1.a. Full Title: Interaction of folate intake and the paraoxonase Q192R polymorphism with risk of acute coronary events, strokes and atherosclerosis: the Atherosclerosis Risks in Communities Study

b. Abbreviated Title (Length 26 characters): PON192, folate intake and CVD risk

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

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3. Timeline:

A manuscript will be submitted for review by the ARIC Publications Committee within one year of approval of this proposal.

4. Rationale:

During recent years, the role of elevated plasma total homocysteine (tHcy) in heart diseases has been intensively studied. Homocysteine (Hcy) is a sulphur-containing amino acid, formed during normal metabolism of the essential amino acid methionine. Defects in intracellular Hcy metabolism lead to the elevation of plasma tHcy. These metabolic defects can have a genetic or a nutritional background, i.e. an inadequate intake of folate or vitamin B₆ or B₁₂ that serve as cofactors or substrates for the enzymes involved in Hcy metabolism.¹ Approximately two thirds of the cases with elevated tHcy levels have been attributed to low or moderate plasma/serum concentrations of these vitamins, of which folate is considered the most important.²,³ Few previous epidemiological studies have addressed the link between dietary folate and the risk of cardiovascular diseases (CVD).¹ Those that have researched this issue produced conflicting results. In some studies, subjects with lower circulating folate concentrations or lower dietary intake of folate have had higher risk of coronary events compared with others, although not all studies have detected this association.¹
For reasons outlined below, a gene-nutrient interaction between the paraoxonase (PON) genes and folate intake on the risk of acute coronary events is plausible. In human plasma, Hcy occurs in various forms. Most is oxidized and exists as various disulfides such as Hcy thiolactone, with less than 1% in the reduced (sulfhydryl) form. The remainders are oxidized and exist as various disulphides, such as Hcy thiolactone. Hcy thiolactone is formed in all human cells and because inadvertent reactions of thiolactone with proteins are potentially harmful, the ability to detoxify Hcy thiolactone is essential for biological integrity. H. Jakubowski (2000) reported that the enzyme Hcy thiolactonase, which hydrolyzes Hcy thiolactone to Hcy, could be in fact PON. If this theory is confirmed, then PON could hydrolyze Hcy thiolactone back to Hcy and Hcy could be converted either back to methionine (via a reaction which needs folate and vitamin B12 as co-factors), or condensed with serine to form cystathionine in a reaction that is dependent on vitamin B6 (transsulphuration pathway).

The human serum PON is an antioxidative enzyme present in HDL, which eliminates radicals in the circulation and protects against coronary diseases. PON has been suggested to account for an important part of the antioxidative property of HDL, and it has been shown that PON protects LDL against oxidation. A lowered PON activity or mutations in the PON1 gene have also been reported in patients with atherosclerotic heart disease.

The human PON gene family consists of three members, PON1, PON2, and PON3, aligned next to each other on chromosome 7. The enzyme activity is modulated by two common amino acid polymorphisms at positions Q192R (Gln>Arg) and M55L (Met>Leu) in the paraoxonase gene PON1. Subjects with the Q192Q genotype have lower PON activity than those with the other genotypes. Also, it has been demonstrated that the Q type isozyme is more efficient in protecting against LDL oxidation than the R type, but controversially, the R type hydrolyzes thiolactones more readily than the Q isoform.

The ARIC cohort has been genotyped for the PON 192 polymorphism by Dr. Bray’s laboratory (n=16,095, of which 2879, 17% are QQ genotypes and 6370, 38% RR genotypes). The ARIC participant’s usual dietary intake was assessed twice using a semi-quantitative food frequency questionnaire, at baseline and at the Visit 3 re-examination. This information is available for approximately 14,000 cohort members. Vitamin and mineral supplement users were identified from a medication usage survey at each cohort visit, and from a more detailed survey of vitamins and dietary supplements in the course of ARIC’s visit 3 examination. Plasma tHcy concentration was measured on approximately 700 cohort members selected as cases of coronary heart disease and their controls.

Schimakawa et al. (1997) demonstrated in the ARIC study that intakes of folate, vitamin B6 and vitamin B12 were inversely associated with plasma tHcy. Folsom et al., 1997 reported that after accounting for other risk factors, higher plasma PLP (active form of vitamin B6) was associated with lower coronary heart disease incidence, but plasma tHcy or folate was not.

As folate-PON interactions are not studied extensively, we propose to assess the association between folate intake, PON192 polymorphism and the risk of acute coronary event in the ARIC Study. The Follow-up for analysis of this study should be limited also to the end of year 1996, prior to the establishment of the folic acid fortification program initiation in the US in1997-98.
5. **Main Hypothesis/Study Questions**: The aim of this work is to quantify the interaction between folate intake and PON 192 polymorphism with the risk of acute coronary events (n=1081 from Visit 1 through 2001), stroke (n = 356 from Visit 1 through 2001), and with the extent of carotid atherosclerosis measured as intima-media thickness and also by the presence of plaque. The rationale supporting this hypothesis is that both low folate intake and low PON activity could affect to plasma tHcy levels. The main hypothesis is that study subjects with both low folate intake and low PON activity genotype (QQ) are at highest risk of atherosclerotic thromboembolic events and a higher burden of subclinical atherosclerosis. A sub-analysis will apply censoring at the time of the full implementation of the folate fortification program.

6. **Data (variables, time window, source, inclusions/exclusions)**: This manuscript is based on nutrition data collected in ARIC’s Visit 1 examinations. Data needed are food-frequency data (specifically intake of folate, vitamin B6, vitamin B12, total energy, dietary fiber, whole grain, fruit and vegetables), and risk factors for acute coronary event as potential confounders (examination year, sex, age, race, BMI, serum lipids, blood pressure, smoking status, education, physical activity, and field center), IMT and plaque/shadowing, incident coronary heart disease and incident stroke updated through 2002, and length of follow-up period.

**Exclusions are:** Missing prevalent CHD (at Visit 1), missing prevalent stroke (at Visit 1), missing ultrasound data, missing dietary information at visit 1, missing exposure information and missing information on potential confounders.

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.csec.unc.edu/ARIC/search.php](http://www.csec.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)?

None evident

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.

References: