Association of a Fasting Glucose Genetic Risk Score With Subclinical Atherosclerosis

The Atherosclerosis Risk in Communities (ARIC) Study

Laura J. Rasmussen-Torvik,[1] Man Li,[2] Wen H. Kao,[2] David Couper,[3] Eric Boerwinkle,[4] Suzette J. Bielinski,[5] Aaron R. Folsom,[6] and James S. Pankow[6]

OBJECTIVE—Elevated fasting glucose level is associated with increased carotid intima-media thickness (IMT), a measure of subclinical atherosclerosis. It is unclear if this association is causal. Using the principle of Mendelian randomization, we sought to explore the causal association between circulating glucose and IMT by examining the association of a genetic risk score with IMT.

RESEARCH DESIGN AND METHODS—The sample was drawn from the Atherosclerosis Risk in Communities (ARIC) study and included 7,260 nondiabetic Caucasian individuals with IMT measurements and relevant genotyping. Components of the fasting glucose genetic risk score (FGGRS) were selected from a fasting glucose genome-wide association study in ARIC. The score was created by combining five single nucleotide polymorphisms (SNPs) (rs780094 [*GCKR*], rs560887 [*G6PC2*], rs4607517 [*GCK*], rs13266634 [*SLC30A8*], and rs10830963 [*MTNR1B*]) and weighting each SNP by its strength of association with fasting glucose. IMT was measured through bilateral carotid ultrasound. Mean IMT was regressed on the FGGRS and on the component SNPs, individually.

RESULTS—The FGGRS was significantly associated ($P = 0.009$) with mean IMT. The difference in IMT predicted by a 1 SD increment in the FGGRS (0.0048 mm) was not clinically relevant but was larger than would have been predicted based on observed associations between the FFGRS, fasting glucose, and IMT. Additional adjustment for baseline measured glucose in regression models attenuated the association by about one third.

CONCLUSIONS—The significant association of the FGGRS with IMT suggests a possible causal association of elevated fasting glucose with atherosclerosis, although it may be that these loci influence IMT through nonglucose pathways.
Diabetes **60:331–335, 2011**

From the [1]Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; the [2]Division of Epidemiology, Johns Hopkins School of Public Health, Baltimore, Maryland; the [3]Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, North Carolina; the [4]Human Genetics Center, University of Texas Health Science Center at Houston, Houston, Texas; the [5]Division of Epidemiology, Mayo Clinic, Rochester, Minnesota; and the [6]Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, Minnesota.

Corresponding author: Laura J. Rasmussen-Torvik, ljrtorvik@northwestern.edu.

Received 16 June 2010 and accepted 18 October 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 29 October 2010. DOI: 10.2337/db10-0839.

Elevated fasting glucose level is associated with increased carotid intima-media thickness (IMT) (1,2), a measure of subclinical atherosclerosis. However, it is still unclear if this relation is causal, due to unmeasured confounding by other cardiovascular risk factors, or due to the metabolic derangements of diabetes—a disease defined by fasting glucose level.

Several recent fasting glucose genome-wide association studies (GWAS) (3–5) and a large GWAS meta-analysis (6) have identified multiple genetic variants with strong associations to fasting plasma glucose level. A recent GWAS in the Atherosclerosis Risk in Communities (ARIC) study found five variants significantly associated with fasting glucose after correction for genome-wide testing (7). Consistent associations for all five of the variants have been reported in other fasting glucose GWAS (6). We demonstrated that these variants are much more strongly associated with fasting glucose in the normal or prediabetic range than in the diabetic range (7).

The discovery of genetic variants reproducibly associated with fasting glucose provides the opportunity to investigate a causal association between fasting glucose and cardiovascular disease (CVD) using the theory of Mendelian randomization. Because of random assortment of alleles at the time of gamete formation, genetic variants should not be associated with known and unknown confounders in association analyses. Genetic variants can also be measured very accurately and are thus subject to little measurement error. Finally, genetic variants are also not susceptible to issues of reverse causality (8). Therefore, the proxy use of single nucleotide polymorphisms (SNPs) significantly associated with a trait instead of the trait itself in association analyses can help to explore a causal association between a trait and disease (9). This technique was recently used in a meta-analysis to examine the causal relationship of C-reactive protein to heart disease (10). In this paper, we applied principles of Mendelian randomization to explore whether there is a causal relation between fasting glucose in the nondiabetic range and subclinical atherosclerosis. In order to reduce problems with multiple testing, to create a genetic variable that accounted for a substantive amount of variation in fasting glucose, and to attempt to account for pleiotropic effects of individual SNPs, a composite genetic risk score was used. However, Mendelian randomization results from single SNPs associated with fasting glucose are also presented in the online appendix available at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0839/DC1.

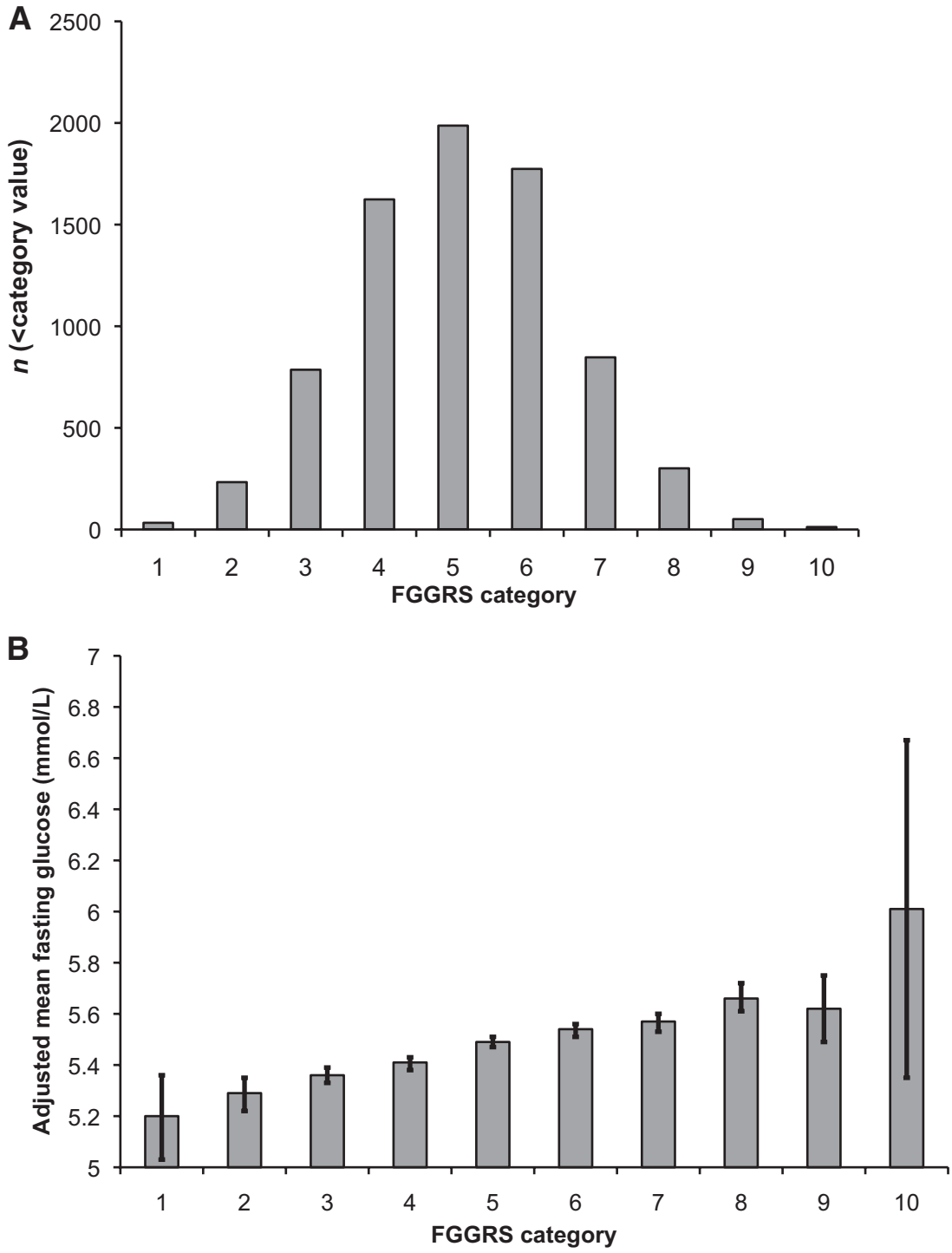

FIG. 1. Distribution of the FGGRS (*A*) and mean fasting glucose by FGGRS (*B*). FGGRS = {[(no. of rs780094 risk alleles) × 0.0463] + [(no. of rs560887 risk alleles) × 0.0685] + [(no. of rs4607517 risk alleles) × 0.0673] + [(no. of rs13266634 risk alleles) × 0.0433] + [(no. of rs10830963 risk alleles) × 0.0796]}/0.061. To create these plots, all individuals with an FGGRS <1 were included in the first category, all individuals with a FGGRS ≥1 but <2 were included in the second category, etc. Means of fasting glucose in *B* are adjusted for age, sex, and ARIC study center and presented with 95% CI.

RESEARCH DESIGN AND METHODS

The ARIC study is a multicenter prospective cohort study focused on CVD occurrence (11). Caucasian and African American men and women aged 45–64 years at baseline were recruited from four U.S. communities. A total of 15,792 individuals participated in the baseline examination in 1987–1989. The study was approved by the institutional review board at each center, and all participants gave informed consent.

