ARIC Manuscript Proposal # 1468

PC Reviewed: 01/13/09	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Genetic variants are associated cross-sectionally and longitudinally with multiple measures of fasting glucose: the ARIC Study

b. Abbreviated Title (Length 26 characters): GWAS genes and glucose change

2. Writing Group:

Writing group members: Laura Rasmussen-Torvik, Alvaro Alonso, Mandy Li, Linda Kao, Anna Kottgen, Yuer Yan, David Couper, Eric Boerwinkle, Sue Bielinski James Pankow, for the ARIC Diabetes GWAS working group

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

First author: Laura Rasmussen-Torvik
Address: 1300 S. Second St, Suite 300
Minneapolis, MN 55454

Phone: 612-626-7921 Fax: 612-624-0315
E-mail: rasm0218@umn.edu

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).
Name: James Pankow
Address: 1300 S. Second St, Suite 300
Minneapolis, MN 55454

Phone: 612-624-2883 Fax: 612-624-0315
E-mail: panko001@umn.edu

3. Timeline: Analyses to begin immediately upon approval of manuscript. Expect first draft to authors within 4 months and submission to the publications committee within 6 months

4. Rationale:

This manuscript has two objectives: (1) to present GWAS results for fasting glucose in ARIC; and (2) to evaluate (in ARIC) cross-sectional and longitudinal associations between selected candidate SNPs identified by previous GWAS of diabetes and fasting glucose.

There have been several recent genome wide association studies (GWAS) of type 2 diabetes (1-7). These studies have identified at least 18 gene variants reproducibly associated with type 2 diabetes (8). In addition, GWAS have identified at least 4 variants associated cross-sectionally with fasting glucose in non-diabetic individuals (9-11). Interestingly, not all variants associated with prevalent type 2 diabetes are similarly associated with fasting glucose in non-diabetic individuals, and not all variants associated with fasting glucose in non-diabetic individuals are similarly associated with prevalent type 2 diabetes, suggesting that different genes may be involved in regulation of glucose in normal and diseased states.

The ARIC study is participating in MAGIC (Meta-Analyses of Glucose and Insulin-related Traits Consortium), an international collaboration seeking to identify genes associated with diabetes-related quantitative traits through GWAS studies. ARIC data from visit 1 will serve as an *in silico* replication sample for selected candidate SNPs identified by MAGIC's meta-analysis of fasting glucose and other diabetes-related quantitative traits (insulin, HOMA-IR, HOMA-B). However, because ARIC joined the consortium too late to participate in MAGIC's primary meta-analysis of fasting glucose, we wish to present the ARIC fasting glucose GWAS results independently.

The ARIC study is different from most previous fasting glucose GWAS studies in that glucose was measured 4 times over a period of nine years. Therefore, in ARIC we have the ability to identify candidate genes in the GWAS not only through selecting those genes with the smallest p-values at any one visit, but also by selecting genes consistently associated with the trait. Furthermore, we have the ability to complete a GWAS with a measure of average glucose (to perhaps reduce some natural variability associated with assay). In the Framingham GWAS of fasting glucose, many SNPs were found to be more highly associated with a measure of average fasting glucose than a single measure of glucose (11).

The multiple measures of fasting glucose in the ARIC study provide an interesting opportunity to follow-up on previous GWAS of type 2 diabetes and fasting glucose. The allelic effect of diabetes and fasting glucose GWAS candidate genes can be determined for fasting glucose measured at visits 1-4 and for average fasting glucose. In ARIC an analysis of fasting glucose change can be undertaken, and the association of the candidate genes with glucose change tested. It is not known if these candidate genes influence diabetes and fasting glucose level by raising fasting glucose level throughout the life course, or by accelerating the increase of fasting glucose over time; the analysis of change in fasting glucose can begin to answer this question.

5. Main Hypothesis/Study Questions:

GWAS Analyses:

1. In the white participants without diabetes, which of the 2.5 million genotyped or imputed SNPs in the ARIC GWAS are associated ($p < 5 \times 10^{-8}$) cross-sectionally with fasting glucose (at visits 1, 2, 3, and 4)? What SNPs in the GWAS are significantly associated with average fasting glucose?

