1.a. Full Title:

b. Abbreviated Title (Length 26 characters):
   Gamma-fibrinogen and CHD

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

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3. Timeline:
   Laboratory measurements will take about 2 months, data verification and analysis 1 month, manuscript preparation 1 month.

4. Rationale:
   Heart disease is the leading cause of death in the United States, and stroke is the third leading cause. The incidence of heart disease and stroke have been correlated with several risk factors, including plasma fibrinogen levels (Maresca et al., 1999). One such study that clearly shows a strong association of plasma fibrinogen levels with coronary heart disease is the Atherosclerosis Risk in Communities (ARIC) study (Folsom et al., 2000). The mechanism by which elevated fibrinogen levels contribute to heart disease is unclear. The most common form of fibrinogen consists of three polypeptide chains, $\alpha$, $\beta$, and $\gamma$, arranged as a dimer with the stoichiometry $(\alpha, \beta, \gamma)_2$. In approximately 10% of fibrinogen molecules, one of the $\gamma$ chains, termed $\gamma'$ (Wolfenstein-Todel & Mosesson, 1981), has a twenty amino acid sequence substituted for the carboxyl terminal four amino acids found in the more common $\gamma$ chain, termed $\gammaA$, and is referred to as $\gammaA/\gamma'$ fibrinogen. Previous studies have shown that $\gammaA/\gamma'$ fibrinogen forms clots that are more extensively crosslinked by factor XIIIa, a plasma transglutaminase, and are therefore
resistant to breakdown by fibrinolytic enzymes, including tissue-type plasminogen activator (Falls and Farrell, 1997). In addition, the binding of thrombin to γA/γ fibrin may provide an additional source of clot-bound thrombin (Meh et al., 1996) that can convert more fibrinogen to fibrin in a "feed-forward" reaction. Clot-bound thrombin is active even in the presence of heparin, since clot-bound thrombin is resistant to heparin-catalyzed inhibition by antithrombin III (Hogg & Jackson, 1989; Weitz et al., 1990). These facts provided the impetus to investigate whether γA/γ fibrinogen is a risk factor for coronary artery disease. Since in vitro data showed that γ fibrinogen forms fibrin clots that are resistant to fibrinolysis, we hypothesized that elevated levels of γA/γ fibrinogen may constitute an independent risk factor for thrombosis. We therefore developed a monoclonal antibody to the γ 20 amino acid carboxy peptide that specifically recognizes the γA/γ form of fibrinogen. Using this antibody, we developed an ELISA to measure the amount of γA/γ fibrinogen in plasma. From preliminary case-control (91 CHD cases and 120 controls) study, it appears that high levels of γA/γ fibrinogen may be a risk factor for coronary artery disease, independent of the levels of total fibrinogen. However, these results were obtained in a small pilot case/control study, rather than in a more definitive prospective study.

We therefore propose to use this ELISA assay to measure the amount of γA/γ fibrinogen in a population of well-characterized individuals from the ARIC study to see if γA/γ fibrinogen truly constitutes an independent risk factor for thrombosis. The results of this research will extend our knowledge of the risk factors affecting thrombosis, and will provide crucial preliminary data for future NIH-funded investigations into the mechanism of thrombosis. In addition, the results may lead to new screening tests for thrombotic risk in humans.

References


5. Main Hypothesis/Study Questions:

Our hypothesis is that elevated levels of γA/γ fibrinogen are a risk factor for thrombosis, independent of the total plasma levels of fibrinogen. The strength of the hypothesis rests on reproducible in vitro data that elevated γA/γ fibrinogen levels give rise to clots that resist lysis
(Falls & Farrell, 1997), and therefore provide a firm physiological rationale for the proposed epidemiology studies.

6. Data (variables, time window, source, inclusions/exclusions):
   Approximately 1000 plasma samples will be used for measurement of gamma fibrinogen, from the new post Visit 2 CHD and CRS list.

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?  ___ Yes    _X_ 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”?  ___ 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/stdy/studymem.html]

   ___x__ Yes    _______ No