

Racial/Ethnic Differences in Association of Fasting Glucose–Associated Genomic Loci With Fasting Glucose, HOMA-B, and Impaired Fasting Glucose in the U.S. Adult Population

QUANHE YANG, PHD¹
TIEBIN LIU, MSPH¹
PETER SHRADER, MS²
AJAY YESUPRIYA, MPH¹
MAN-HUEI CHANG, MPH¹
NICOLE F. DOWLING, PHD¹

RENÉE M. NED, MMSC, PHD¹
JOSÉE DUPUIS, PHD³
JOSE C. FLOREZ, MD, PHD⁴
MUIJ J. K HOURY, MD, MPH¹
JAMES B. MEIGS, MD, MPH⁵
THE MAGIC INVESTIGATORS*

OBJECTIVE — To estimate allele frequencies and the marginal and combined effects of novel fasting glucose (FG)-associated single nucleotide polymorphisms (SNPs) on FG levels and on risk of impaired FG (IFG) among non-Hispanic white, non-Hispanic black, and Mexican Americans.

RESEARCH DESIGN AND METHODS — DNA samples from 3,024 adult fasting participants in the National Health and Nutrition Examination Survey (NHANES) III (1991–1994) were genotyped for 16 novel FG-associated SNPs in multiple genes. We determined the allele frequencies and influence of these SNPs alone and in a weighted genetic risk score on FG, homeostasis model assessment of β -cell function (HOMA-B), and IFG by race/ethnicity, while adjusting for age and sex.

RESULTS — All allele frequencies varied significantly by race/ethnicity. A weighted genetic risk score, based on 16 SNPs, was associated with a 0.022 mmol/l (95% CI 0.009–0.035), 0.036 mmol/l (0.019–0.052), and 0.033 mmol/l (0.020–0.046) increase in FG levels per risk allele among non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, respectively. Adjusted odds ratios for IFG were 1.78 for non-Hispanic whites (95% CI 1.00–3.17), 2.40 for non-Hispanic blacks (1.07–5.37), and 2.39 for Mexican Americans (1.37–4.14) when we compared the highest with the lowest quintiles of genetic risk score ($P = 0.365$ for testing heterogeneity of effect across race/ethnicity).

CONCLUSIONS — We conclude that allele frequencies of 16 novel FG-associated SNPs vary significantly by race/ethnicity, but the influence of these SNPs on FG levels, HOMA-B, and IFG were generally consistent across all racial/ethnic groups.

Diabetes Care 33:2370–2377, 2010

From the ¹Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, Georgia; the ²General Medicine Division, Massachusetts General Hospital, Boston, Massachusetts; the ³Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts; the ⁴Center for Human Genetic Research and Diabetes Research Center (Diabetes Unit), Massachusetts General Hospital, Program in Medical and Population Genetics, Broad Institute, Department of Medicine, Harvard Medical School, Boston, Massachusetts; and the ⁵General Medicine Division, Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Corresponding author: Quanhe Yang, qay0@cdc.gov.

Received 10 May 2010 and accepted 17 August 2010. Published ahead of print at <http://care.diabetesjournals.org> on 30 August 2010. DOI: 10.2337/dc10-0898.

*The MAGIC Investigators are listed in the online appendix, available at <http://care.diabetesjournals.org/cgi/content/full/dc10-0898/DC1>.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Fasting glucose (FG) is associated with future risk of type 2 diabetes and cardiovascular disease (1–3). Impaired FG (IFG) (FG between 5.6 and 7.0 mmol/l), which is the high end of the nondiabetic FG distribution, is also a risk factor for type 2 diabetes and cardiovascular disease (1,2,4,5). The prevalence of type 2 diabetes and the complications associated with the disease vary significantly by race/ethnicity (6). Researchers conducting recent genetic association studies have used homeostasis model assessment of β -cell function (HOMA-B) to identify several loci that influence levels of FG and β -cell function (7–9). A meta-analysis of genome-wide association data, by the Meta-Analysis of Glucose and Insulin-Related Traits Consortium (MAGIC), recently confirmed 16 common single nucleotide polymorphisms (SNPs) associated with FG levels (10). Index SNPs were in or near *PROX1*, *G6PC2*, *GCKR*, *ADCY5*, *SLC2A2*, *DGKB*, *GCK*, *SLC30A8*, *GLIS3*, *ADRA2A*, *TCF7L2*, *MTNR1B*, *FADS1*, *CRY2*, *MADD*, and *C2CD4B* (10). These associations were discovered in white people of European ancestry; the influence of these SNPs on FG levels in nonwhite populations and their allele frequencies in general-population samples are unknown. The frequencies of common disease-associated alleles discovered by candidate gene or genome-wide association studies (GWASs) can differ significantly across racial/ethnic groups (<http://hapmap.ncbi.nlm.nih.gov>), and in some cases, the risk allele within a locus will differ on the basis of race/ethnicity (11,12). Other studies suggest that despite the substantial variations in allele frequencies, the genetic effects on common diseases are largely consistent across racial/ethnic groups (13). We addressed these concerns by genotyping 16 confirmed FG-associated SNPs in adults who completed the Third National Health and Nutrition Examination Survey (NHANES III; 1991–1994),