Candidate SNP Analyses:

2. In white participants without diabetes, are 22 SNPs identified from previous GWAS of prevalent diabetes or fasting glucose associated cross-sectionally with fasting glucose at visits 1, 2, 3, and 4? What are the allelic effects of each SNP on fasting glucose?

3. In white participants without diabetes, are 22 SNPs identified from previous GWAS of prevalent diabetes or fasting glucose associated with average fasting glucose across visits? What are the allelic effects of each SNP on average fasting glucose?

4. Among white participants without diabetes at visit 1, are 22 SNPs identified from previous GWAS analyses of prevalent diabetes or fasting glucose associated with change in fasting glucose over 9 years of follow-up? What are the allelic effects of each SNP on change in fasting glucose?

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).

GWAS analyses (study question 1)

Study design: Four cross sectional analyses (at each visit) and one analysis with a trait derived from the average of glucose measurements across visits.

Inclusions: Caucasian participants in the ARIC study will be included.

Exclusions: Individuals who did not wish to participate in genetic research will be excluded. At each visit, individuals who did not fast for at least 8 hours prior to blood draw will be excluded, as will individuals with diagnosed diabetes, on diabetes treatment, or with fasting glucose greater than 126mg/dl.

Exposure variables: ~2.5 million genotyped and imputed SNPs from the Affy 6.0 array

Outcome variable: Fasting glucose measured at visits 1, 2, 3, and 4, average of fasting glucose across visits

Covariates: age, sex, center

Statistical model: The association of all typed and imputed SNPs remaining after data cleaning will be assessed with fasting glucose measures at visits 1, 2, 3, and 4. An additive genetic model will be used employing a 1 d.f. trend test. For the GWAS using average fasting glucose, the same procedures will be employed.

Preliminary data: Based on preliminary analyses, there are three distinct areas of the genome associated ($p< 5 \times 10^{-8}$) with fasting glucose in white participants without diabetes in ARIC. All three regions have been discovered in previous GWAS of fasting glucose, including the primary meta-analysis for MAGIC. Because these findings are not novel, they may be difficult to publish in a stand alone manuscript.

Candidate SNP analyses (study questions 2-4)

Study design: Cross sectional and longitudinal study design. The longitudinal design will be employed to study the association of selected genotypes with fasting glucose change.

Inclusions: All Caucasian participants of ARIC

Exclusions: Any individual not fasting more than 8 hours. Any individual not wishing to participate in genetic research. Any individual with diagnosed diabetes, on diabetes treatment, or with fasting glucose greater than 126mg/dl—for cross-sectional and average analyses.

Exposures: 22 SNPs in several genes identified through previous GWAS of type 2 diabetes or fasting glucose (see table below). Only 1 SNP per gene was selected. A table of the SNP identifiers and source of genotypes is included below. We will engage in conversation with the MAGIC consortium for permission to include additional novel SNPs that may be discovered in their meta-analysis of fasting glucose.

Gene	SNP	Source	Identified through T2DM GWAS or fasting glucose GWAS?
TCF7l2	rs7903146	ARIC DNA lab	T2DM
KCNJ11	rs5219	ARIC DNA lab	T2DM
CDKN2A/2B	rs10811661	ARIC DNA lab	T2DM
PPARG	rs1801282	ARIC DNA lab	T2DM
WFS1	rs10010131	Affy chip	T2DM
CDKN2A/2B	rs564398	Affy chip	T2DM
IGF2BP2	rs4402960	ARIC DNA lab	T2DM
FTO	rs8050136	ARIC DNA lab	T2DM
CDKAL1	rs109463398	ARIC DNA lab or Affy Chip	T2DM
SLC30A8	rs13266634	ARIC DNA lab	T2DM
TCF2	rs757210	Affy chip	T2DM
HHEX	rs1111875	ARIC DNA lab	T2DM
ADAM30/NOTCH2	rs2641348	Affy chip	T2DM
TSPAN8/LGR5	rs7961581	Affy chip	T2DM
CDC123	rs12779790	Affy chip	T2DM
ADAMTS9	rs4607103	Affy chip	T2DM
THADA	rs7578597	Affy chip	T2DM
JAZF1	rs864745	Affy chip	T2DM
G6PC2	rs560887	Affy chip	Fasting glucose
MTNR1B	rs10830963	Affy chip	Fasting glucose
GCK	rs4607517	Affy chip	Fasting glucose
GCKR	rs780094	Affy chip or ARIC DNA lab	Fasting glucose

