, Ulrike</td>
<td>WHI</td>
<td><a href="mailto:upeters@fhcrc.org">upeters@fhcrc.org</a></td>
<td>N</td>
</tr>
<tr>
<td>Pendergrass, Sarah</td>
<td>EAGLE</td>
<td><a href="mailto:sarah.pendergrass@chgr.mc.vanderbilt.edu">sarah.pendergrass@chgr.mc.vanderbilt.edu</a></td>
<td></td>
</tr>
<tr>
<td>Pankow, Jim</td>
<td>CALiCo</td>
<td><a href="mailto:Panko001@umn.edu">Panko001@umn.edu</a></td>
<td></td>
</tr>
<tr>
<td>Cheng, Iona</td>
<td>MEC</td>
<td><a href="mailto:icheng@crch.hawaii.edu">icheng@crch.hawaii.edu</a></td>
<td></td>
</tr>
<tr>
<td>Schumacher, Fred</td>
<td>MEC</td>
<td><a href="mailto:Schumacher_f@ccnt.usc.edu">Schumacher_f@ccnt.usc.edu</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NHGRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matise, Tara</td>
<td>Coordinating Center</td>
<td><a href="mailto:Matise@dls.rutgers.edu">Matise@dls.rutgers.edu</a></td>
<td>N</td>
</tr>
</tbody>
</table>

Names, affiliations, email address of non-PAGE investigators proposed as co-authors:

<table>
<thead>
<tr>
<th>N, N</th>
<th>university</th>
<th>email</th>
<th>Junior Investigator?</th>
</tr>
</thead>
<tbody>
<tr>
<td>university</td>
<td>email</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>N, N</td>
<td>Email</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

II. SCIENTIFIC RATIONALE
Inflammation is an important health outcome related to many diseases and endpoints, and is commonly implicated in the pathogenesis of atherosclerosis (1). Several circulating inflammatory biomarkers can be measured in plasma (2), with C-reactive protein (CRP) being the most studied. Inflammatory biomarker levels have been shown to be associated with a large number of outcomes, such as obesity (3), stroke (4), cognitive impairment (4), type 2 diabetes (5), fitness level and body composition (6), cancer (7), and chronic obstructive pulmonary disorder (8, 9), among others. Genetic variants have also been associated with each particular health outcome. However, it has not been well studied whether these genetic variants may also be associated with inflammation. Because inflammation is potentially involved in so many disease outcomes, there is interest in understanding the biological underpinnings for inter-individual variability in systemic inflammatory biomarkers. This study is designed to evaluate whether genetic variants which have been previously associated with inflammation-related outcomes, such as obesity or type 2 diabetes, are also associated with inflammatory biomarker levels, which would make inflammation an intermediate endpoint of interest.

Inflammation levels are influenced by both genetic and environmental factors. Clinical covariates have been shown to explain a substantial amount of the variation in inflammation. A report from the Framingham Heart Study, for example, demonstrated that twelve clinical covariates accounted for 26% of the total variance of serum CRP levels, with body mass index (BMI) alone explaining 15% of the variance (14). As such, gene-environment interactions likely play a significant role in inflammatory biomarker levels. This study provides the opportunity to explore the relationship between environmental factors and inflammatory levels, as well as the effect of gene-environment interactions.

Genetic complexities in serum inflammatory levels also require further elucidation. One twin study indicated pleiotropy for CRP variants with BMI, leptin, triglycerides and systolic blood pressure (15). It is possible that such influences may be true in the other direction as well, where variants in genes associated with cardiovascular disease or stroke may also influence levels of inflammatory biomarkers such as CRP or IL-6. Epistasis has been demonstrated for particular combinations of TH and ADRB2 alleles, which predict plasma CRP levels (15). Genetic variants related to different health outcomes may also interact in various ways to influence inflammation levels. Multiple genes are involved in regulating CRP levels, such as IL-6, IL-1, and TNF-α (10), and SNPs in one or more regulatory genes influence serum CRP concentrations (15, 16). It thus seems likely that inflammatory biomarker levels are influenced and regulated by more than a single gene or pathway, and could involve variants previously associated with other health outcomes. This study provides the opportunity to further evaluate how SNPs related to inflammation-related disease are associated with certain inflammatory marker levels in multi-ethnic populations, both by themselves and through interaction with other gene variants.