which allowed us to collect data from a large, representative sample of the U.S. population that included non-Hispanic whites, non-Hispanic blacks, and Mexican Americans. We tested the hypotheses that there is significant racial/ethnic variation in 1) SNP risk (FG raising) allele frequencies, 2) the marginal and combined effects of these 16 SNPs on FG levels and HOMA-B, and 3) the combined effect of these 16 risk SNPs on risk of IFG.

RESEARCH DESIGN AND METHODS

The NHANES III used a stratified multistage probability design to obtain a nationally representative sample of the civilian, noninstitutionalized U.S. population. In the NHANES III, each survey participant had a household interview and a physical examination; a subset of individuals had fasting blood sampling. Blood specimens for DNA collection were obtained from the NHANES III Phase 2 (1991–1994) participants aged ≥ 12 years. DNA lysates were created from cell lines generated using Epstein-Barr-transformed lymphocytes from these blood specimens. We limited our analyses to nondiabetic participants who were aged ≥ 20 years and had fasted between 8 and 23 h before blood collection. Of 3,517 eligible participants, we excluded 162 participants who self-identified their race/ethnicity as “other” and also excluded 331 participants who either self-reported diabetes or had a FG level ≥ 7.0 mmol/l or who had an oral glucose tolerance test (OGTT) ≥ 11.1 mmol/l, which left 3,024 participants in the study.

FG, HOMA-B, IFG, and diabetes definitions

Plasma glucose levels were measured by hexokinase (COBAS MIRA; Roche Diagnostics, Montclair, NJ). We used HOMA as a surrogate measure of β -cell function (HOMA-B; calculated as $[20 \times \text{fasting insulin } \{\mu\text{IU/ml}\} / \text{FG } \{\text{mmol/l}\} - 3.5]$) (14).

We defined IFG as having a fasting plasma glucose ≥ 5.6 mmol/l, but < 7.0 mmol/l and diabetes as use of antidiabetes medications (including insulin) or a fasting plasma glucose ≥ 7.0 mmol/l (4).

SNP genotyping

We genotyped 16 SNPs that were confirmed as associated with FG levels among nondiabetic people of European ancestry in the MAGIC GWAS (10). We used SNPs rs573225 as a proxy ($r^2 = 1.0$ in CEU) for SNP rs560887 in *G6PC2* and SNP rs11558471 as a proxy ($r^2 = 1.0$ in CEU)

for SNP rs13266634 in *SLC30A8*. Genotyping was performed with the use of iPLEX (Sequenom) (15). The minimum call rate was 98.1%, and all SNPs were in Hardy-Weinberg equilibrium (HWE) according to the National Center for Health Statistics criterion (if $P < 0.01$ in two or more race/ethnicity groups, HWE is rejected).

Genetic risk score

We constructed a weighted genetic risk score (GRS) to examine the combined effect of 16 SNPs on FG levels, HOMA-B, and risk for IFG. We used the β -coefficients from the published European ancestry GWASs of these FG-associated SNPs (10). We multiplied these β -coefficients by 0, 1, or 2, according to the number of risk alleles carried by each individual, and then summed them. To facilitate the comparison with the simple GRS (summing the number of risk alleles), we divided the score by 0.948 (twice the sum of the β -coefficients) and multiplied by 32 (total number of alleles) (16). Although a number of SNPs did not show significant association with FG or HOMA-B in the NHANES III, we assumed, on the basis of GWAS results, that each SNP is independently associated with FG for computation of a weighted GRS. This assumption might not be appropriate if an index SNP is correlated with the causal SNP in the discovery population but not so in other racial/ethnic groups due to differences in linkage disequilibrium patterns (17). We used an additive genetic model for each SNP and applied a linear weighting of 0, 1, or 2 to genotypes containing 0, 1, or 2 risk alleles (16). We fit the weighted GRS as a continuous variable and categorized it into quintiles in multivariate analyses. In presenting the results, we rounded the weighted GRS to the whole number.

