1. **Full Title**: CRP genetic variation predicts (does not predict) CRP levels and risk of incident CHD and ischemic stroke: the ARIC study

b. **Abbreviated Title (Length 26 characters)**: CRP predicts incident CHD/Stroke

2. **Writing Group**: Kelly Volcik, Christie Ballantyne, Aaron Folsom, Jim Pankow, Eric Boerwinkle

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**: CRP levels are available for a stratified random sample of the ARIC cohort (Table 4). Genotyping for the CRP triallelic polymorphism (rs3091244) is complete for the entire ARIC cohort. Statistical analyses will begin immediately, with a first draft manuscript prepared by June 2007 (initial plans are to submit as a letter to a peer-reviewed journal).

4. **Rationale**:  
C-reactive protein (CRP), a classic acute-phase reactant, is a marker of systemic inflammation and has been associated with cardiovascular events in numerous prospective and case-control studies.\(^1\)\(^1\)\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\) However, whether CRP is merely a marker of inflammation or plays a direct role in atherogenesis has been debated. CRP may be the direct link between chronic inflammation and cardiovascular disease, but many other inflammatory markers have also been determined to be indicators of cardiovascular risk (e.g. fibrinogen, vWF, IL-6 and white blood cell count).\(^7\)\(^13\)\(^14\)\(^15\)

Recent genetic studies have focused on associations between a triallelic polymorphism in the promoter region of the CRP gene (rs3091244) and CRP levels, as well as associations between this SNP and risk of cardiovascular disease. Miller and colleagues evaluated the CRP triallelic SNP in the Women's Health Study (WHS), the Pravastatin Inflammation/CRP Evaluation (PRINCE), and the Physician’s Health Study (PHS).\(^16\) Both minor alleles of the triallelic SNP were associated with higher CRP levels.\(^16\) Findings from this study support the hypothesis that the CRP
trialelic SNP is a functional SNP that modulates transcription factor binding.\textsuperscript{16,17} However, further investigation of the relationship between the CRP triallelic SNP and cardiovascular endpoints (MI and ischemic stroke) showed no associations with either of these diseases.\textsuperscript{16} Kathiresan and colleagues evaluated thirteen SNPs in the CRP gene in \textasciitilde3300 participants of the Framingham Heart Study (FHS) and found the CRP triallelic SNP to be the strongest variant associated with CRP levels.\textsuperscript{18} No associations were detected for the CRP triallelic SNP and prevalent or incident CVD.\textsuperscript{18} A third study investigated the CRP triallelic SNP in individuals from the Third National Health and Nutrition Examination Survey (NHANES III).\textsuperscript{19} This study found the CRP triallelic SNP to be associated with serum CRP levels in non-Hispanic blacks and Mexican Americans.\textsuperscript{19} In analyses of prevalent CHD, the CRP triallelic AA genotype was positively associated with prevalent CHD in non-Hispanic whites, while the AT genotype was inversely associated with prevalent CHD in non-Hispanic whites.\textsuperscript{19} The authors noted the very small number of cases with the AA or AT genotype (each n<5) compared to the CC genotype (n=52), therefore prompting caution in the interpretation of results.\textsuperscript{19}

We propose to evaluate the association between the CRP triallelic SNP and incident CHD and stroke, as well as the association between this polymorphism and hsCRP levels, in the large bi-racial ARIC cohort.

5. Main Hypothesis/Study Questions:

1. Estimate the frequency distribution of the CRP triallelic SNP in the ARIC cohort. \textit{If allele frequencies are markedly disparate between whites and African Americans, all of the following analyses will be conducted separately by race.}

2. Utilizing Cox regression, evaluate the ability of the CRP triallelic SNP to predict incident CHD. Models will be adjusted for age, gender, race, BMI, HDL and total cholesterol, smoking, diabetes and hypertension status.

3. Utilizing Cox regression, evaluate the ability of the CRP triallelic SNP to predict incident ischemic stroke. Models will be adjusted for age, gender, race, smoking, diabetes and hypertension status.

4. Evaluate whether the CRP triallelic SNP is associated with hsCRP levels. These analyses will be evaluated utilizing the SUDAAN software package to adjust for the sampling strategy. Age, gender, race and case status (CHD and stroke) will be included as covariates.

5. The survival analyses described above (\#s 2 and 3) for incident CHD and stroke will be repeated including hsCRP levels as a covariate. The method of Barlow will be used to adjust for the sampling strategy. Covariates will be the same as listed above in \#s 2 and 3.

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).

The CRP triallelic SNP (rs3091244) has been genotyped on the entire ARIC cohort and will be evaluated for associations with incident CHD and stroke, as well as evaluated for correlations with hsCRP levels. CRP levels are available for only a subset (stratified random sample) of the ARIC cohort. Therefore, analyses involving CRP levels will be evaluated utilizing the SUDAAN software package / method of Barlow to adjust for the sampling strategy where appropriate. Goodness of fit to Hardy-Weinberg expectations will be carried out using a chi-square test.

ARIC’s incident CHD and ischemic stroke case status will be the primary dependent variables. The usual DNA restriction, ethnic group and missing data exclusion criteria will be used. Exclusions will include the following: 1) positive or unknown history of prevalent CHD or stroke or history of TIA/stroke, 2) prohibited use of DNA, 3) ethnic background other than white or African American, as well as African Americans not from Jackson or Forsyth. For incident CHD analyses, we will use the variable IN_03SP. For incident ischemic stroke analyses, we will use the variable
IN03ISC. Independent variables include but are not limited to CRP genotype status and traditional risk factors such as age, gender, smoking, diabetes and hypertension status.

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
8.a. Will the DNA data be used in this manuscript?  __X__ Yes   ____ No
   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.  __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)?

MS#1029: CRP gene polymorphism and risk of CHD and stroke (JS Pankow) – this proposal is investigating a different CRP SNP (rs2794521) in the promoter region of the gene, and Dr. Pankow is part of our writing group

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  ___ Yes   __X__ No
   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)* ________________________)

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.  Agreed

References: