ARIC Manuscript Proposal # 1906

PC Reviewed: 2/14/12	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Genome-wide association analysis of fetuin-A levels

b. Abbreviated Title (Length 26 characters): GWAS of fetuin-A

2. Writing Group:
Writing group members:

This is a consortium analysis involving CHARGe and other cohorts. Jim Pankow is responsible for overseeing analysis in ARIC and will handle communications with the ARIC Publications and Steering Committees. ARIC is tentatively allotted three authors for this manuscript.

ARIC: Jim Pankow, Ron Hoogeveen, Maria Ines Schmidt

CHS: David Siscovick, Bruce Psaty, Richard A. Jensen, Joe Zumada, Jerome I. Rotter, Ida Chen, Mark O. Goodarzi, Alice Arnold (Anne Newman) - and fetuin-A working group (Multi-PI R01 for CHS): Kenneth Mukamal, Luc Djousse, Jorge Kizer, Susan Zieman, Joe Ix

Nurses' Health Study: Katryn Rexrode, Eric Rimm, Qi Sun, Majken K. Jensen

FHS: Vasan Ramanchandran, 2 others

MESA: Ronit Katz, Xiuqing Guo, (Yongmei Liu, Jim Pankow, Joe Ix, David Siscovick)

Health ABC: Yongmei Liu, Anne Newman, 1 more (Joe Ix)

The EPIC Potsdam Study, the KORA study, and the Heart and Soul Study have also been contacted. These cohorts have fetuin-A levels, but not GWAS data. Each has tentatively agreed to provide SNP look-ups.

NOTE: The co-author list is partial at this time and may not be a complete/final list, especially as additional replication cohorts are added, etc.

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

First author: Majken K. Jensen (Nurses' Health Study)

Address:

Phone: Fax:
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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

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3. Timeline: Analysis to begin in ARIC to begin as soon as approval is granted
First draft by June 2012

4. Rationale:

Fetuin-A is a protein that is produced almost exclusively by hepatocytes and secreted into the bloodstream.[1,2] Fetuin-A directly binds and inhibits the insulin receptor on adipocytes and skeletal muscle cells. When bound, it decreases the rate of insulin receptor tyrosine kinase activity, and downstream intracellular signaling cascades, resulting in insulin resistance.[7, 8] This function is conserved in multiple mammalian models *in vitro*[9-12] and *in vivo*.[7, 8, 12] The fetuin-A knock-out mouse has improved insulin sensitivity by euglycemic clamp experiments, lower triglyceride and FFA levels, resistance to weight gain, and less adiposity.[13, 14] We[15] and others[16, 17] have shown that higher fetuin-A is cross-sectionally associated with insulin resistance and longitudinally associated with incident diabetes in older adults.[18,19]

The gene encoding fetuin-A (*AHSG*) is located on Chr 3 (3q27); a locus that has been linked with phenotypes of the metabolic syndrome and T2DM.[20,21] A French group conducted a detailed candidate gene association study of the *AHSG* gene by directly sequencing the gene-encoding exons and the flanking regions. They identified 9 single nucleotide polymorphisms (SNPs) with a minor allele frequency of 5% or more, and showed strong linkage disequilibrium (LD) among the SNPs in the entire region. In a case-control study, they found that one SNP (rs1071592) had a significantly higher prevalence among those with T2DM compared to normoglycemic controls. Two other

SNPs that were in almost complete LD (rs4917 and rs2248690) showed the same tendencies, although associations with these SNPs with T2DM did not reach statistical significance.[22]

In the literature, variation in the *AHSG* gene is associated with fetuin-A levels. Carriers of the major alleles of rs2248690 (A; frequency in Caucasians ~74%), rs4917 (C; ~77%), and rs4918 (T ~66%) have higher levels of circulating fetuin-A, compared to carriers of the variant alleles.[23-25] However, it is not known whether other genetic loci can be identified that play a role in the regulation of fetuin-A concentrations. So far, no genome-wide analyses have been performed to identify genetic determinants of fetuin-A. Hence, the current proposal aims to include ARIC GWAS data in a meta-analysis of cohorts with fetuin-A levels available in the CHARGE consortium (FHS, CHS, ARIC), the Health ABC cohort, and the Nurses' Health Study.

5. Main Hypothesis/Study Questions:

To identify the common genetic determinants of fetuin-A levels.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

In ARIC, fetuin-A concentrations will be taken from measurements performed on visit 1 plasma samples in the random subcohort selected for the "Inflammatory Precursors of Type 2 Diabetes" ancillary study.

SAMPLES
Men and Women.

EXCLUSIONS
1. Missing genotype data
2. Missing data on fetuin-A concentrations

STRATIFICATION OF SAMPLE
Analysis will be conducted separately for Caucasians and African-Americans.

TRAIT OF INTEREST
Fetuin-A (g/L), untransformed.

COVARIATES
Age, age^2, sex, field center, and principal components, where appropriate.

Brief analysis plan and methods:

Genetic model: additive model

Fetuin-A (untransformed) = age +age^2 +sex + principal components/field center +SNP

QC methods at cohort levels as previously described at

http://depts.washington.edu/chargeco/wiki/QCprocedures

Meta-analysis:

Cohort-specific results will be meta-analyzed using MetABEL or Metal software.

a. Fixed Effects
b. Significance threshold: $p < 5x10^{-8}$
c. Final QC step (e.g. filtering MAF at 1%) at meta-analysis stage

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 ICTDER03 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 ICTDER03 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 ICTDER03 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)?

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

11.b. If yes, is the proposal

_x__ 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)* _____ _____ _____)

2006.03 (Stampede and Geneva genotype funding in Caucasians)
2007.02 (CARe, genotyping in African Americans)
1995.09 (Inflammatory Precursors of Type 2 Diabetes)

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

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

References:

1. Denecke B, Graber S, Schafer C, Heiss A, Woltje M, Jahnen-Dechent W. Tissue distribution and activity testing suggest a similar but not identical function of fetuin-B and fetuin-A. *Biochem J.* Nov 15 2003;376(Pt 1):135-145.
2. Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M, Whooley MA. Fetuin-A and kidney function in persons with coronary artery disease--data from the heart and soul study. *Nephrol Dial Transplant.* Aug 2006;21(8):2144-2151.
3. Heiss A, DuChesne A, Denecke B, et al. Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. *J Biol Chem.* Apr 11 2003;278(15):13333-13341.
4. Ketteler M, Schlieper G, Floege J. Calcification and Cardiovascular Health. New Insights Into an Old Phenomenon. *Hypertension.* Apr 17 2006.
5. Schinke T, Amendt C, Trindl A, Poschke O, Muller-Esterl W, Jahnen-Dechent W. The serum protein alpha2-HS glycoprotein/fetuin inhibits apatite formation in vitro and in mineralizing calvaria cells. A possible role in mineralization and calcium homeostasis. *J Biol Chem.* Aug 23 1996;271(34):20789-20796.

6. Schafer C, Heiss A, Schwarz A, et al. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest.* Aug 2003;112(3):357-366.
7. Auberger P, Falquerho L, Contreres JO, et al. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. *Cell.* Aug 25 1989;58(4):631-640.
8. Rauth G, Poschke O, Fink E, et al. The nucleotide and partial amino acid sequences of rat fetuin. Identity with the natural tyrosine kinase inhibitor of the rat insulin receptor. *Eur J Biochem.* Mar 1 1992;204(2):523-529.
9. Srinivas PR, Wagner AS, Reddy LV, et al. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. *Mol Endocrinol.* Nov 1993;7(11):1445-1455.
10. Mathews ST, Srinivas PR, Leon MA, Grunberger G. Bovine fetuin is an inhibitor of insulin receptor tyrosine kinase. *Life Sci.* 1997;61(16):1583-1592.
11. Grunberger G MS, Deutsch DD. *Tyrosine Kinase Inhibitors. In Insulin Signaling: From Cultured Cells to Animal Models, Frontiers in Animal Diabetes Research*. New York: Taylor and Francis; 2002.
12. Cintron VJ, Ko MS, Chi KD, et al. Genetic mapping and functional studies of a natural inhibitor of the insulin receptor tyrosine kinase: the mouse ortholog of human alpha2-HS glycoprotein. *Int J Exp Diabetes Res.* 2001;1(4):249-263.
13. Mathews ST, Rakhade S, Zhou X, Parker GC, Coscina DV, Grunberger G. Fetuin-null mice are protected against obesity and insulin resistance associated with aging. *Biochem Biophys Res Commun.* Nov 17 2006;350(2):437-443.
14. Mathews ST, Singh GP, Ranalletta M, et al. Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. *Diabetes.* Aug 2002;51(8):2450-2458.
15. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. *Circulation.* Apr 11 2006;113(14):1760-1767.
16. Mori K, Emoto M, Yokoyama H, et al. Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects. *Diabetes Care.* Feb 2006;29(2):468.
17. Stefan N, Hennige AM, Staiger H, et al. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. *Diabetes Care.* Apr 2006;29(4):853-857.
18. Stefan N, Fritsche A, Weikert C, et al. Plasma fetuin-A levels and the risk of type 2 diabetes. *Diabetes.* Oct 2008;57(10):2762-2767.
19. Ix JH, Wassel CL, Kanaya AM, et al. Fetuin-A and incident diabetes mellitus in older persons. *JAMA.* Jul 9 2008;300(2):182-188.
20. Vionnet N, Hani EH, Dupont S et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites:evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet. 2000;67(6):1470-80.

21. Kissebah AH, Sonnenberg GE, Myklebust J et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. PNAS 2000;97(26):14478-83.
22. Siddiq A, Lepretre F, Hercberg S, Froguel P, Gibson F. A synonymous coding polymorphism in the alpha2-Heremans-schmid glycoprotein gene is associated with type 2 diabetes in French Caucasians. Diabetes. 2005;54(8):2477-81.
23. Fisher E, Stefan N, Saar K et al. Association of AHSG gene polymorphisms with fetuin-A plasma levels and cardiovascular diseases in the EPIC-Potsdam study. Circ Cardiovasc Genet 2009; 2:607-613.
24. Verduijn M, Prein RA, Stenvinkel P, Carrero JJ, le CS, Witasp A, Nordfors L, Krediet RT, Boeschoten EW, Dekker FW. Is fetuin-A a mortality risk factor in dialysis patients or a mere risk marker? A Mendelian randomization approach. Nephrol Dial Transplant 2011; 26:239-245.
25. Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, Barany P, Lindholm B, Jogestrand T, Heimburger O, Holmes C, Schalling M, Nordfors L. Low fetuin-A levels are associated with cardiovascular death: Impact of variations in the gene encoding fetuin. Kidney Int 2005; 67:2383-2392,