Statistical analysis

We calculated weighted allele frequencies and their 95% CIs, stratified by race/ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American) using the NHANES sample weights. We carried out HWE tests for each genetic variant for each racial/ethnic group. To test for differences in allele frequencies, we pooled all racial/ethnic groups and used χ^2 to test the differences across the racial/ethnic groups. We used linear regression modeling to evaluate the relationship between 16 SNPs and FG levels and HOMA-B, adjusted for age and sex.

We examined the adjusted marginal effect on FG by including one SNP at a time in the model for each racial/ethnic group. SNPs were coded as 0, 1, or 2 for those who were noncarriers, heterozygous, or homozygous for the risk (FG raising) alleles, respectively. We modeled the GRS in a similar fashion by testing associations of a per-risk allele increase with FG. For the GRS models, we calculated adjusted R^2 , with and without the GRS to determine the explained variance, by adding the GRS in the models for each racial/ethnic group. We tested for homogeneity of effects of these SNPs in marginal and GRS models across race/ethnicity by including the interaction terms of SNP or GRS with race/ethnicity in the regression models using the pooled dataset and reported the associated P value.

We used age- and sex-adjusted logistic regression to evaluate the association of the GRS with prevalence of IFG for each racial/ethnic group. We modeled the GRS to test the association of a per-risk allele increase with odds of IFG. We also categorized the GRS into quintiles to examine the trend of effect on risk of IFG. We tested for homogeneity of the GRS effect on IFG across race/ethnicity by including the interaction term using the pooled dataset. Statistical tests, using linear or logistic regression modeling, were based on Satterthwaite adjusted-F statistics. All tests were two sided, and a P value of < 0.05 at each locus or the GRS was considered significant. To avoid nonresponse bias among NHANES III participants for whom DNA were available, the sample weights were recalculated for the NHANES III DNA dataset by using previously described methods (18). We used SUDAAN (version 10.0) and SAS (version 9.2; SAS Institute, Cary, NC) statistical software for our analyses to account for the complex sampling design.

RESULTS— There were 3,024 individuals available for this study, including 1,225 non-Hispanic white, 897 non-Hispanic black, and 902 Mexican American participants. Compared with other groups, FG was higher among Mexican Americans, and HOMA-B was lower among non-Hispanic whites (Table 1).

Allele frequencies of FG genetic variants

All allele frequencies (16 SNPs) were significantly different across racial/ethnic groups ($P < 0.05$). Risk allele frequencies varied from 17.1% (rs4607517, *GCK*) to

Table 1—Characteristics of participants by race/ethnicity, NHANES III DNA Bank (1991–1994)

Variables	Weighted distribution by race/ethnicity*			P value†
	Non-Hispanic white	Non-Hispanic black	Mexican	
<i>n</i>	1,225	897	902	
FG (mmol/l)				
Mean (95% CI)	5.17 (5.14–5.19)	5.16 (5.11–5.21)	5.30 (5.24–5.36)	<0.001
HOMA-B (<i>n</i> = 3,017)				
Mean (95% CI)	121.5 (109.4–133.6)	155.1 (144.3–165.9)	144.0 (134.6–153.4)	0.008
Age (years)				
Mean (SEM)	43.7 (0.7)	39.6 (0.7)	35.5 (0.7)	<0.001
Sex (% [95% CI])				
Male	48.6 (44.9–52.3)	44.3 (41.2–47.4)	53.0 (50.7–55.3)	
Female	51.4 (47.7–55.1)	55.7 (52.6–58.8)	47.0 (44.7–49.3)	0.001
BMI (kg/m ²) (% [95% CI])				
<25	45.0 (41.9–48.2)	33.4 (29.7–37.4)	32.2 (28.5–36.2)	
25 to <30	34.4 (30.9–38.1)	36.4 (33.6–39.3)	41.6 (37.7–45.7)	
≥30	20.6 (17.9–23.6)	30.2 (27.2–33.2)	26.1 (22.8–29.8)	<0.001
Proportion of non-Hispanic white, non-Hispanic black, and Mexican Americans (95% CI)	81.9 (77.7–85.4)	12.1 (9.0–16.0)	6.0 (4.3–8.3)	

*For continuous variables, the means were adjusted for age and sex. †P values were tests for the differences across race/ethnicity groups and are based on Satterthwaite adjusted F statistics for the continuous variables and χ^2 for the categorical variables.