We included only participants with cleaned genotype information available from the Affymetrix Genome-Wide Human SNP Array 6.0 (n = 9,345 Caucasians). Individuals with prevalent diabetes or missing information about diabetes at baseline were excluded (n = 829). Prevalent diabetes was defined as the presence of any of the following: a fasting serum glucose of ≥126 mg/dl (7.0 mmol/l), a nonfasting serum glucose level of ≥200 mg/dl (11.1 mmol/l), self-reported physician diagnosis of diabetes, or self-reported pharmacological treatment of diabetes in the past 2 weeks. Additionally, we excluded individuals with prevalent CVD (n = 753), defined as a self-reported history of physician-diagnosed myocardial infarction or stroke or prior myocardial infarction detected by electrocardiogram or prior self-reported cardiovascular

TABLE 1
Associations of IMT with measured fasting glucose and the FGGRS

	Models* adjusted for age, sex, study center				Model* additionally adjusted for baseline fasting glucose	
	Association per 1 SD increment (1.4) in FGGRS	*P*	Association per 1 SD increment (0.50 mmol/l) in measured fasting serum glucose	*P*	Association per 1 SD increment (1.4) in FGGRS	*P*
Baseline fasting glucose (mmol/l)	β = 0.0854 (SE 0.0054)	1.07E−54	—	—	—	—
IMT (mm)	β = 0.0048 (SE 0.0019)	0.009	β = 0.0103 (SE 0.0019)	8.01E−8	β = 0.0032 (SE 0.0019)	0.09

*Linear regression.

surgery or coronary angioplasty. Finally, we excluded any individual not fasting at least 8 h at baseline (n = 125) and anyone with either a missing or extremely large (greater than 1.7 mm) IMT measurement at baseline (n = 378). After all exclusions, there were 7,260 individuals in the sample.

Phenotypic measurements. Glucose was measured in serum by a hexokinase/glucose-6-phosphate dehydrogenase method on a Coulter DACOS device (Beckman Coulter, Fullerton, CA). BMI was calculated from participants' heights and weights measured in scrub suits. Carotid artery IMT was determined by high-resolution B-mode ultrasound, as described previously (12,13). Trained technicians scanned the extracranial carotid arteries bilaterally, and scans were read according to a standardized protocol at a centralized facility. Missing measurements were imputed from sex- and race-specific multivariate linear models of mean IMT, as described elsewhere (2). The variable used for analysis was the average IMT of the far wall across three segments in the left and right carotid arteries: carotid bifurcation, common carotid, and internal carotid (six sites total).