Outcome: Fasting glucose measured at visits 1, 2, 3, and 4. Average of fasting glucose measured across visits. Change in fasting glucose over visits 1 – 4.

Confounders: age, sex, center, BMI

Data analysis: Tests of association will be completed in SAS. We will use an additive genetic model employing a 1 d.f. trend test. Because all SNPs have demonstrated previous highly significant associations with diabetes or fasting glucose GWAS, we will report p-values and genotype specific means for all associations, rather than focusing on a specific threshold for significance. We will report associations both adjusted and not adjusted for BMI. We will also consider performing analyses stratified by BMI.

For association analysis with change in fasting glucose we will use repeated measures analysis of variance (Proc MIXED in SAS). Additionally, to take into consideration the glucose-lowering effect of anti-diabetic medication among subjects diagnosed and treated at later visits (while including the maximum number of participants), we will add a constant to the glucose level in those individuals taking anti-diabetic medicine. Based on

published literature, we estimate that individuals taking anti-diabetic medication, had they not been taking medication, would have a plasma glucose level 20 mg/dL higher than the observed (12). The use of this analytical approach, adding a constant to the observed quantitative outcome, has proven to be relatively unbiased and more efficient than other methods in the study of quantitative traits (13). We will also repeat the change analyses excluding individuals when they are diagnosed as diabetic or start diabetic medication (and at every subsequent visit), to see how sensitive the results are to the use of the medication adjustment.

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

8.c. If yes, is the author aware that the participants with RES_DNA = 'not for profit' restriction must be excluded if the data are used by a for profit group?
__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)?

ARIC Manuscript Proposal # 1409-- Genome-wide Association Study of Diabetes-Related Quantitative Traits in ARIC Whites. Dr. Pankow, the lead author on this manuscript is a coauthor on this proposal. This is one of several manuscripts that is expected to result from the GWAS of diabetes-related traits.

ARIC Manuscript Proposal # 1273-- Genetic risk score for type 2 diabetes. Dr. Pankow, the lead author on this proposal, is a coauthor on this manuscript. There is no overlap as we do not intend to create a risk score for fasting glucose, nor do we intend to look at the association of any genetic variants with diabetes.

ARIC Manuscript Proposal # 1141-- Transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes In this paper, the association of fasting glucose (at all 4 visits) with TCF7L2 will be reported (using a GEE method). Therefore, for the cross-sectional analyses with candidate genes, we will reference this result for TCF7L2. Dr. Yan, the lead author on this manuscript is a coauthor on this proposal and we will work with her to assure that there is no overlap between the papers.

ARIC Manuscript Proposal # 1380-- Association of the single nucleotide polymorphism rs780094 in the glucokinase regulator gene (*GCKR*) and metabolic phenotypes in the ARIC Study. In this paper the association between visit 1 fasting glucose and rs780094 will be reported. Therefore, for the cross-sectional analyses with candidate genes, we will reference this result for GCKR. Dr. Kottgen, the lead author on this manuscript, is a coauthor on this proposal and we will work with her to assure that there is no overlap between the papers.

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)

*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.