86.1% (rs10885122, ADRA2) among non-Hispanic whites, from 7.9% (rs10830963, MTNR1B) to 93.7% (rs7944584, MADD) among non-Hispanic blacks, and from 18.0% (rs7903146 (TCF7L2) to 86.5% (rs11920090, SLC2A2) among Mexican Americans (Table 2) (online appendix Table 1, available at <http://care.diabetesjournals.org/cgi/content/full/dc10-0898/DC1>).

Marginal effect of genetic variants on FG and HOMA-B

We observed three SNPs (rs573225, rs780094, and rs11558471) that were significantly associated with FG levels among non-Hispanic whites, one SNP (rs10830963) among non-Hispanic blacks, and three variants (rs573225, rs4607517, and rs10830963) among Mexican Americans (online appendix Tables 2 and 3).

However, the majority of β -coefficients (37 of 48) showed positive associations in the expected direction with FG levels among all three racial/ethnic groups. We found no evidence of heterogeneity effects of these SNPs on FG levels across racial/ethnic groups, except for rs11885122 for which non-Hispanic whites had lower FG and non-Hispanic blacks and Mexican Americans had higher FG levels ($P = 0.009$). Gener-

Table 2—Weighted-risk (FG raising) allele frequencies of 16 FG-associated SNPs by race/ethnicity, NHANES III DNA Bank (1991–1994)

SNP	Chromosome	Nearest gene	Risk allele (effect/other)†	Risk allele frequency % (95% CI)*		
				Non-Hispanic white	Non-Hispanic black	Mexican American
rs340874	1	PROX1	C/T	52.4 (48.9–55.9)	21.9 (19.2–24.8)	41.2 (38.2–44.2)
rs573225	2	G6PC2	A/G	69.9 (67.7–72.1)	92.6 (90.9–93.9)	82.6 (79.8–85.1)
rs780094	2	GCKR	C/T	58.3 (55.9–60.6)	80.8 (78.7–82.7)	65.1 (62.1–67.9)
rs11708067	3	ADCY5	A/G	77.8 (75.4–80.0)	85.3 (83.2–87.2)	72.4 (69.8–75.0)
rs11920090	3	SLC2A2	T/A	85.4 (83.8–86.8)	66.0 (63.8–68.1)	86.5 (84.0–88.7)
rs2191349	7	DGKB	T/G	54.7 (52.7–56.6)	57.9 (55.8–59.9)	41.9 (38.2–45.7)
rs4607517	7	GCK	A/G	17.1 (15.3–18.9)	9.4 (7.6–11.7)	21.8 (19.8–23.8)
rs11558471	8	SLC30A8	A/G	69.0 (65.6–72.2)	88.7 (86.9–90.3)	72.9 (70.7–75.0)
rs7034200	9	GLIS3	A/C	51.3 (48.9–53.7)	62.6 (60.3–64.8)	52.3 (50.4–54.2)
rs10885122	10	ADRA2A	G/T	86.1 (84.7–87.4)	34.9 (32.6–37.2)	82.9 (80.8–84.9)
rs7903146	10	TCF7L2	T/C	29.1 (26.8–31.6)	26.7 (25.3–28.2)	18.0 (16.3–19.8)
rs10830963	11	MTNR1B	G/C	28.0 (25.8–30.3)	7.9 (6.4–9.8)	21.1 (18.5–24.0)
rs174550	11	FADS1	T/C	66.5 (63.9–69.0)	89.8 (87.6–91.6)	39.2 (34.5–44.1)
rs11605924	11	CRY2	A/C	48.3 (45.5–51.2)	85.4 (83.3–87.3)	50.0 (46.5–53.6)
rs7944584	11	MADD	A/T	71.4 (68.9–73.8)	93.7 (91.7–95.3)	84.3 (82.0–86.3)
rs11071657	15	C2CD4B	A/G	64.2 (62.0–66.4)	86.4 (83.7–88.6)	49.2 (46.1–52.3)

*All SNPs were in HWE. †FG-raising allele (effect) is denoted first.

Table 3—Adjusted mean FG levels and HOMA-B by quintiles of weighted genetic risk score and race/ethnicity, NHANES III DNA Bank (1991–1994)