### III. OBJECTIVES AND PLAN

**Study Questions/Hypotheses.**

Inflammation is a complex trait which influences, and is influenced by, many factors. Some of these factors may be the same factors that cause inflammation-related disease. This study proposes to elucidate these factors through the analysis of genetic variants and environmental data among the diverse PAGE study population. The specific aims of this study are to:

1) Evaluate if SNPs known to be associated with various outcomes related to inflammation, such as stroke, cardiovascular disease, obesity, or type 2 diabetes, are also associated
with inflammatory markers, as a potential intermediate outcome within multi-ethnic study populations.

2) Explore if variants found to be associated in aim 1 interact with environmental factors, such as sex, smoking, physical activity, NSAID use, or diet (gene-environment interactions).

3) Explore if variants found to be associated in aim 1 interact with each other (gene-gene interactions).

**Study populations, study design for each**
The following PAGE studies will be included to answer these questions:
- Women’s Health Initiative (WHI)
- Multiethnic Cohort (MEC)
- Epidemiologic Architecture for Genes Linked to the Environment (EAGLE)/the National Health and Nutrition Examination Surveys
- Genetic Epidemiology of Causal Variants Across the Life Course Consortium (CALICO), which includes Atherosclerosis Risk in Communities (ARIC), Coronary Artery Risk in Young Adults (CARDIA), Cardiovascular Heart Study (CHS), Hispanic Community Health Study, and the Strong Heart Study.

**Variant/SNPs (Specify)**
Our analysis will include all variants of year one related to cardiovascular disease, myocardial infarction, stroke, type-2 diabetes, obesity and body mass index. For a comprehensive list please see file “snp_studies_v5” on the PAGE website (we will include all SNPs listed for these outcomes). We will not be using the SNPs for inflammation (specifically for CRP, IL6, IL6R, PVRL2, HNF1A, and 12q23), as EAGLE will propose a single study on the relationship of inflammatory SNPs with inflammatory biomarkers.

**Phenotype(s) (Specify)**
Phenotypes of interest are concentrations of inflammatory biomarkers E-Selectin, IL-1 beta, IL-6, CRP, SIAM, TNF-α, TNFR1, TNFR2, and VCAM-1. These biomarkers have been previously associated as intermediate outcomes involved in the pathogenesis of further disease such as coronary heart disease, hypertension, stroke, venous thromboembolism, and others.

**Covariates (Specify)**
Covariates of interest include age, sex, race/ethnicity, socioeconomic status, BMI/obesity, alcohol use, physical activity, smoking status, NSAIDs, hormone use, and dietary factors, including antioxidant vitamins and omega-3 fatty acid intake, based on exposure working group recommendations for these. We are also interested in further health outcomes, such as cardiovascular disease, type-2 diabetes, stroke, obesity, and myocardial infarction (based on working group definitions).

**Main statistical analysis methods**
Genotype data will be coded assuming additive genetic models, coding each SNP as 0, 1, 2 for the minor allele count. We will model concentrations of inflammatory biomarkers as continuous variables in linear regression models to test for associations between these traits and the SNPs listed previously. To harmonize inflammatory markers between studies, we will utilize quantile normalization. For the CRP analysis we will do stratified analysis of measurements with and without high sensitivity. Covariates as listed above will be included in the model. Gene-gene and gene-environment interactions will be assessed by testing for the
statistical significance of interaction terms by the -2 log likelihood ratio test. SNPs listed in the file “snp_studies_v5” on the PAGE website will be adjusted for multiple comparisons using the Bonferroni adjustment (0.05 / x). If studies agree we will include SNPs that overlap at least two studies, as well as SNPs that were only included in a single study. To investigate the impact of diseases associated with inflammation, we will stratify by cardiovascular disease, type-2 diabetes, stroke, obesity, and myocardial infarction.

Ancestry and how it is used in the analyses
In order to adjust for potential population stratification, analyses will be done either using ethnicity as a covariate or in groups stratified by ethnicity. Any additional analyses will follow the recommendations of the ancestry working group, including recommendations of using AIMs as they become available.

IV. SOURCE OF DATA TO BE USED (Provide rationale for any data whose relevance to this manuscript is not obvious):

Check all that apply:

a. Aggregate/summary data to be generated by investigators of the study(ies) mentioned:

[X] EAGLE; [X] CALiCO; [X] MEC; [X] WHI; [ ] CC; [ ] Other: ___________________
If CALiCo, specify [ ] ARIC; [ ] CARDIA; [ ] CHS; [ ] SHS-Fam; [ ] SHS-Cohort; [ ] SOL

If Other: Study/Organization contributing aggregate data to analyses for this manuscript:

I, Jonathan Kocarnik, affirm that this proposal has been reviewed and approved by all listed investigators.

V. REFERENCES


