ARIC Manuscript Proposal # 1033

PC Reviewed: _09/03/04	**Status: __A__**	**Priority: __2__**
SC Reviewed: _09/03/04_	**Status: _A___**	**Priority: __2_**

1.a. Full Title: Association of polymorphisms in endothelial genes involved in arachidonic acid metabolism and nitric oxide synthesis with non-invasively measured atherosclerotic burden and risk of adverse cardiovascular events

b. Abbreviated Title (Length 26 characters): Endothelial polymorphisms and CVD

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

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Writing group members: Samuel Arbes, Molly S. Bray, David Couper, Gerardo Heiss, Kari E. North, Darryl C. Zeldin (others are welcome)

3. Timeline:

- Anticipate data will be available: Immediately
- Analysis to be completed: November, 2004
- First draft anticipated: January, 2005

4. Rationale:

Arachidonic acid is oxidatively metabolized in endothelial cells to epoxyeicosatrienoic acids (EETs) by cytochromes P450 *CYP2J2* and *CYP2C8*. The EETs are hydrolyzed to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH, also called *EPHX2*). Arachidonic acid is also metabolized by cyclooxygenase-1 (COX-1, also called *PTGS1*) and cyclooxygenase-2 (COX-2, also called *PTGS2*) in the endothelium to produce prostaglandin H_2 (PGH_2), which is further metabolized by thromboxane A_2 synthase (TXA_2S, also called *CYP5A1*) and prostacyclin synthase (PGI_2S, also called *CYP8A1*) to produce thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), respectively. Both the EETs and prostaglandins possess potent physiological effects important to endothelial function.

Endothelial dysfunction has been proposed to play an important role in the pathogenesis of atherosclerotic cardiovascular disease. Endothelial dysfunction is manifest by reductions in endothelial-dependent vasodilatation, primarily due to functional impairments in endothelial nitric oxide synthase (eNOS, also called *NOS3*) and nitric oxide availability. However,

alterations in the biosynthesis and metabolism of EETs and/or prostaglandins may also contribute to the development and progression of atherosclerotic cardiovascular disease.

We hypothesize that (1) endothelial EET biosynthesis by CYP2J2 or CYP2C8, (2) endothelial EET hydrolysis by sEH, and (3) prostaglandin synthesis by COX-1, COX-2, TXA_2S, and PGI_2S play an integral role in the maintenance of normal endothelial function. We also hypothesize that the biological activity of EETs and prostaglandins are even more critical in patients with established atherosclerotic disease and a dysfunctional nitric oxide synthesis (eNOS) system.

Numerous single nucleotide polymorphisms have been discovered in the genes encoding these important endothelial proteins, some of which are associated with significant changes in enzyme expression and activity. However, associations between the presence of certain polymorphisms in these genes, as well as interactions between genes, and atherosclerotic cardiovascular disease have not been well characterized.

5. Main Hypothesis/Study Questions:

As part of the ARIC ancillary study entitled "Role of Cytochrome P450-Derived Eicosanoids in Endothelial Dysfunction and Cardiovascular Disease" (AS# 2003.02), genotype data was collected on multiple polymorphisms in the genes encoding CYP2J2, CYP2C8, sEH, COX-1, COX-2, TXA_2S, PGI_2S, and eNOS. The specific polymorphisms included in this ancillary study are listed in Table 1 below.

Hypothesis #1 – Polymorphisms in the genes encoding CYP2J2, CYP2C8, sEH, COX-1, COX-2, TXA_2S, PGI_2S, and eNOS are significantly associated with non-invasively measured atherosclerotic burden and/or risk of adverse cardiovascular events in individuals enrolled in the Atherosclerosis Risk In Communities (ARIC) Study.

Association analyses for polymorphisms in each gene will be conducted using case-cohort or case-control analyses, per the design selected for each study end point (see Section 6). These analyses will be guided by specific hypotheses generated from existing preclinical and clinical data related to the selected polymorphisms in each gene, since each polymorphism listed in Table 1 was selected due to its high potential for biological relevance. Because of the commonalities between the study end points and the evaluation of multiple polymorphisms for each study endpoint, we realize the large scope of analyses that could be conducted on this dataset. In consideration of this potential, analysis of these data will be tested in a first set of parsimonious analyses guided by specific hypotheses related to the respective biological pathways and the known functional relevance of each polymorphism. Moreover, construction of haplotypes will be completed to consolidate the genotyping data and allow simultaneous evaluation of associations between multiple polymorphisms in each gene and each study endpoint. We believe this approach will attenuate the potential for the limitations associated with multiple testing and exploratory analyses.

These analyses will be run by C. Lee in collaboration with K. North and D. Couper, using the analysis programs provided by the Coordinating Center. The results will be reviewed by the writing group to guide additional analyses and determine whether several manuscripts are warranted.

Hypothesis #2 – Interactions between polymorphisms in the genes encoding CYP2J2, CYP2C8, sEH, COX-1, COX-2, TXA_2S, PGI_2S, and eNOS significantly predict non-invasively measured atherosclerotic burden and/or adverse cardiovascular outcomes in individuals enrolled in the ARIC study.

