ARIC MANUSCRIPT PROPOSAL FORM

Manuscript #215

1. Title:
Role of apolipoprotein C-III gene locus in hypertriglyceridemia

2. Writing Group:
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3. Timeline:
DNA from 50 normolipidemic individuals, 48 subjects with elevated plasma cholesterol, 47 subjects with elevated plasma triglyceride (TG), and 123 subjects with both elevated plasma cholesterol and TG has been extracted (see Ms. 113). To contrast the frequency of the Sst-I polymorphism and apoC-III promoter haplotypes in subjects with high TG alone and those with high TG and high cholesterol, the original high TG group needs to be increased from 47 subjects to 123 subject. Only white subjects may be used since the RFLP differs by race. Initial subject selection was performed with the help of Drs. Sharrett and Chambless. The same selection criteria and exclusion criteria (detailed in Mx. 113, specifications are attached) will be used for enrichment of the hypertriglyceridemic sample to have a comparable group of HT and HT-hypercholesterolemic subjects. Thawing of one plasma-aliquot from visit one for each of the hypertriglyceridemic subjects is requested to measure plasma apoC-II, apoC-III, apoE, apoA-II, apoA-IV, and apoC-II and apoC-III in VLDL and HDL. These measurements have already been performed in the hypercholesterolemic-hypertriglyceridemic group. The determination of the Sst-I RFLP and the polymorphisms in the apoC-III gene promoter in the 268 DNA specimens available can begin immediately. DNA sequence analysis will be used for verification of results. After identification of 80 additional hypertriglyceridemic subjects, DNA will be extracted and similar analyses will be performed

4. Rationale:
In ARIC-Manuscript 113, currently under review by the publication committee, we found no differences in allele frequencies at the apoA-I/C-III/A-IV gene locus -- as defined by a variant XmnI polymorphic site 2.5 kilobase pairs upstream of the apoA-I gene transcription start site -- among four lipid phenotypes, i.e. subjects with normal cholesterol and triglyceride (TG) levels, elevated cholesterol and normal TG, elevated cholesterol and elevated TG, and normal cholesterol and elevated TG. In the hypercholesterolemic-hypertriglyceridemic group, subjects possessing the rare 6.6-kbp allele exhibited a greater carotid artery intimal-medial thickness than subjects homozygous for the 8.3-kbp allele. Furthermore, the 6.6-kbp allele was associated with distinct changes in lipid transport, i.e., differences in the plasma ratios of apoC-II to apoC-III, apoE to apoC-III, apoC-II to apoA-IV, and apoE to apoA-IV.

There are now numerous studies showing a relationship of a variant Sst-I site in the 3' untranslated region of the apoC-III gene with hypertriglyceridemia. Most recently, this Sst-I site was shown to be in very strong linkage disequilibrium with sequence variation in the promoter region of the apoC-III gene. This study was conducted in 78 normolipidemic adults and 79 adults with hypertriglyceridemia (Proc. Natl. Acad. Sci U.S.A. 90, 4562-4566, 1993). In this study population, three communion haplotypes were identified in the apoC-III gene promoter. One of the three haplotypes appeared to protect from hypertriglyceridemia

(relative risk 0.28; $p = 0.005$), while one haplotype showed a very strong association with hypertriglyceridemia (relative risk 3.14; $p < 0.0025$).

5. Main hypotheses/issues to be addressed:
1) The frequency of the Sst-I polymorphic site differs among lipid phenotypes, i.e. the highest frequency will be observed in hypertriglyceridemic subjects.

2) The Sst-I polymorphic site is in strong linkage disequilibrium with sequence variation in the apoC-III gene promoter as shown by allele-specific oligonucleotide hybridization and increased plasma apoC-III levels.

3) The Sst-I site is in linkage equilibrium with the XmnI site (studied in Ms. #113) and may or may not be associated with increased wall thickness in any of the lipid phenotypes studied (no previous date on this, therefore no apriori hypothesis).

4) The apolipoprotein abnormality, most likely associated with the Sst-I polymorphism, differs from that described for the XmnI polymorphism. (This information will allow us to focus better on the genetic defect that is associated with the XmnI polymorphic site in hypertriglyceridemic-hypercholesterolemic subjects.)

6. Data Requirements:
Selection of the additional 80 subjects with elevated triglycerides only using the attached specifications will be performed with the help of Drs. Sharrett and Chambless. Data analysis will be performed by Dr. Boerwinkle. Data on the Sst-I
polymorphism and sequence heterogeneity in the apoC-III gene promoter as well as plasma apoprotein levels in the hypertriglyceridemic group will be collected in the ARIC Lipid Laboratory. The list of other variables is the same as for manuscript 113.