Manuscript #190

1. Title:
Apo[a] phenotype and Lp[a] plasma concentrations in subjects with CAD or asymptomatic carotid atherosclerosis.

2. Writing Group:
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3. Timeline:
The study will require identification of 171 cases with ARIC-defined prevalent coronary artery disease, similar with regard to sex, age within ten years, and visit date with a window of six months to 171 cases with carotid thickening already studied with the PPL protocol, for analysis of Lp[a] phenotypes. A time span of 6 months between the Lp[a] measurement and the clinical CAD event should be included in selection criteria, since Lp[a] may behave as acute phase protein, which would confound the relationship among apo[a] phenotypes and Lp[a] plasma concentrations. Thus, the diagnosis of CAD should be based on visit 1 data, while analyses will be performed in visit 2 specimens. Subjects, in whom a CAD event occurred within the 6 months prior to visit 2 specimen collection, can be excluded later from analyses. Only white participants will be used to avoid potentially confounding influences of race on the relationship between apo[a] phenotype and Lp[a] levels. Exclusion criteria are the same as for the PPL study, i.e., diabetes mellitus (self-reported, use of insulin or oral antidiabetics), kidney disease, use of thyroid hormone, use of sex hormones, use of nicotinic acid or neomycin as lipid lowering drugs (perhaps all lipid altering drugs depending on the number of subjects which would have to be excluded). The sample size of 171 subjects with prevalent disease is optimized for comparison with our previous analyses of 171 white carotid cases. In addition, data have been collected in 271 ultrasound controls who were group matched to carotid cases. Furthermore, previous studies have found apo[a] phenotype differences between coronary cases and controls in sample sizes much smaller than the one proposed. In our 171 carotid cases the frequency of polymorphs 1-5 was 0.26 using a single allele count for subjects homozygous for one polymorph. We expect the frequency of polymorphs 1-5 to be 0.40 or higher in CAD cases (80% power and 5% significance level).

As soon as subjects are identified, Lp[a] phenotype analysis will be performed. This will require thawing of 1 plasma aliquot stored at the Lipid Laboratory. Visit 2 aliquots will be used for this purpose since visit 2 aliquots were also used for carotid cases. Analyses will take 2 months.
4. Rationale:
Lp[a] comprises a unique class of plasma lipoproteins. Its two most abundant protein are two high molecular weight glycoproteins, apoB-100 and apo[a]. Apo[a] is covalently linked to apoB-100 by a disulfide-bridge and exists in several polymorphs distinguished by their molecular weights. The molecular basis for the variation in apo[a] size lies primarily in multiple apo[a] alleles which code for 9 to 35 kringle type 2 (plasminogen kringle type 4) repeats.

Family studies have shown that more than 90% of the variability of Lp[a] plasma levels is determined by the apo[a] size locus. An inverse associations exists between Lp[a] plasma levels and apo[a] size. Apo[a] size explains 19 - 70% of the variance in Lp[a] plasma levels in different populations. Hence, the sequences that encode the number of apo[a] kringle 2 units as well as additional sequence variation at the apo[a] gene locus are responsible for much of the control of plasma Lp[a] levels.

In ARIC manuscript #98 just completed (the manuscript has been forwarded to the publication Committee), cases with carotid disease, but free of prevalent CAD, had higher Lp[a] levels than controls. However, the distribution of apo[a] phenotypes was not different between carotid cases and controls. Rather, Lp[a] levels were higher in carotid cases for most of the phenotypes which were present in more than 3 cases and 3 controls. This finding was somewhat unexpected since previous studies in subjects with symptomatic CAD showed an increased prevalence of phenotypes with small apo[a] polymorphs.

We believe that these differences between our carotid cases and the cases with prevalent coronary artery disease studied by others could be of major pathophysiologic significance, as described below.

5. Main Hypothesis/Issues to be Addressed:
Small apo[a] polymorphs are more frequent in CAD cases than in cases with carotid thickening, but Lp[a] plasma concentrations are similar for the two case groups. Stated in a simplified form, the frequency of polymorphs 1-5 will be higher in CAD cases than in carotid cases.

There is ample information in the literature that Lp[a] is both atherogenic and thrombogenic. Our hypothesis states that the Lp[a] plasma concentration, irrespective of apo[a] size, is the main determinant of the atherogenic property of Lp[a] that is relevant in early atherosclerosis characterized by intima-media thickening without occlusive thrombosis. In contrast, small apo[a] polymorphs, typically present in elevated concentrations, are the main determinant of the thrombogenic potential of Lp[a] that may be implicated in the precipitation of clinical events. This hypothesis can be tested by