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Obesity and associated lifestyles modify the effect of glucose metabolism-related genetic variants on impaired glucose homeostasis among postmenopausal women

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ABSTRACT

Purpose: Impaired glucose metabolism-related genetic variants likely interact with obesity-modifiable factors in response to glucose intolerance, yet their interconnected pathways have not been fully characterized.

Methods: With data from 1,027 postmenopausal participants of the Genomics and Randomized Trials Network study and 15 single-nucleotide polymorphisms (SNPs) associated with glucose homeostasis, we assessed whether obesity, physical activity, and high dietary fat intake interact with the SNP–glucose variations. We used regression analysis plus stratification and graphic approaches.

Results: Across carriers of the 15 SNPs, fasting levels of glucose, insulin, and homeostatic model assessment-insulin resistance (HOMA-IR) were higher in obese, inactive, and high fat-diet women than in their respective counterparts. Carriers within subgroups differently demonstrated the direction and/or magnitude of the variants' effect on glucose-relevant traits. Variants in GCKR, GCK, DGKB/TMEM195 (P for interactions = 0.02, 0.02, and 0.01), especially, showed interactions with obesity: obese, inactive, and high fat-diet women had greater increases in fasting glucose, insulin, and HOMA-IR levels. Obese carriers at TCF7L2 variant had greater increases in fasting glucose levels than nonobese carriers (P for interaction = 0.04), whereas active women had greater decreases in insulin and HOMA-IR levels than inactive women (P for interaction = 0.02 in both levels).

Conclusions: Our data support the important role of obesity in modifying glucose homeostasis in response to glucose metabolism–relevant variants. These findings may inform research on the role of glucose homeostasis in the etiology of chronic disease and the development of intervention strategies to reduce risk in postmenopausal women.

KEYWORDS
glucose metabolism–related genetic variant, high-fat diet, obesity, physical activity, postmenopausal women

1 | INTRODUCTION

Glucose intolerance is thought to be central in the development of chronic diseases such as type 2 diabetes mellitus (T2DM), metabolic syndrome, and cardiovascular disease (CVD) (Ainsworth, Haskell, & Whitt, 2000; Arcidiacono, Iiritano, & Nocera, 2012; Belkina & Denis, 2010; McCarthy, 2010). In particular, hyperinsulinemia and insulin resistance (IR) are key determinants of many obesity-relevant cancers, including reproductive (e.g. breast, endometrial, prostatic, ovarian) and nonreproductive (e.g. colorectal, kidney, esophageal, gastric, pancreatic, liver) cancers (Boesgaard et al., 2010; Bookman, Din-Lovinescu, & Worrall, 2013; Clayton et al., 2011). Besides its importance in glucose homeostasis, insulin is an essential hormone in anabolic processes in early cell growth and development, directly through the insulin receptor and indirectly through the insulin-like growth factor receptor (IGFR), and via their main downstream signaling pathways (Boesgaard et al., 2010; Clayton et al., 2011). Insulin receptors that are mainly found

in adipose tissues, muscle, and liver cells are overexpressed in tumor cells, resulting in the enhanced anabolic state necessary for tumor growth and development, via downstream insulin signaling (Arcidiacono et al., 2012; Booth, Roberts, & Laye, 2012). In particular, activated insulin receptors and IGFRs upregulate insulin response substrate-1, a crucial mediator leading to oncogenic potential, enhancing growth factor-dependent proliferation and affecting cell metabolism (Arcidiacono et al., 2012; Boesgaard et al., 2010). Thus, impaired glucose metabolism, such as IR, leading to hyperglycemia and hyperinsulinemia, contributes to abnormal multiple cellular signaling cascades, overexpression of these receptors, and hyperactive signaling pathways, and therefore may be associated with carcinogenesis.

Impaired glucose metabolism is a heterogeneous disorder reflecting both genetic and lifestyle factors that jointly influence susceptibility (Arcidiacono et al., 2012; McCarthy, 2010). Conditions related to glucose intolerance are energy imbalance and obesity (particularly visceral adiposity) (Ainsworth et al., 2000; Arcidiacono et al., 2012; Belkina & Denis, 2010; Boesgaard et al., 2010; Clayton et al., 2011). The high-fat Western diet and physical inactivity contribute to energy imbalance and are thus important in glucose homeostasis (Ainsworth et al., 2000; Boesgaard et al., 2010; Buettner, Scholmerich, & Bollheimer, 2007). By using fasting levels of glucose, insulin, and homeostatic model assessment (HOMA), the Meta-Analyses of Glucose and Insulin-Related Traits Consortium conducted a metaanalysis of genome-wide association (GWA) studies that revealed 17 loci associated with fasting glucose homeostasis (Boesgaard et al., 2010; Chan, Huang, & Meng, 2014; Clayton, Banerjee, Murray, & Renehan, 2011; Dupuis et al., 2010). Previous studies evaluating genetic polymorphism variations in glucose metabolism–relevant genes have not yet provided clear links to the traits related to glucose homeostasis. The effects of these variants are modest and do not account for all the genetic variance in glucose intolerance.

These genetic variants can interact with correlated lifestyle factors such as obesity, physical activity, and diet; e.g. the risk of such variants for glucose intolerance may be reduced by desirable lifestyle modifications. Studying gene–lifestyle interactions is undoubtedly important, but research incorporating molecular, individual, social, and environmental determinants is limited (Dupuis, Langenberg, & Prokopenko, 2010). Moreover, the few population-based or trial data have yielded replicated results suggesting limitation in the studies, such as small sample sizes, racial differences, and adjustment for limited sets of physiologic and behavioral factors. Interaction studies that include more comprehensive covariates can reduce potential confounding effect. In addition, interaction studies are complicated to model for testing hypothesis-driven questions. For example, most studies evaluating effects of gene–lifestyle interaction on relevant traits have depended on only *P*-values of effect size for the interaction, but a more comprehensive analytic approach, such as a stratification and graphic evaluation, is needed to reveal apparent differences between subgroups in direction and magnitude of the effect size (Jung, 2014).

We conducted this study among postmenopausal women, a population highly susceptible to obesity and increased morbidity and mortality, to understand genetic variation in glucose tolerance–related traits. Using fasting levels of glucose, insulin, and HOMA-IR, we evaluated whether the same variants influence glucose variations in subgroups stratified by effect modifiers (i.e., obesity, measured by body mass index [BMI], waist circumference, and waist/hip ratio [w/h]; physical activity; and high-fat diet). We hypothesized that the extent to which genetic risk variants influence glucose intolerance is greater among participants who are obese, less physically active, or have higher dietary fat intake than among their respective counterparts. Identifying such gene–lifestyle interactions will provide insights into the role of glucose intolerance in the development of chronic disease including obesity-related cancer, and suggest strategies to reduce the risk in postmenopausal women.

2 | MATERIALS AND METHODS

2.1 | Study population

The study included postmenopausal women who were enrolled in the Genomics and Randomized Trials Network (GARNET) study, a substudy of the Women's Health Initiative (WHI), which aims to identify genetic variants associated with response to treatments for conditions of clinical or public health significance (Hale, Lopez-Yunez, & Chen, 2012; Haskell, Lee, & Pate, 2007). Details of that study's rationale and design have been described elsewhere (Hong, Chung, & Cho, 2014; Howe, Subbaramaiah, Hudis & Dannenberg, 2013; The Women's Health Initiative Study Group, 1998). WHI study participants were recruited from 40 clinical centers nationwide between 1993 and 1998; eligible women were 50–79 years old, postmenopausal (i.e. having had a hysterectomy or no menstrual bleeding for the previous 6 months [if age $\geq$ 56] or 12 months [if age 50–55]), expected to live near the clinical centers for at least three years after enrollment, and able to provide written consent. WHI-GARNET participants were enrolled in the WHI Hormone Therapy (HT) trial, met eligibility requirements for submission to the database of Genotypes and Phenotypes, and provided DNA samples. Of 4,949 participants who were invited to the WHI-GARNET study, 4,894 underwent genotyping as part of WHI-GARNET, a GWA study (Haskell et al., 2007; Hong et al., 2014; Howe et al., 2013; Ingelsson, Langenberg, & Hivert, 2010). For this study, we included women with fasting glucose and/or insulin concentrations available at baseline (i.e. at screening or first annual visit [AV1]) and

excluded women whose blood was collected after fewer than 8 hours' fasting and those with unreliable glucose concentrations (i.e. < 4 mg/dl), resulting in 1,241 participants. Additionally, we excluded 214 without information on covariates, which left a final total of 1,027 women (83% of the 1,241). We obtained approval from the institutional review board of the University of California, Los Angeles.