Genotyping. ARIC GWAS genotyping has been described in detail previously (7). SNPs were selected for inclusion in the fasting glucose genetic risk score (FGGRS) based on five regions of the genome that exceeded a genome-wide threshold for significance (14) in a previously-published GWAS of baseline fasting glucose among nondiabetic subjects in the ARIC study (7). A single SNP from each region was selected for inclusion in the score based on the SNP that reached the greatest level of significance for the region in fasting glucose or diabetes GWAS meta-analyses (6,15). The selected SNPs were rs780094 (*GCKR*), rs560887 (*G6PC2*), rs4607517 (*GCK*), rs13266634 (*SLC30A8*), and rs10830963 (*MTNR1B*). In cases where the selected SNP was imputed (rs4607517, rs13266634), the most likely predicted genotype was used.

Statistical analysis. All analyses were performed in SAS v9.1 (Cary, NC). To create the FGGRS, the number of risk (glucose-increasing) alleles from SNPs rs10830963, rs560887, rs4607517, rs780094, and rs13266634 was summed. To account for the differing effect sizes of the SNPs, the number of risk alleles from each SNP was multiplied by the predicted effect size from regression of baseline fasting glucose on that SNP (7). The total score was then divided by the average effect size to rescale the score with a possible range of 0–10 (the range of the possible number of glucose-increasing alleles for each individual). An equation for the calculation of the risk score can be found in Fig. 1.

Untransformed IMT was regressed on the FGGRS, and both variables were modeled continuously. Regression was weighted by the number of observed (versus imputed) IMT measurements per person. Predicted associations between FGGRS and IMT were estimated by multiplying the observed difference in baseline fasting glucose for 1 SD increase of FGGRS (0.0854 mmol/l) by the observed difference in IMT per 1 SD increase in baseline fasting glucose (0.0103 mm per 0.50 mmol/l of glucose). The predicted association between FGGRS and IMT was then compared with the observed association between FGGRS and IMT for the same 1 SD increase in FGGRS.

RESULTS

Of the 7,260 individuals in the study sample, 44.5% were male. The average age in the study sample was 53.9 years, and the average BMI was 26.5 kg/m^2. The mean fasting glucose measurement was 5.5 mmol/l. Figure 1*A* demonstrates the distribution of FGGRS in the sample. The FGGRS was normally distributed with a mean of 4.6 and an SD of 1.4. Figure 1*B* shows the mean serum fasting glucose by FGGRS category, adjusted for age, sex, and ARIC study center. Adjusted mean fasting glucose increased for each successive category of the FGGRS except between categories 8 and 9 in which the mean decreased by 0.04 mmol/l.

Table 1 shows the associations of IMT with fasting glucose and the FGGRS. The FGGRS was very significantly associated with fasting glucose in ARIC ($P = 1.1 \times 10^{-54}$). Adjusted for age, sex, and study center, the FGGRS accounted for an additional 2.9% of the variance in fasting glucose. The FGGRS was significantly associated (P = 0.009) with mean IMT, although the difference in IMT predicted by 1 SD increment of the FGGRS (0.0048 mm) was small. The IMT and FGGRS association was attenuated by approximately one third (β = 0.0032 mm) when fasting glucose was also included as a covariate in the regression model. Supplementary Table 1 in the online appendix details the association of individual SNPs in the FGGRS with fasting glucose. No single SNP accounted for more than an additional 0.86% of the variance in fasting glucose. Supplementary Table 2 lists the associations of the individual SNPs in the FGGRSs with IMT before and after adjustment for baseline fasting glucose. Only one SNP (rs560887) was significantly (P = 0.0055) associated with IMT when analyzed individually.