Characteristics	β-Coefficient*	P value†	Quintiles of the 16 SNP weighted genetic risk score					P value‡	R ² s	
			Q1	Q2	Q3	Q4	Q5		Without score	With score
Non-Hispanic white										
n			286	131	300	251	186			
GRS range			0–15	16	17–18	19–20	≥21			
FG (mmol/l)	0.022 (0.009–0.035)	0.002	5.10 (5.05–5.14)	5.18 (5.08–5.27)	5.18 (5.10–5.25)	5.20 (5.13–5.26)	5.28 (5.20–5.35)	0.005	0.208	0.229
HOMA-B	–3.54 (–7.21 to 0.13)	0.058	136.8 (106.9–166.8)	120.3 (100.7–139.9)	117.0 (106.8–127.2)	128.4 (108.5–148.2)	102.1 (92.1–112.1)	0.055	0.013	0.031
Non-Hispanic black										
n			160	114	178	258	111			
GRS range			0–16	17	18	19–20	≥21			
FG (mmol/l)	0.036 (0.019–0.052)	<0.001	5.04 (4.96–5.11)	5.06 (4.95–5.17)	5.06 (4.95–5.17)	5.15 (5.07–5.24)	5.26 (5.19–5.33)	<0.001	0.115	0.133
HOMA-B	–4.98 (–8.32 to –1.64)	0.005	170.6 (152.0–189.2)	161.9 (131.3–192.4)	170.7 (150.4–191.0)	154.2 (135.0–173.4)	133.9 (119.3–148.6)	0.003	0.064	0.078
Mexican American										
n			209	106	139	205	186			
GRS range			0–15	16	17	18–19	≥20			
FG (mmol/l)	0.033 (0.020–0.046)	<0.001	5.11 (5.00–5.21)	5.27 (5.19–5.36)	5.24 (5.13–5.34)	5.22 (5.10–5.34)	5.37 (5.29–5.45)	<0.001	0.135	0.165
HOMA-B	–4.10 (–7.17 to –1.03)	0.011	160.5 (139.9–181.2)	153.5 (142.1–164.8)	160.5 (130.8–190.1)	141.1 (120.4–161.7)	127.2 (117.9–136.6)	0.005	0.043	0.054

Data and 95% CIs in parentheses. *β-Coefficients (95% CIs) of linear regression models adjusted for age and sex. †P values for β-coefficients are based on Satterthwaite adjusted F test. ‡P values for testing difference in FG levels or HOMA-B across quintiles of weighted genetic risk score are based on Satterthwaite adjusted F statistics. §Adjusted R² for regression models with and without (age and sex only) weighted genetic risk score. ||P = 0.291 and P = 0.637 for testing heterogeneity of weighted genetic risk score on FG levels and HOMA-B, respectively, across racial/ethnic groups are based on Satterthwaite adjusted F statistics.

ally, similar patterns of associations of these 16 SNPs were seen for HOMA-B (online appendix Table 2).

Combined effects of genetic variants on FG and HOMA-B

The number of risk alleles at 16 SNPs ranged from 10 to 27 (median 19), 12 to 26 (median 20), and 10 to 26 (median 18) among non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, respectively. For the weighted GRS, the corresponding numbers were 8–27 (median 17), 10–24 (median 18), and 9–26 (median 17), respectively. The GRS was normally distributed in all three racial/ethnic groups (Kolmogorov-Smirnov *P* < 0.001) (online appendix Fig. 1). FG increased significantly across the quintile of GRS for all racial/ethnic groups. Each additional risk allele in the score was associated with a 0.022, 0.036, and 0.033 mmol/l increase in FG among non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, respectively (Table 3). There was no evidence of a different effect of the GRS on FG levels or HOMA-B across race/ethnicity (*P* = 0.291 and 0.637 for testing heterogeneity of the GRS on FG levels and HOMA-B across race/ethnicity, respectively). In a model including age and sex, the GRS explained more of the total variation in FG in Mexican Americans and in non-Hispanic blacks than in non-Hispanic whites (Table 3). Generally, similar patterns of associations of these 16 SNPs were seen for HOMA-B (Table 3).

Combined effects of genetic variants on IFG

The prevalence of IFG, adjusted for age and sex, was lowest among non-Hispanic blacks and highest among Mexican Americans. The adjusted odds ratios for IFG were highest among Mexican Americans, comparing people in the highest with those in the lowest quintiles of the GRS for all racial/ethnic groups (Table 4) (online appendix Fig. 2). There was no evidence of a different effect of the GRS on risk for IFG across race/ethnicity (*P* = 0.365 for testing heterogeneity across race/ethnicity). Each additional allele was associated with a significant increased risk of IFG for all racial/ethnic groups.