References

1. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, de Bakker PI, Abecasis GR, Almgren P, Andersen G, Ardlie K, Bostrom KB, Bergman RN, Bonnycastle LL, Borch-Johnsen K, Burtt NP, Chen H, Chines PS, Daly MJ, Deodhar P, Ding CJ, Doney AS, Duren WL, Elliott KS, Erdos MR, Frayling TM, Freathy RM, Gianniny L, Grallert H, Grarup N, Groves CJ, Guiducci C, Hansen T, Herder C, Hitman GA, Hughes TE, Isomaa B, Jackson AU, Jorgensen T, Kong A, Kubalanza K, Kuruvilla FG, Kuusisto J, Langenberg C, Lango H, Lauritzen T, Li Y, Lindgren CM, Lyssenko V, Marvelle AF, Meisinger C, Midthjell K, Mohlke KL, Morken MA, Morris AD, Narisu N, Nilsson P, Owen KR, Palmer CN, Payne F, Perry JR, Pettersen E, Platou C, Prokopenko I, Qi L, Qin L, Rayner NW, Rees M, Roix JJ, Sandbaek A, Shields B, Sjogren M, Steinthorsdottir V, Stringham HM, Swift AJ, Thorleifsson G, Thorsteinsdottir U, Timpson NJ, Tuomi T, Tuomilehto J, Walker M, Watanabe RM, Weedon MN, Willer CJ, Wellcome Trust Case Control Consortium, Illig T, Hveem K, Hu FB, Laakso M, Stefansson K, Pedersen O, Wareham NJ, Barroso I, Hattersley AT, Collins FS, Groop L, McCarthy MI, Boehnke M, Altshuler D: Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet.* 40:638-645, 2008

2. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP,

Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, Wellcome Trust Case Control Consortium (WTCCC), McCarthy MI, Hattersley AT: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science.* 316:1336-1341, 2007

3. 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.* 81:338-345, 2007

4. 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.* 316:1341-1345, 2007

5. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN,

Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science.* 316:1331-1336, 2007

6. Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 447:661-678, 2007

7. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorradottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Ostaptchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet.* 39:770-775, 2007

8. Lango H, UK Type 2 Diabetes Genetics Consortium, Palmer CN, Morris AD, Zeggini E, Hattersley AT, McCarthy MI, Frayling TM, Weedon MN: Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. *Diabetes.* 57:3129-3135, 2008

9. Bouatia-Naji N, Rocheleau G, Van Lommel L, Lemaire K, Schuit F, Cavalcanti-Proenca C, Marchand M, Hartikainen AL, Sovio U, De Graeve F, Rung J, Vaxillaire M, Tichet J, Marre M, Balkau B, Weill J, Elliott P, Jarvelin MR, Meyre D, Polychronakos C, Dina C, Sladek R, Froguel P: A polymorphism within the G6PC2 gene is associated with fasting plasma glucose levels. *Science.* 320:1085-1088, 2008

10. Chen WM, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orru M, Grazia Piras M, Bonnycastle LL, Willer CJ, Lyssenko V, Shen H, Kuusisto J, Ebrahim S, Sestu N, Duren WL, Spada MC, Stringham HM, Scott LJ, Olla N, Swift AJ, Najjar S, Mitchell BD, Lawlor DA, Smith GD, Ben-Shlomo Y, Andersen G, Borch-Johnsen K, Jorgensen T, Saramies J, Valle TT, Buchanan TA, Shuldiner AR, Lakatta E, Bergman RN, Uda M, Tuomilehto J, Pedersen O, Cao A, Groop L, Mohlke KL, Laakso M, Schlessinger D, Collins FS, Altshuler D, Abecasis GR, Boehnke M, Scuteri A, Watanabe RM: Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. *J Clin Invest.* 118:2620-2628, 2008

11. Meigs JB, Manning AK, Fox CS, Florez JC, Liu C, Cupples LA, Dupuis J: Genome-wide association with diabetes-related traits in the framingham heart study. *BMC Med Genet.* 8 Suppl 1:S16, 2007

12. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G, ADOPT Study Group: Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med.* 355:2427-2443, 2006

13. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR: Adjusting for treatment effects in studies of quantitative traits: Antihypertensive therapy and systolic blood pressure. *Stat Med.* 24:2911-2935, 2005