

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]**

First author: Suzette J. Bielinski
Address: University of Minnesota
1300 South Second Street, Suite 300
Minneapolis, Minnesota 55116

Phone: 612-624-1899 Fax: 612-624-0315
E-mail: Bielinski@epi.umn.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

Aaron Folsom
Address: University of Minnesota
1300 South Second Street, Suite 300
Minneapolis, Minnesota 55116

Phone: 612-626-8862 Fax: 612-624-0315
E-mail: folsom@epi.umn.edu

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 Indians¹⁻¹⁶. 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 birth¹⁷. This premature death has been associated with the absence of a proliferative compartment in the gut and loss of the secretory neuroendocrine cell¹⁷. *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 remodeling¹⁸. Moreover, transcriptional activation of Tcf-4 turns on the NF- κ B signaling pathway, which regulates inflammatory signaling pathways¹⁸. 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 signaling¹⁹. A promoter SNP in this gene has been associated with levels of soluble CD14²⁰, myocardial infarction²¹, and IgA nephropathy²². *TLR4* is located at 9q32-q33 and encodes for the TLR4 protein that is activated by the LPS-lipopolysaccharide binding protein (LBP)-CD14 complex to induce inflammatory gene expression through NF- κ B and MAPK signaling²³. Common polymorphisms in *TLR4* are associated with differences in LPS sensitivity²⁴. *TLR2* maps to 4q32 and mediates the production of interleukin-12²⁵. Several polymorphisms in *TLR2* have been discovered including several SNPs, R677W²⁶ and R753Q²⁷, and a microsatellite in intron 2²⁸. *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 oxide²⁹ and a translocation of the *MPO* gene to chromosome 15 is associated with acute promyelocytic leukemia³⁰. 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 treatment³¹.

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 PROC SURVEYREG 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.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)?

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 ☒ 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.csc.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.

REFERENCES

1. Cauchi S, El Achhab Y, Choquet H, Dina C, Kremler F, Weitgasser R, Nejjari C, Patsch W, Chikri M, Meyre D, Froguel P. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med.* 2007.
2. Chandak GR, Janipalli CS, Bhaskar S, Kulkarni SR, Mohankrishna P, Hattersley AT, Frayling TM, Yajnik CS. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. *Diabetologia.* 2007;50:63-67.
3. Dancott CM, Pollin TI, Reinhart LJ, Ott SH, Shen H, Silver KD, Mitchell BD, Shuldiner AR. Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes.* 2006;55:2654-2659.
4. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med.* 2006;355:241-250.
5. Granhall C, Park HB, Fakhrai-Rad H, Luthman H. High-resolution quantitative trait locus analysis reveals multiple diabetes susceptibility loci mapped to intervals <800 kb in the species-conserved Niddm1 of the GK rat. *Genetics.* 2006;174:1565-1572.
6. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadóttir A, Stykarsdóttir U, Magnusson KP, Walters GB, Palsdóttir E, Jonsdóttir T, Gudmundsdóttir T, Gylfason A, Saemundsdóttir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdóttir U, Gulcher JR, Kong A, Stefansson K. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet.* 2006;38:320-323.
7. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, Hitman GA, Walker M, Wiltshire S, Hattersley AT, McCarthy MI. Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes.* 2006;55:2640-2644.
8. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S. Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population. *Diabetologia.* 2007;50:980-984.
9. Mayans S, Lackovic K, Lindgren P, Ruikka K, Agren A, Eliasson M, Holmberg D. TCF7L2 polymorphisms are associated with type 2 diabetes in northern Sweden. *Eur J Hum Genet.* 2007;15:342-346.
10. Munoz J, Lok KH, Gower BA, Fernandez JR, Hunter GR, Lara-Castro C, De Luca M, Garvey WT. Polymorphism in the transcription factor 7-like 2 (TCF7L2) gene is associated with reduced insulin secretion in nondiabetic women. *Diabetes.* 2006;55:3630-3634.
11. Parra EJ, Cameron E, Simmonds L, Valladares A, McKeigue P, Shriver M, Wachter N, Kumate J, Kittles R, Cruz M. Association of TCF7L2 polymorphisms with type 2 diabetes in Mexico City. *Clin Genet.* 2007;71:359-366.
12. Salonen JT, Uimari P, Aalto JM, Pirskanen M, Kaikkonen J, Todorova B, Hypponen J, Korhonen VP, Asikainen J, Devine C, Tuomainen TP, Luedemann J, Nauck M, Kerner W, Stephens RH, New JP, Ollier WE, Gibson JM, Payton A, Horan MA, Pendleton N, Mahoney W, Meyre D, Delplanque J, Froguel P, Luzzatto O, Yakir B, Darvasi A. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. *Am J Hum Genet.* 2007;81:338-345.
13. Saxena R, Gianniny L, Burt NP, Lyssenko V, Guducchi C, Sjogren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes.* 2006;55:2890-2895.
14. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, Duren WL, Chines PS, Stringham HM, Erdos MR, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Collins FS, Boehnke M. Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. *Diabetes.* 2006;55:2649-2653.
15. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW,

- Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science*. 2007;316:1341-1345.
16. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007;445:881-885.
 17. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, Clevers H. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet*. 1998;19:379-383.
 18. Wang X, Xiao Y, Mou Y, Zhao Y, Blankesteyn WM, Hall JL. A role for the beta-catenin/T-cell factor signaling cascade in vascular remodeling. *Circ Res*. 2002;90:340-347.
 19. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*. 1990;249:1431-1433.
 20. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol*. 1999;20:976-983.
 21. Shimada K, Watanabe Y, Mokuno H, Iwama Y, Daida H, Yamaguchi H. Common polymorphism in the promoter of the CD14 monocyte receptor gene is associated with acute myocardial infarction in Japanese men. *Am J Cardiol*. 2000;86:682-684, A688.
 22. Yoon HJ, Shin JH, Yang SH, Chae DW, Kim H, Lee DS, Kim HL, Kim S, Lee JS, Kim YS. Association of the CD14 gene -159C polymorphism with progression of IgA nephropathy. *J Med Genet*. 2003;40:104-108.
 23. Bochkov VN, Kadl A, Huber J, Gruber F, Binder BR, Leitinger N. Protective role of phospholipid oxidation products in endotoxin-induced tissue damage. *Nature*. 2002;419:77-81.
 24. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet*. 2000;25:187-191.
 25. Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, Maitland M, Norgard MV, Plevy SE, Smale ST, Brennan PJ, Bloom BR, Godowski PJ, Modlin RL. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science*. 1999;285:732-736.
 26. Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol*. 2001;31:53-58.
 27. Schroder NW, Diterich I, Zinke A, Eckert J, Draing C, von Baehr V, Hassler D, Priem S, Hahn K, Michelsen KS, Hartung T, Burmester GR, Gobel UB, Hermann C, Schumann RR. Heterozygous Arg753Gln polymorphism of human TLR-2 impairs immune activation by *Borrelia burgdorferi* and protects from late stage Lyme disease. *J Immunol*. 2005;175:2534-2540.
 28. Boraska Jelavic T, Barisic M, Drmic Hofman I, Boraska V, Vrdoljak E, Peruzovic M, Hozo I, Puljiz Z, Terzic J. Microsatellite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. *Clin Genet*. 2006;70:156-160.
 29. Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusa AJ, Nauseef WM, White CR, Freeman BA. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science*. 2002;296:2391-2394.
 30. Weil SC, Rosner GL, Reid MS, Chisholm RL, Lemons RS, Swanson MS, Carrino JJ, Diaz MO, Le Beau MM. Translocation and rearrangement of myeloperoxidase gene in acute promyelocytic leukemia. *Science*. 1988;240:790-792.
 31. Makela R, Dastidar P, Jokela H, Saarela M, Punnonen R, Lehtimäki T. Effect of long-term hormone replacement therapy on atherosclerosis progression in postmenopausal women relates to myeloperoxidase promoter polymorphism. *J Clin Endocrinol Metab*. 2003;88:3823-3828.