CONCLUSIONS— Our findings show that risk allele frequencies of the included SNPs discovered in populations of European ancestry differed significantly by racial/ethnic group, consistent with

Table 4—Adjusted prevalence and odds ratio for IFG by quintiles of weighted genetic risk score and race/ethnicity, Third National Health and Nutrition Examination Survey DNA Bank (NHANES III 1991–1994)

Characteristics	GRS as continuous variable	P value*	Quintiles of the weighted genetic risk score					
			Q1	Q2	Q3	Q4	Q5	
Non-Hispanic white†								
Number of cases	289		56	39	76	61	57	
GRS range	8–27		8–15	16	17–18	19–20	≥21	
IFG prevalence adjusted for age and sex	20.4 (16.8–24.0)	0.041	16.7 (10.7–22.7)	20.6 (12.5–28.7)	23.1 (17.4–28.8)	18.9 (11.6–26.3)	25.1 (17.0–33.1)	
Odds ratio adjusted for age and sex	1.07 (1.01–1.14)	0.023	1	1.34 (0.66–2.71)	1.57 (0.97–2.56)	1.18 (0.51–2.76)	1.78 (1.00–3.17)	
Non-Hispanic black†								
Number of cases	145		20	16	30	51	28	
GRS range	10–24		10–16	17	18	19–20	≥21	
IFG prevalence adjusted for age and sex	18.2 (15.4–21.0)	0.119	14.1 (7.3–20.9)	13.0 (7.4–18.6)	14.6 (8.8–20.4)	21.4 (16.1–26.7)	26.7 (19.9–33.6)	
Odds ratio adjusted for age and sex	1.16 (1.04–1.30)	0.008	1	0.90 (0.45–1.81)	1.04 (0.44–2.44)	1.74 (0.80–3.76)	2.40 (1.07–5.37)	
Mexican American†								
Number of cases	227		41	28	39	55	64	
GRS range	9–27		9–15	16	17	18–19	≥20	
IFG prevalence adjusted for age and sex	24.6 (20.1–29.1)	0.206	18.4 (11.7–25.1)	23.6 (17.6–29.7)	27.5 (18.9–36.1)	23.8 (12.5–35.1)	33.0 (26.4–39.7)	
Odds ratio adjusted for age and sex	1.10 (1.04–1.16)	0.001	1	1.42 (0.73–2.76)	1.78 (0.86–3.68)	1.44 (0.83–2.47)	2.39 (1.37–4.14)	

Data and 95% CI are in parentheses. *For prevalence of IFG, P values for trend test across quintiles of weighted genetic risk score; for adjusted odds ratio, P values for using weighted genetic risk score as a continuous variable in logistic regression models are based on Satterthwaite adjusted F test. †P = 0.365 for testing heterogeneity of weighted genetic risk score as a continuous variable on risk for IFG across racial/ethnic groups is based on Satterthwaite adjusted F statistic.

the findings of other studies and HapMap estimates (11,12). However, despite significant variations in allele frequencies, the patterns of influence of these SNPs on FG levels, HOMA-B, and IFG were generally consistent across racial/ethnic groups. A GRS derived by the combination of these 16 SNPs was weakly, yet significantly, associated with an increase in FG levels, a decrease in HOMA-B, and an increase in risk for IFG in all racial/ethnic groups. Our findings suggest that the genetic variants at these glycemic loci, discovered in the white population of European ancestry, also contribute to the elevated FG and reduced HOMA-B among non-Hispanic blacks and Mexican Americans.

The 16 FG-associated SNPs are located in or near genes involved in multiple biological pathways (10). Some of these SNPs were also associated with type 2 diabetes (19–21), and a few have been replicated in non-European populations (22,23). Our study includes the most updated SNPs associated with FG and estimates their frequencies and effects in a nationally representative sample of the U.S. population. Our results suggest that with an adequate sample size (not necessarily as large as for a GWAS), these FG-associated SNPs would likely be replicated among non-Hispanic blacks and Mexican Americans.

Although FG levels seem tightly regulated within a narrow range by a feedback mechanism that targets a particular FG set point for each person (24), FG levels vary substantially among nondiabetic populations, and an estimated 25–40% of the variation may be explained by genetic factors (7). Many studies have suggested that elevated FG levels are associated with multiple health conditions, including risk for type 2 diabetes and cardiovascular diseases (1,2,4,5). These diseases represent a major burden of disease in many populations (25). Identification of populations at high risk of developing type 2 diabetes has great public health importance. Our findings suggest that a GRS, on the basis of these FG-associated SNPs, is significantly associated with IFG, but it is unclear if these SNPs can improve predictions for risks of type 2 diabetes or cardiovascular disease in the general population. Further studies are also needed to examine the possible associations of these SNPs with type 2 diabetes and cardiovascular diseases in different races and ethnicities.