To further investigate the association between IMT and the FGGRS, we undertook several sensitivity analyses. Excluding individuals with a FGGRS <1 or >8.8 did not attenuate the association (β = 0.0049 mm, P = 0.009). Excluding individuals near the fasting glucose threshold for diabetes at baseline (fasting glucose >6.66 mmol/l, individuals who might be at very high risk of shortly developing diabetes or who may have been misclassified as not having diabetes) attenuated the association only slightly (β = 0.0046 mm, P = 0.01). Adjusting the regression model for 10 principal components derived from GWAS data to account for differences in genetic ancestry within the sample did not attenuate the association (β = 0.0048 mm, P = 0.009). Using a different FGGRS derived from the results of the Dupuis et al. (6) GWAS meta-analysis of fasting glucose, which included 16 SNPs and was weighted based on the effect sizes taken from the meta-analysis replication sample, attenuated the association of score and IMT slightly (P = 0.025).

Figure 2 compares the observed association of the FGGRS and IMT with the predicted association. The observed association (0.0048 mm of IMT per 1.4 unit difference in score) was over two times larger than the predicted association (0.0018 mm of IMT per 1.4 unit difference in score). Supplementary Fig. 1*A*–*E* in the online appendix compares observed with predicted associations for the five component SNPs of the FGGRS. For all SNPs (except rs780094, a SNP with known opposing pleiotropic

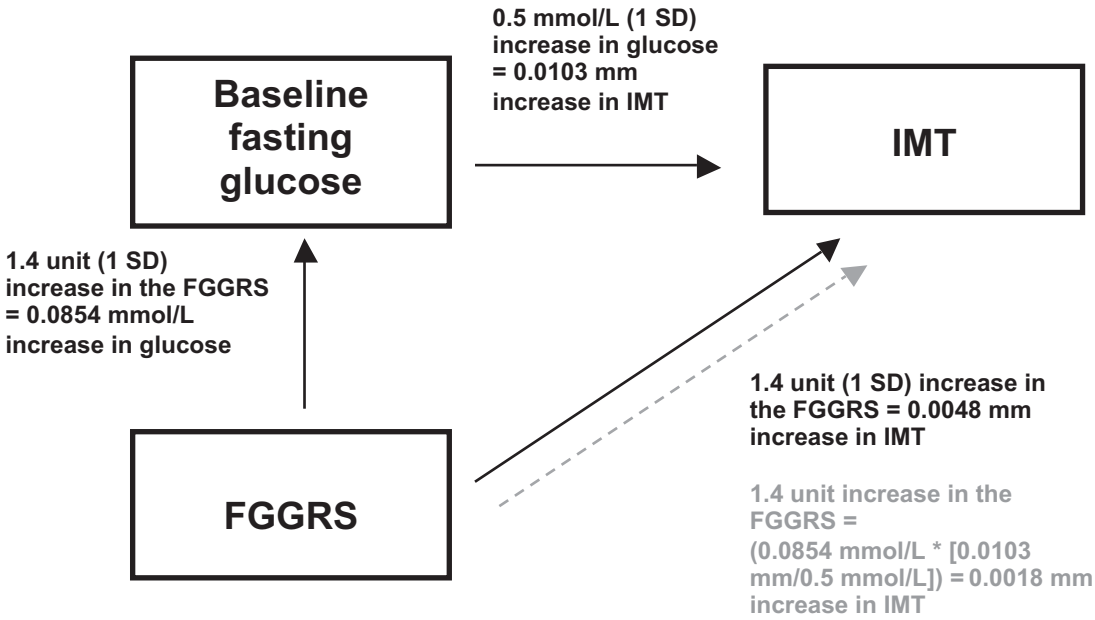

FIG. 2. Observed versus expected associations between the FGGRS and IMT. Associations determined from observed regression equations are described in black. All regressions adjusted for age, sex, and center. Predicted associations are described in gray.

associations to other cardiovascular risk factors), the observed association with IMT was larger than that predicted—although the magnitude of the difference varied considerably between SNPs.

