1.a. Full Title: Association of the human KLOTHO KL-VS allele with occurrence of atherosclerosis and incident coronary artery disease (CAD) in African Americans and whites from the Atherosclerosis Risk in Communities Study

1.b. Abbreviated title: KLOTHO KL-VS allele and CAD

2. Writing group:
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3. Timeline:
Genotype data collection will begin immediately upon approval at the ARIC DNA core laboratory which is finalizing cost estimates. If logistically the genotyping cannot be done under this arrangement, approval from the lab committee will be obtained for genotyping at Hopkins. Data analysis will begin by June 2004, and drafts of the manuscripts will be distributed for internal circulation by December 2004.

4. Rationale:
We previously identified an allele of the gene KLOTHO (MIM 604824), termed KL-VS, that is prevalent in the general population (frequency =0.157) and in homozygosity is associated with reduced human longevity (Arking et al. 2002). This allele is characterized by two amino acid substitutions in complete linkage disequilibrium, F352V and C370S. Transient transfection assays demonstrated that secreted levels of klotho harboring V352 are reduced 6-fold, whereas extracellular levels of the S370 form are increased 2.9-fold. The V352/S370 double mutant exhibits an intermediate phenotype (1.6-fold increase), demonstrating intragenic complementation in cis. The remarkable evolutionary conservation of F352 among homologous proteins suggested that this amino acid may also be functionally important. The corresponding substitution, F289V, in the closest human klotho paralog with a known substrate, cBGL1, completely eliminated its ability to cleave a target substrate, providing further evidence for the functional significance of the F352V SNP.
Klotho-deficient mice display extensive and accelerated arteriosclerosis in association with medial calcification of the aorta and both medial calcification and intimal thickening of medium-sized muscular arteries (Kuro-o et al. 1997). Additionally, they exhibit impaired endothelium-dependent vasodilation, decreased nitric oxide metabolites (NO₂ and NO₃) in urine, and impaired angiogenesis, suggesting that klotho protein may protect the cardiovascular system through endothelium derived NO production (Saito et al. 1998; Saito et al. 2000; Nagai et al. 2000; Fukino et al. 2002). Remarkably, in a rat model with multiple atherogenic risk factors including hypertension, diabetes, obesity, and hyperlipidemia, in vivo klotho gene delivery can ameliorate vascular endothelial dysfunction and prevent medial hypertrophy and perivascular fibrosis (Shiraki-Iida et al. 2000).

Recent work has demonstrated that klotho is a novel beta-glucuronidase capable of hydrolyzing steroid beta-glucoronides (Tohyama et al. 2004). While the specific biological targets of klotho have not been identified, klotho likely plays a role in calcium homeostasis (Yoshida et al. 2002), and klotho levels have been demonstrated to alter gene expression of ACE (Yang et al. 2003) and PAI-1 (Takeshita et al. 2002)—genes involved in coronary artery disease (CAD) (Crisan and Carr 2001; Margaglione 1998).

To determine whether klotho influences atherosclerotic risk in humans, we performed cross-sectional studies to assess the association between the KL-VS allele and occult coronary artery disease (CAD) in two independent samples of apparently healthy siblings of individuals with early-onset (<60 years) CAD. In SIBS-I, the KL-VS allele conferred a relative odds of 1.90 (95% C.I. 1.21-2.98) for occult CAD, after adjusting for familial intraclass correlations (P<0.005). Logistic regression modeling, incorporating known CAD risk factors, demonstrated that the KL-VS allele is an independent risk factor (P<0.019), and that the imposed risk of KL-VS allele status is influenced by modifiable risk factors. Hypertension (P<0.022) and increasing HDL-C levels (P<0.022) mask or reduce the risk conferred by the KL-VS allele, respectively, while current smoking (P<0.004) increases the risk. Remarkably concordant effects of the KL-VS allele and modifying factors on the risk for occult CAD were seen in SIBS-II.

We purposefully studied apparently healthy siblings of individuals with premature incident CAD in order to enrich for subjects with a genetic predisposition to CAD. While this study design was successful, the extent to which the KL-VS allele contributes to atherosclerotic CAD in the general population remains to be elucidated. Therefore, we propose to study the KL-VS allele in the ARIC cohort, to assess its role in incident CAD in a population-based cohort.

5. **Main Issues/Hypotheses to be addressed:**
   a. Ability of the KL-VS allele to predict carotid artery disease case status (as measured by wall thickness), both individually and after considering the predictive ability of traditional risk factors
   b. Ability of the KL-VS allele to predict incident CAD in the ARIC study, both individually, and after considering the predictive ability of traditional risk factors
   c. The effect of the KL-VS allele on CAD is influenced by HDL-C levels, blood pressure, and/or smoking
d. Are the F352V and C370S mutations always in cis (present together), and if not, do they have varying effects on CAD?

e. The KL-VS allele is associated with serum Ca levels at baseline. Race-specific associations will be examined; however, we do not believe true causal associations would differ between these groups.

6. **Data (variables, timeline, source, inclusion/exclusion):**
   Genotype data will be collected in all ARIC participants included in the ARIC incident CHD case-cohort study. Variables to be included in the analysis are: age, sex, race, hypertension and diabetes status, blood lipid levels, systolic and diastolic blood pressure, serum calcium levels, and carotid intimal-medial thickness.
   All analyses will assume the following steps:
   1. Hardy-Weinberg equilibrium among genotypes will be checked by calculating expected frequencies of genotypes and using the chi-square goodness-of-fit test.
   2. All analyses will first be stratified by ethnicity to test for interaction. If no interaction is detected, pooled analyses will be performed.
   3. Case-cohort analysis will be performed by using the SAS macro by Barlow. This will be used to assess the independent effect of the KL-VS allele on risk of incident CHD from traditional cardiovascular risk factors at baseline. A recessive model will be assumed since our previous results indicate that homozygosity of the KL-VS allele confers risk of reduced life span and premature CAD.

7.a. **Will the data be used for non-CVD analysis in this manuscript?**  __Yes  _X_ No

7.b. **If Yes, is the author aware that the file ICTER02 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  _X_ No
   (This file ICTDER01 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  _X_ No

8.b. **If Yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER01 must be used to exclude those with the value RES_DNA = “No use/storage DNA”?**  _X_ Yes  _X_ 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](http://bios.unc.edu/units/csc/ARIC/stdy/studymem.html)
   __X__ Yes  ______ No