However, the combined effects of

these SNPs explain only a small percentage of the total variations in FG levels and HOMA-B (<3%), suggesting that additional loci exist. These loci may be common genetic variants with small effects, rare variants with larger effects, or variants that strongly interact with each other or with environmental factors (in which the number and effect size remain unknown). These interactions are undoubtedly important but complicated to model; their exploration is beyond the scope of our study.

The major strengths of our study include the availability of FG and HOMA-B measurements from a nationally representative sample of the U.S. population with multiple racial/ethnic groups and the genotyping of the 16 most updated FG-associated SNPs. There are several limitations of our study. First, these FG-associated SNPs were discovered among populations of European ancestry and may be proxies for the causal variants. It is well known that linkage disequilibrium patterns vary significantly by race/ethnicity (17), and it is unclear if linkage disequilibrium might break down for some of these SNPs in other racial/ethnic groups (online appendix Fig. 3). Additional fine mapping in all three race/ethnic groups is needed. Second, we replicated a limited number of SNPs—even among non-Hispanic whites—that were significantly associated with FG levels or HOMA-B in the NHANES III. As indicated in the power calculation (online appendix), we had limited sample size to detect an effect size of <0.07 mmol/l in FG per allele for each individual SNP. The most likely explanation for the lack of significant association was the limited sample size of fasting individuals from the NHANES III. Since these were GWAS-confirmed, FG-associated SNPs (at least among non-Hispanic whites), we included all SNPs in our analysis. However, using the GRS as a continuous variable, we have adequate power to detect an effect size as low as 0.016 mmol/l per risk allele. When including only the 11 SNPs that showed the expected direction of effects for FG and HOMA-B, the association of the GRS appeared to be stronger, though the patterns remained unchanged (results not shown). At least for non-Hispanic whites, a larger sample size would not likely change the conclusions since the majority of the SNPs' effects are in the expected direction, consistent with studies in European populations (10). Third, we calculated the weighted GRS

for the three racial/ethnic groups on the basis of the published β -coefficients from populations of European ancestry because of a lack of estimates for other racial/ethnic groups (10). These β -coefficients might not be appropriate for other populations. However, the consistent effects of the GRS on FG, HOMA-B, and IFG across racial/ethnic groups suggest that the weighting was not totally inappropriate in these populations. In addition, the pattern of association between weighted and unweighted GRS on FG, HOMA-B, and IFG were consistent (online appendix Tables 4 and 5).

In summary, our results suggest that the allele frequencies of FG-associated SNPs varied significantly by race/ethnicity. However, the patterns of combined effects of these SNPs on FG levels and HOMA-B were consistent across the different racial/ethnic groups. A GRS that was based on 16 SNPs was significantly associated with risk of IFG among all racial/ethnic groups, which may make it useful for the identification of people who are at high risk for developing diabetes.

Acknowledgments—This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) R01 DK078616 (to J.B.M.), NIDDK K24 DK080140 (to J.B.M.), a NIDDK Research Career Award K23 DK65978 (to J.C.F.), a Massachusetts General Hospital Physician Scientist Development Award, and a Doris Duke Charitable Foundation Clinical Scientist Development Award (to J.C.F.).

J.B.M. currently has research grants from GlaxoSmithKline and serves on a consultancy board for Interleukin Genetics. No other potential conflicts of interest relevant to this article were reported.

Q.Y., T.L., P.S., and J.B.M. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Q.Y., J.B.M., and M.J.K. contributed to the study concept and design. J.B.M. contributed to the acquisition of data. Q.Y., T.L., P.S., J.B.M., J.D., and J.C.F. contributed to the analysis and interpretation of the data. Q.Y., T.L., P.S., A.Y., M.-H.C., N.F.D., R.M.N., J.D., J.C.F., M.J.K., and J.B.M. contributed to the critical revision of the manuscript for important intellectual content. Q.Y., T.L., P.S., A.Y., and J.D. contributed to statistical expertise. J.B.M., N.F.D., and M.J.K. contributed to administrative, technical, and material support. J.B.M. and Q.Y. contributed to study supervision.

Parts of this study were presented in abstract form at the 70th Scientific Sessions of

the American Diabetes Association, Orlando, Florida, 25–29 June 2010.

The data are from the NHANES III genetic datasets (<http://www.cdc.gov/nchs/nhanes/genetics/genetic.htm>).

We thank Sekar Kathiresan, MD, for assistance in obtaining the NHANES III DNA that we used for genotyping.

