1.a. Full Title:

b. Abbreviated Title (Length 26 characters): Interleukin-6 gene, inflammation and incident cardiovascular disease

2. Writing Group (list individual with lead responsibility first):

   Lead:     Yongmei Liu  
   Address:  2024 E. Monument St.  
             Suite 2-516  
             Baltimore, MD 21205  
   Phone:    410-502-8896  
   Fax:      410-614-9793  
   E-mail:   yoliu@jhsph.edu

   Writing group members:  
   Josef Coresh, Eric Boerwinkle, Christy Ballantyne, Ron Hoogeveen, others welcome.

3. Timeline: 10/03-1/04

4. Rationale:

   Evolving evidence illustrates a strong association between inflammation and cardiovascular disease (CVD). However, it is unknown whether inflammation is atherogenic or a marker of atherosclerosis. The association of genetic susceptibility to inflammation with a higher risk of CVD is free of this problem since genotypes precede atherosclerosis and do not change over time. Interleukin-6 (IL-6), as a major pro-inflammatory cytokine, plays a central role to the inflammatory response inducing the synthesis of major acute-phase proteins and inhibiting the production of albumin. Therefore, more light has shed on a candidate gene, \textit{IL6}.

   Two polymorphisms in the promoter region of the \textit{IL6} gene, -572G/C and -174G/C, have been studied as potential functional variants in Whites. The –572G/C polymorphism had no association with CVD\cite{1,2}. The –174 C allele was associated with higher risk of CVD or mortality in some studies\cite{1-4}, but not in others\cite{5-7}. This association is thought to be partly mediated though the effect on blood pressure\cite{1,8} and lipid proteins\cite{9}. However, the effect of the 2 polymorphisms on circulating concentrates of IL-6 is rather mixed\cite{10-12}. Our recent study on the \textit{IL6} gene revealed that 2 novel variants, Pro32Ser (12% in Blacks) and Asp162Val (1%) in Whites) in the coding region, and the previously reported variant, –174G/C in the promoter region, affected inflammation and risk of CVD among a cohort of dialysis patient.

   To replicate these findings in the general population, we propose a case-cohort study on the associations of the \textit{IL6} polymorphisms with inflammatory markers, traditional CVD risk factors, and risks of coronary heart disease (CHD). \textit{IL6} genotyping was proposed as part of Table 4 genetic study and IL-6 and TNF-alpha levels were measured in Dr. Ballantyne’s laboratory as part of Table 3 study.
5. **Main Hypothesis/Study Questions:**

To test the hypothesis that *IL6* polymorphisms affect or modify:

A. IL-6 levels, TNF-alpha, C-reactive protein (CRP), fibrinogen, white blood cell count, and serum albumin (only in subgroups who have both measurements of *IL6* genotype and its levels)

B. Traditional CVD risk factors: blood pressure, total cholesterol, Lp(a) and LDL-cholesterol

C. The risk of Incident CHD or stroke. Much of this risk may be mediated through inflammatory marker levels.

D. The association of environmental factors, particularly smoking, on inflammatory markers and CVD. In particular, we hypothesize that *IL6* genotypes will be most associated with inflammation and CVD in non-smokers and least associated in current smokers.

To test the hypothesis that serum IL-6 and TNF-alpha affect:

E. The risk of Incident CHD.

6. **Data (variables, time window, source, inclusions/exclusions):**

The proposed study will include two case-cohort study groups. One comprises 1081 incident CHD and 356 incident stroke cases occurred between baseline and 31Dec2000, and a subset of 1081 participants who were randomly sampled with stratification by age, race, and sex (Table 4 participants). The other one includes 406 incident CHD cases occurred between visit 2 and 31Dec1995, and a subset of 486 participants who were randomly sampled with stratification by age, race, and sex (Table 3 participants).

Exclusion criteria: (1) neither White nor African American, (2) prevalent CVD, or (3) missing the *IL6* genotype result.

Exposure variables: 4 of *IL6* polymorphisms, -572G/C, –174G/C, Pro32Ser and Asp162Val, serum IL-6 and TNF-alpha levels,

Outcomes:

F. Traditional CVD risk factors: systolic and diastolic blood pressure, total cholesterol, and LDL-cholesterol

G. Incident CVD, defined as (1) a definite or probable MI, (2) a silent MI between examinations as ascertained by ECG, (3) a definite fatal CHD death, (4) a coronary revascularization, or (5) stroke

Covariates: age, race gender, smoking, alcohol consumption, baseline body-mass-index and CVD risk factors, statins use

Analysis:

1. Genotype analysis and haplotype analysis. Haplotypes will be estimated using PHASE. Both genotypes & haplotypes will be assumed to act co-dominantly.

2. Cross-sectional analysis using multi-variate linear regression for aims A & B

3. Survival analysis using a weighted proportional hazards regression (Barlow Macro) for aims C & D & E

Limitation:
Given that two case-cohort study participants are involved for measurements of IL6 genotyping and its levels, we understand the power will be very limited to examine this very important association. We will revisit this issue later.

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://bios.unc.edu/units/csc/ARIC/study/studymem.html

___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)? Dr. Eric Boerwinkle

11. 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.

Reference List


