

ARIC Manuscript Proposal # 1033

PC Reviewed: 09/03/04

Status: A

Priority: 2

SC Reviewed: 09/03/04

Status: A

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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):

Lead: Craig R. Lee

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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 H₂ (PGH₂), which is further metabolized by thromboxane A₂ synthase (TXA₂S, also called *CYP5A1*) and prostacyclin synthase (PGI₂S, also called *CYP8A1*) to produce thromboxane A₂ (TXA₂) and prostacyclin (PGI₂), 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, TXA₂S, and PGI₂S 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, TXA₂S, PGI₂S, 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, TXA₂S, PGI₂S, 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, TXA₂S, PGI₂S, 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, TxB₂, tPA antigen, etc.).

7.a. Will the data be used for non-CVD analysis in this manuscript? Yes 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 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”? 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.csc.unc.edu/ARIC/search.php>**

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