References

- Levitan EB, Song YQ, Ford ES, Liu SM. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies *Arch Intern Med* 2004;164:2147–2155
- Meigs JB, Nathan DM, D'Agostino RB Sr, Wilson PW. Fasting and postchallenge glycemia and cardiovascular disease risk: the Framingham Offspring Study. *Diabetes Care* 2002;25:1845–1850
- Tirosh A, Shai I, Tekes-Manova D, Israeli E, Pereg D, Shochat T, Kochba I, Rudich A. Normal fasting plasma glucose levels and type 2 diabetes in young men. *N Engl J Med* 2005;353:1454–1462
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;32(Suppl. 1):S62–S67
- de Vegt F, Dekker JM, Jager A, Hienkens E, Kostense PJ, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. Relation of impaired fasting and postload glucose with incident type 2 diabetes in a Dutch population: the Hoorn Study. *JAMA* 2001;285:2109–2113
- Black SA. Diabetes, diversity, and disparity: what do we do with the evidence? *Am J Public Health* 2002;92:543–548
- 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* 2008;118:2620–2628
- 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, Crispioni L, de Geus EJ, Dehghan A, Delou

- kas 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, Orru 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
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* 2008;320:1085–1088
 10. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Magi 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, Sparso T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proenca 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, Roccascaccia 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, Bottcher 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, Gyllenstein U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanal 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, Jorgensen T, Julia 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, Martinez-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, Orru M, Palyz 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, Sigurdsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvanen AC, Tanaka T, Thorand B, Tichet J, Tonjes 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, Borecki IB, Loos RJ, Meneeton 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-Rios 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, 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, Ruukonen 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
 11. Hinds DA, Stuve LL, Nilsen GB, Halperin E, Eskin E, Ballinger DG, Frazer KA, Cox DR. Whole-genome patterns of common DNA variation in three human populations. *Science* 2005;307:1072–1079
 12. Adeyemo A, Rotimi C. Genetic variants associated with complex human diseases show wide variation across multiple populations. *Public Health Genomics* 2009;13:72–79
 13. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. *Nat Genet* 2004;36:1312–1318
 14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419
 15. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice N, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science* 2002;296:2225–2229
 16. Cornelis MC, Qi L, Zhang C, Kraft P, Manson J, Cai T, Hunter DJ, Hu FB. Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. *Ann Intern Med* 2009;150:541–550
 17. McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JPA, Hirschhorn JN. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet* 2008;9:356–369
 18. Lohr SL. *Sampling: Design and Analysis*. Pacific Grove, CA, Duxbury Press, 1999
 19. Florez JC. Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: where are the insulin resistance genes? *Diabetologia* 2008;51:1100–1110
 20. Simonis-Bik AM, Nijpels G, van Haeften TW, Houwing-Duistermaat JJ, Boomsma DI, Reiling E, van Hove EC, Diamant M, Kramer MH, Heine RJ, Maassen JA, Slagboom PE, Willemsen G, Dekker JM, Eekhoff EM, de Geus EJ, t Hart LM. Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic β -cell function. *Diabetes* 2010;59:293–301
 21. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, Sparso T, Holmkvist J, Marchand M, Delplanque J, Lobbens S, Rocheleau G, Durand E, De Graeve F, Chevre JC, Borch-Johnsen K, Hartikainen AL, Ruukonen A, Tichet J, Marre M, Weill J, Heude B, Tauber M, Lemaire K, Schuit F, Elliott P, Jorgensen T, Charpentier G, Hadjadj S, Cauchi S, Vaxillaire M, Sladek R, Visvikis-Siest S, Balkau B, Levy-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
22. Chambers JC, Zhang WH, Zabaneh D, Sehmi J, Jain P, McCarthy MI, Froguel P, Ruokonen A, Balding D, Jarvelin MR, Scott J, Elliott P, Kooner JS. Common genetic variation near melatonin receptor MTNR1B contributes to raised plasma glucose and increased risk of type 2 diabetes among Indian Asians and European Caucasians. *Diabetes* 2009;58:2703–2708
23. Takeuchi F, Katsuya T, Chakrewarthy S, Yamamoto K, Fujioka A, Serizawa M, Fujisawa T, Nakashima E, Ohnaka K, Ikegami H, Sugiyama T, Nabika T, Kasturiratne A, Yamaguchi S, Kono S, Takayanagi R, Yamori Y, Kobayashi S, Ogihara T, de Silva A, Wickremasinghe R, Kato N. Common variants at the GCK, GCKR, G6PC2-ABC11 and MTNR1B loci are associated with fasting glucose in two Asian populations *Diabetologia* 2010;53:299–308
24. Mason CC, Hanson RL, Knowler WC. Progression to type 2 diabetes characterized by moderate then rapid glucose increases. *Diabetes* 2007;56:2054–2061
25. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001;414:782–787