2.2 | Data collection

Standardized written protocols and periodic quality assurance (QA) visits from the coordinating center were used to assure uniform data collection. As additional data QA, when reporting or data-entry errors were detected, they were corrected or treated as missing data; corrections for discrepancies between answers to main-sub questions or among relevant variables were also made according to data QA procedures. Participants completed self-administered questionnaires, providing demographic, and socioeconomic information, medical, and reproductive histories, and lifestyle behaviors. Trained staff obtained anthropometric measurements, including height, weight, and waist and hip circumferences. We used the data from the screening visit (or AV1). Of 46 variables initially selected from a literature review for their association with obesity and glucose intolerance, 26 were finally selected for this study after multicollinearity test and univariate and/or stepwise regression analyses.

Demographic and socioeconomic characteristics included age, race, education, and marital status; medical histories included cancer, CVD, DM, hypertension, high cholesterol (requiring pills ever), and family history of DM. Reproductive histories included pregnancy history, history of hysterectomy, or oophorectomy, ages at menarche and menopause, oral contraceptive use, and exogenous estrogen use (both opposed and unopposed). Lifestyle variables included dietary intake, physical activity, and depressive symptom. Dietary intake was evaluated with the Food Frequency Questionnaire; after stepwise analysis, we included total calories, dietary total sugars, and percentages of calories from protein, monounsaturated fatty acids (MFA), and polyunsaturated fatty acids (PFA). Physical activity was measured via metabolic equivalent task (MET); recreational physical activity, combining walking, and mild, moderate, and strenuous physical activity, was assigned a MET value corresponding to intensity. The total MET·hours·week^{-1} was calculated by multiplying the MET level for the activity by the hours exercised per week and summing the values for all activities (Haskell et al., 2007; Kirchhoff, Machicao, & Haupt, 2008; Lam, Chang, Rogers, Khoury, & Schully, 2015). Total physical activity values stratified participants into two groups, with 10 METs as the cutoff, according to current American College of Sports Medicine and American Heart Association recommendations (Haskell et al., 2007).

2.3 | Genotyping and outcome variable

Genotyping for WHI-GARNET participants was performed with the HumanOmni1-Quad single–nucleotide polymorphism (SNP) platform (Illumina, Inc., San Diego, CA). Genotyping QA was ensured using a standardized protocol at the University of Washington GARNET data coordinating center (Haskell et al., 2007; Hong et al., 2014). SNPs were included on the basis of missing call rate (<2%), number of discordant calls (<1%), and Hardy–Weinberg Equilibrium ($P \geq$ 1e-4). Fifteen SNP candidates available for this study were selected by their association ($P <$ 5e-8) with fasting glucose and/or insulin concentrations in the previous metaanalysis with independent replication (Chan et al., 2014; Dupuis et al., 2010; Manning, Hivert, & Scott, 2012).

Outcome variables were fasting levels of glucose, insulin, and HOMA-IR. Glucose was analyzed using the hexokinase method on a Hitachi 747 (Boehringer Mannheim Diagnostics, Indianapolis, IN). Most insulin testing was by radioimmunoassay (RIA) method (Linco Research, Inc., St. Louis, MO); later testing used an automated ES300 method. The two methods yielded comparable results for insulin levels. Coefficients of variation for glucose and insulin were 1.28% and 10.93%, respectively. HOMA-IR, as a surrogate of IR, was estimated as glucose (unit: mg/dl) × insulin (unit: μIU/ml)/405 (Matthews et al., 1985).

2.4 | Statistical analysis

Differences in participants' characteristics by effect modifiers (BMI, waist circumference, w/h, physical activity, and high-fat diet) were assessed using unpaired 2-sample t tests for continuous variables and chi-square tests for categorical variables. If continuous variables were skewed or had outliers, Wilcoxon's rank-sum test was implemented. Multicollinearity was evaluated by using coefficient of multiple determination, tolerance, and variance-inflation factor for each exposure variable, using remaining covariates as its predictors; no significant multicollinearity was present.

Multiple linear regression with an additive model was performed to produce effect sizes and 95% confidence intervals (CIs) of the exposure (glucose metabolism–relevant SNP, treating the genotypes as ordinal or categorical variables) to predict the outcomes of interest (fasting glucose, insulin, and HOMA-IR levels). For the regression assumptions that were met, while glucose levels were analyzed without transformation, insulin, and HOMA-IR levels were natural log–transformed.

