ARIC Manuscript Proposal # 1290

PC Reviewed: _10/09/07	**Status: _A___**	**Priority: _2__**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: The effects of polymorphisms of *TCF7L2*, *CD14*, *MPO*, *TLR2*, and *TLR4* on monocyte activation: The Atherosclerosis Risk in Communities (ARIC) MRI Study

b. Abbreviated Title (Length 26 characters): Genetic effects on monocytes

2. Writing Group:

Writing group members: Suzette J. Bielinski, Jennifer Hall, Aaron Folsom, James S. Pankow, Eric Boerwinkle, Nevenka Matijevic-Aleksic

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _SB__ **[please confirm with your initials electronically or in writing]**

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3. Timeline:

Starting Analyses: November 2007
First Draft: February 2007
Submission for Publication: April 2007

4. Rationale:

Type 2 diabetes is a risk factor for coronary artery disease. Variants in the gene TCF7L2 have recently been identified to be associated with increased risk for type 2 diabetes in multiple cohorts from Scandinavia, Poland, the USA, France, Japan, West Africa, Mexican Americans, and Indians[1-16]. The magnitude of the risk conferred by TCF7L2 variants (~40% increased risk per allele) is greater than for any previously described common variant. The *TCF7L2* SNP rs7903146 remains the most highly associated with increased risk of type 2 diabetes (Odds Ratio [OR] 1.40; $P = 6.7 \times 10^{-20}$). The *TCF7L2* SNP rs7903146 resides within an intron, and the biological mechanism through which this SNP confers increased risk for type 2 diabetes remains unknown.

The gene *TCF7L2* is located on chromosome 10 and encodes the transcription factor Tcf-4. *TCF7L2* is a member of the Tcf/Lef family of high mobility group box transcription factors. *TCF7L2* is best known for its role as a transcription factor in the Wnt signaling pathway that regulates cellular growth, differentiation, and development. *TCF7L2* contains an HMG box serving as the DNA binding domain, which binds to the A/T A/T CAAAG consensus sequence in multiple targets. Deletion of *TCF7L2* in the mouse results in death shortly after birth[17]. This premature death has been associated with the absence of a proliferative compartment in the gut and loss of the secretory neuroedocrine cell[17]. *TCF7L2* is expressed in several cell types and tissues including monocytes and muscle (www.genecards.org). Of particular interest, a specific role for Tcf-4 has been shown in the process of vascular remodeling[18]. Moreover, transcriptional activation of Tcf-4 turns on the NF-kB signaling pathway, which regulates inflammatory signaling pathways[18]. Thus, several lines of pre-clinical evidence provided the rationale for testing whether the *TCF7L2* SNP rs7903146 was associated with altered inflammatory phenotypes in monocytes.

Variants of genes monocyte differentiation antigen (*CD14*), toll-like receptor 4 (*TLR4*), toll-like receptor 2 (*TLR2*), and myeloperoxidase (*MPO*) that encode for monocyte proteins are important monocyte phenotypes. The *CD14* gene is located at 5q31.1 and encodes for a membrane protein that is critical for lipopolysaccharide (LPS) dependent signaling[19]. A promoter SNP in this gene has been associated with levels of soluble CD14[20], myocardial infarction[21], and IgA nephropathy[22]. *TLR4* is located at 9q32-q33 and encodes for the TL4 protein that is activated by the LPS-lipopolysaccharide binding protein (LBP)-CD14 complex to induce inflammatory gene expression through NF-kappa-B and MAPK signaling[23]. Common polymorphisms in *TLR4* are associated with differences in LPS sensitivity[24]. *TLR2* maps to 4q32 and mediates the production of interleukin-12[25]. Several polymorphisms in *TLR2* have been discovered including several SNPs, R677W[26] and R753Q[27], and a microsatellite in intron 2[28]. *MPO* maps to 17q23.1 and functions as part of the host defense system. MPO has been shown to modulate the vasodilatory and vascular signaling functions of nitric oxide[29] and a translocation of the MPO gene to chromosome 15 is associated with acute promyelocytic leukemia[30]. The effects of long-term hormone replacement therapy (HRT) on progression of atherosclerosis were found to differ by MPO genotype with carriers of the GG genotype of the -463 polymorphism benefiting from HRT treatment[31].

The aim of this project is to test the hypothesis that the variants in these genes are associated with altered monocyte inflammatory phenotypes in a biracial cohort of adults from the ARIC Carotid MRI study.

5. Main Hypothesis/Study Questions:

Polymorphisms of *TCF7L2*, *CD14*, *MPO*, *TLR2*, and *TLR4* are associated with increased levels of monocyte activation.

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

Outcome: the 14 monocyte flow cytometry variables (%gated and MFI)
(P3MONOCI2P, P3MONOCI2XD, P3MONOCP2P, P3MONOCP2XD, P4MONOCI2P, P4MONOCI2XD, P4MONOCK2XD, P4MONOCK2YD, P4MONOCL2P, P4MONOCL2XD, P5MONONP, P5MONONXD, P6MONOL2XD, P6MONOL2YD)

Exposure: Variants of *TCF7L2*, *CD14*, *MPO*, *TLR2*, and *TLR4*

Covariates include, but are not limited to, traditional risk factors including age, sex, race, lipid levels, blood pressure medication use, smoking status and amount, and physical activity.

Analysis Plan (Data analysis to be conducted by the coordinating center)

1. Hardy Weinberg equilibrium among genotypes will be calculated using the chi-square test on race-specific datasets
2. An additive genetic model will be assumed unless indicated otherwise by the results. Therefore, genotypes will be coded as 0 (0 copies of candidate allele), 1 (1 copy), or 2 (2 copies). If appropriate given the results, a dominant model combining homozygotes and heterozygotes will be used.
3. Linear regression will be carried out using PROCSURVEYREG within SAS 9.1 weighted by the inverse of the sampling fractions in the 8 sampling strata to test the null hypothesis that the phenotypic levels are the same across genotypes.

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?
____ 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)?

1. Manuscript #1141 Transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes
2. Manuscript #1235 *TCF7L2* SNPs, cardiovascular disease, and all-cause mortality: The Atherosclerosis Risk in Communities (ARIC) Study
3. Manuscript #1219 Peripheral blood monocyte myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2) levels and carotid artery plaque presence/progression (ARIC CAR MRI Study)
4. Manuscript #1218 Peripheral blood monocyte toll-like receptors TLR-2 and TLR-4 expression and carotid artery atherosclerosis (ARIC CAR MRI Study)
5. Manuscript #1207 Association of monocyte markers with peripheral arterial disease (PAD)
6. Manuscript #1205 Association of platelet and monocyte markers with peripheral arterial disease (PAD)
7. Manuscript #1217 Circulating blood platelet-leukocyte aggregates and leukocyte PSGL-1, and carotid artery atherosclerosis (ARIC CAR MRI Study)
8. Manuscript #1243 Cell markers and carotid remodeling
9. Manuscript #1206 Association of risk factors with blood platelet and monocyte cell-markers and cell aggregates (ARIC MRI)

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes _X__ No

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _____)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. 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.

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