Based on the results of the association analyses outlined above and published data on the biological effects associated with each pathway of interest, specific gene-gene and gene-environment interaction testing will be completed in relation to association with specific study endpoints (see Section 6). All interaction testing will be hypothesis-driven based on biologically plausible interactions between pathways, and not be conducted in an exploratory manner.

These analyses will be run by C. Lee in collaboration with K. North and D. Couper, using the analysis programs provided by the Coordinating Center. Again, the results will be reviewed by the writing group to guide additional analyses and determine whether several manuscripts are warranted.

6. Data (variables, time window, source, inclusions/exclusions):

- *CYP2J2*, *CYP2C8*, *EPHX2*, *PTGS1*, *PTGS2*, *CYP5A1*, *CYP8A1*, and *NOS3* genotype data collected as part of the ARIC ancillary study entitled, "Role of Cytochrome P450-Derived Eicosanoids in Endothelial Dysfunction and Cardiovascular Disease" (the specific polymorphisms included in this analysis are listed in Table 1 below).
- Case status on four endpoints quantifying subclinical atherosclerosis and clinical atherosclerotic events from 1987-2000, including: (1) incident coronary heart disease (non-fatal and fatal MI, acute coronary syndromes, and revascularization procedures, n=1353), (2) incident cerebrovascular disease (stroke and transient ischemic attacks, n=462), (3) prevalent peripheral arterial disease (ankle-arm blood pressure index assessed by DINAMAP, n=212), and (4) prevalent carotid atherosclerosis (increased carotid intima-media thickness assessed by B-mode ultrasound, n=526),.
- The corresponding control groups, including: (1) random cohort control (n=1062), and (2) intima media thickness prevalent controls (n=868).
- Information on demographic covariates (e.g., age, gender, race, center, etc.)
- Information on cardiovascular disease risk factors to use as covariates (e.g., diabetes, hypertension, smoking, lipid levels, body mass index, nutritional status, medication utilization, etc.).
- Information on certain biomarkers collected in a subset of patients (e.g., fibrinogen, TxB_2, tPA antigen, etc.).

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? __X__ 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.cscc.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

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.**

Table 1. Specific polymorphisms included in this analysis (by gene)

Gene	Polymorphism	Location	Amino Acid Change
CYP2J2	G-50T (rs890293)	Promoter	-
CYP2J2	C150A	Exon 1	R49S
CYP2J2	G342A	Exon 2	V113M
CYP2J2	A376G	Exon 2	N124S
CYP2J2	C14558T	Exon 3	R158C
CYP2J2	T15054A	Exon 4	I192N
CYP2J2	A25687T	Exon 8	N404Y
CYP2J2	G10860A	Intron 2	-
CYP2J2	T18778G	Intron 5	-
CYP2J2	C33291T	3'UTR	-
CYP2J2	A33497G	3'UTR	-
CYP2C8	C792G (rs1058930)	Exon 5	I264M
CYP2C8	A805T	Exon 5	I269F
CYP2C8	A1196G	Exon 8	K399R
CYP2C8	G4776A	Intron 4	-
CYP2C8	A26584T (rs2275620)	Intron 7	-
EPHX2	A164G	Exon 2	K55R
EPHX2	C307T	Exon 3	R103C
EPHX2	G461A	Exon 4	C154Y
EPHX2	1206 insCGT	Exon 13	402-402 insR
EPHX2	T1265C	Exon 14	V422A
EPHX2	A1409G	Exon 16	E470G
EPHX2	G14029C	Intron 4	-
EPHX2	G20675	Intron 5	-
PTGS1	G-1006A	Promoter	-
PTGS1	C10251T (rs1236913)	Exon 2	R8W
PTGS1	C10289T (rs3842787)	Exon 2	P17L
PTGS1	G17013A (rs3842789)	Exon 3	R53H
PTGS1	G20724A	Exon 7	G230S
PTGS1	C20745A (rs5789)	Exon 7	L237M
PTGS1	G16978A (rs3842788)	Exon 3	Q41Q
PTGS1	C20564A (rs5788)	Exon 6	G213G
PTGS1	20812 delA (rs3215925)	Intron 7	-
PTGS2	G-765C (rs20417)	Promoter	-
PTGS2	T-607C (rs20419)	Promoter	-
PTGS2	T7678C (rs5273)	Exon 10	V511A
PTGS2	G3245C (rs5277)	Exon 3	V102V
PTGS2	G5515A (rs2066826)	Intron 6	-
CYP5A1	A179467G (rs5769)	Exon 8	K258E
CYP5A1	G237535A (rs4526)	Exon 11	A430T
CYP8A1	T20307A (rs5622)	Exon 3	S118R
CYP8A1	C55002A (rs6095558)	Exon 8	R373R
NOS3	T-793C (rs2070744)	Promoter	-
NOS3	A-586G (rs3918162)	Promoter	-
NOS3	T-580A (rs3918163)	Promoter	-
NOS3	G12685A (rs3918166)	Exon 3	R112Q
NOS3	G26473T (rs3918201)	Exon 20	R885M
NOS3	G18379T (rs1800782)	Intron 12	-
NOS3	G23972A (rs891511)	Intron 16	-