DISCUSSION

In this analysis we created an FGGRS that accounted for 2.9% of the variance in fasting glucose among nondiabetic adults after adjustment for age, sex, and study center. The FGGRS was significantly and positively associated with carotid IMT. Inclusion of measured baseline glucose in the model attenuated the association between the risk score and IMT by ~33% ($P = 0.09$). Similar results were seen for individual SNPs used to construct the risk score except for rs780094, a SNP having demonstrated opposing associations with other cardiovascular risk factors including triglycerides, and HDL, LDL, and VLDL cholesterol (16,17).

The statistical significance of the association between the FGGRS and IMT offers some support for the hypothesis that fasting glucose is causally related to the development of subclinical atherosclerosis. Additionally, the fact that the FGGRS remains modestly associated with IMT after the inclusion of fasting glucose in the model and the larger observed than predicted association between the FGGRS and IMT suggests that the risk score provides additional information to a single measure of fasting glucose. We hypothesize the FGGRS gives more information than a single measurement of fasting glucose because it provides information about cumulative lifetime exposure to fasting glucose (because those with a high FGGRS would be expected, on average, to have slightly higher levels of fasting glucose over much of the life span). The FGGRS also may give more information because of possible pleiotropic effects of the SNPs.

It is difficult to use Mendelian randomization and this FGGRS to completely discriminate the effects of glucose level from other cardiovascular risk factors on IMT because of the possible pleiotropic effects of SNPs in the FGGRS. However, the significant association of the score (as opposed to just the component SNPs) with IMT is unlikely to be due entirely to pleiotropy because it is not expected that all the SNPs used to construct the score would have similar pleiotropic associations with IMT. Therefore, a causal association between fasting glucose and IMT is a plausible explanation for the significant association of IMT and the FGGRS. The larger than predicted associations between the component SNPs and IMT also suggest that *G6PC2* and *GCK* should be studied further to learn about other potential pathways through which these SNPs may alter IMT.

ACKNOWLEDGMENTS

The ARIC study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022, as well as R01HL087641, R01HL59367, and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health (NIH) contract HHSN268200625226C. Infrastructure was partly supported by grant number UL1RR025005, a component of the NIH and NIH Roadmap for Medical Research. L.J.R.-T. was supported by training grant T32HL07779.

No potential conflicts of interest relevant to this article were reported.

L.J.R.-T. analyzed data and wrote the manuscript. M.L., W.H.K., D.C., E.B., S.J.B., A.R.F., and J.S.P. contributed to discussion and reviewed and edited the manuscript.

The authors thank the staff and participants of the ARIC study for their important contributions. The authors are grateful for resources provided by the University of Minnesota Supercomputing Institute.

REFERENCES

1. Tropeano AI, Boutouyrie P, Katsahian S, Laloux B, Laurent S. Glucose level is a major determinant of carotid intima-media thickness in patients with hypertension and hyperglycemia. J Hypertens 2004;22:2153–2160
2. Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, Cram KB, Hutchinson RG. Relation of carotid artery wall thickness to

diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis risk in communities (ARIC) study investigators. Stroke 1994;25:66–673

3. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orrù M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeber G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, Abecasis GR. Variants in MTNR1B influence fasting glucose levels. Nat Genet 2009;41:77–81
4. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proença C, Sparsø T, Holmkvist J, Marchand M, Delplanque J, Lobbens S, Rocheleau G, Durand E, De Graeve F, Chèvre JC, Borch-Johnsen K, Hartikainen AL, Ruokonen A, Tichet J, Marre M, Weill J, Heude B, Tauber M, Lemaire K, Schuit F, Elliott P, Jørgensen T, Charpentier G, Hadjadj S, Cauchi S, Vaxillaire M, Sladek R, Visvikis-Siest S, Balkau B, Lévy-Marchal C, Pattou F, Meyre D, Blakemore AI, Jarvelin MR, Walley AJ, Hansen T, Dina C, Pedersen O, Froguel P. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nat Genet 2009;41:89–94
5. Chen WM, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orrù 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, Jørgensen 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 2008;118:2620–2628
6. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Mägi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparsø T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proença C, Kumari M, Qi L, Timpson NJ, Gieger C, Zabena C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccasecca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben-Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Böttcher Y, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllensten U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanali N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD, Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jørgensen T, Jula A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martínez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orrù M, Pakyz R, Palmer CN, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvänen AC, Tanaka T, Thorand B, Tichet J, Tönjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC, DIAGRAM Consortium, GIANT Consortium, Global BPgen Consortium, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Ríos M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP, Wichmann HE, Illig T, Rudan I, Wright AF, Stumvoll M, Campbell H, Wilson JF, Anders Hamsten on behalf of Procardis Consortium, MAGIC investigators, Bergman RN, Buchanan TA, Collins FS, Mohlke KL, Tuomilehto J, Valle TT, Altshuler D, Rotter JI, Siscovick DS, Penninx BW, Boomsma DI, Deloukas P, Spector TD, Frayling TM, Ferrucci L, Kong A, Thorsteinsdottir U, Stefansson K, van Duijn CM, Aulchenko YS, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Waterworth DM, Vollenweider P, Peltonen L, Mooser V, Abecasis GR, Wareham NJ, Sladek R, Froguel P, Watanabe RM, Meigs JB, Groop L, Boehnke M, McCarthy MI, Florez JC, Barroso I. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010;42:105–116
7. Rasmussen-Torvik LJ, Alonso A, Li M, Kao W, Kottgen A, Yan Y, Couper D, Boerwinkle E, Bielinski SJ, Pankow JS. Impact of repeated measures and sample selection on genome-wide association studies of fasting glucose. Genet Epidemiol 2010;34:665–673
8. Smith GD, Timpson N, Ebrahim S. Strengthening causal inference in cardiovascular epidemiology through Mendelian randomization. Ann Med 2008;40:524–541
9. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008;27:1133–1163
10. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert JC, Bennett D, Coin L, Ashby D, Tzoulaki I, Brown IJ, Mt-Isa S, McCarthy MI, Peltonen L, Freimer NB, Farrall M, Ruokonen A, Hamsten A, Lim N, Froguel P, Waterworth DM, Vollenweider P, Waeber G, Jarvelin MR, Mooser V, Scott J, Hall AS, Schunkert H, Anand SS, Collins R, Samani NJ, Watkins H, Kooner JS. Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA 2009;302:37–48
11. The Atherosclerosis Risk in Communities (ARIC) study: design and objectives. The ARIC investigators. Am J Epidemiol 1989;129:687–702
12. High-resolution B-mode ultrasound scanning methods in the Atherosclerosis Risk in Communities Study (ARIC). The ARIC study group. J Neuroimaging 1991;1:68–73
13. Chambless LE, Folsom AR, Clegg LX, Sharrett AR, Shahar E, Nieto FJ, Rosamond WD, Evans G. Carotid wall thickness is predictive of incident clinical stroke: The atherosclerosis risk in communities (ARIC) study. Am J Epidemiol 2000;151:478–487
14. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–894
15. 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 2008;57:3129–3135
16. Chasman DI, Pare G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Hamsten A, Kathiresan S, Malarstig A, Ordovas JM, Ripatti S, Parker AN, Miletich JP, Ridker PM. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet 2009;5:e1000730
17. Chasman DI, Paré G, Zee RY, Parker AN, Cook NR, Buring JE, Kwiatkowski DJ, Rose LM, Smith JD, Williams PT, Rieder MJ, Rotter JI, Nickerson DA, Krauss RM, Miletich JP, Ridker PM. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B among 6382 white women in genome-wide analysis with replication. Circ Cardiovasc Genet 2008;1:21–30