To determine whether obesity and associated lifestyles modified the gene–glucose variations, besides performing a formal test using the regression model (including an interaction term such as gene*lifestyle), we stratified participants by the effect modifier and compared between-subgroup effect sizes of the exposure (SNP) on the outcomes of interest.

Additionally, we evaluated the interactions graphically by plotting the means of glucose traits for each genotype of the SNP according to the strata defined by the effect modifier. We used R (v 2.15.1) software and then reanalyzed all data using PLINK (v1.9); all results were comparable. A 2-tailed P value < 0.05 was considered significant. Since all our analyses were essentially exploratory, all P-values we reported were unadjusted for multiple testing.

3 | RESULTS

Participants' characteristics stratified by BMI (<30.0 and ≥30.0) are summarized in Table 1. Obese women were more likely to be younger ($P = 0.01$), black ($P < 0.05$), not currently married ($P = 0.04$), and to have more relatives with DM ($P < 0.05$) and more comorbid conditions such as CVD ($P < 0.05$), DM ($P < 0.05$), and hypertension ($P < 0.05$). In addition to being more prone to having undergone hysterectomy or oophorectomy ($P = 0.01$) and earlier menarche ($P < 0.05$) and menopausal transition ($P = 0.01$), obese women were more likely to be inactive ($P < 0.05$) and to consume more total calories ($P < 0.05$), with higher percentages of calories from MFA ($P < 0.05$) and PFA ($P < 0.05$). Consequently, obese women tended to have greater waist circumferences ($P < 0.05$) and w/h ($P < 0.05$). Further, obese women were more likely to have higher fasting glucose ($P < 0.05$), insulin ($P < 0.05$), and HOMA-IR levels ($P < 0.05$). Using a cutoff value relevant to glucose intolerance (Buettner et al., 2007), we also stratified participants by waist circumference, w/h, physical activity, and high-fat diet, and compared their characteristics (supplementary Table S1.1–4).

Fifteen SNPs in previous GWA studies associated with fasting levels of glucose, insulin, and HOMA-IR were selected to evaluate their associations with glucose-homeostasis traits. The allele frequencies of these SNPs in our population were consistent with the frequencies in a European population (McTiernan, Kooperberg, & White, 2003). No significant differences occurred in allele frequency between strata (obesity, physical activity, and high-fat diet) (Tables 2, S2.1–4).

Glycemic loci associated with fasting levels of glucose, insulin, and HOMA-IR, stratified by obesity status (BMI < 30 vs. ≥ 30; waist ≤ 88 vs. > 88; w/h ≤ 0.85 vs. > 0.85) and obesity-relevant variables (physical activity [MET] ≥10 vs. <10; calories from fat <40 vs. ≥40%).

The fasting glucose, insulin, and HOMA-IR levels of obese, inactive, and high fat–diet women were higher than those of nonobese, active, and low fat–diet women, regardless of genotypes, indicating the glucose-relevant traits proportionally associated with obesity and the relevant variables. In addition, within each locus, the effect of variants on glycemic traits differed by obesity status and obesity-relevant variables, implying that interactions between genetic variants and obesity affect glucose homeostasis. We then sought to characterize these relationships.

Most SNPs demonstrated differences in direction and/or magnitude of the associations with glucose-relevant traits within subgroups (Table 3, supplementary Table S3.1–4). Carriers of GCKR rs780094 G allele overall showed increased glycemic traits in both nonobese and obese women, stratified by obesity status variables (i.e. BMI, waist circumference, and w/h). Among these variables, abdominal adiposity (w/h >0.85; P for interaction = 0.02) was related to a greater allele-dependent increase in fasting glucose levels than was overall obesity (BMI ≥30) (Fig. 1A–C). These carriers in the higher fat–diet group (≥ 40%) also displayed greater allele-dependent increases in fasting levels of glucose ($P = 0.03$), insulin ($P = 0.04$), and HOMA-IR ($P = 0.02$) than in the lower fat–diet group (supplementary Table S3.4, Fig. 1D). Carriers of GCK rs4607517 A allele in obese or inactive women had greater allele-dependent increases in fasting glucose levels than those in nonobese or active women (P for interaction = 0.02 in w/h; $P = 0.01$ in physical activity) (supplementary Fig. S1C, D). Similarly, obese carriers of TCF7L2 rs4506565 T allele showed a greater allele-dependent increase in fasting glucose levels than nonobese carriers (P for interaction = 0.04 in w/h) (supplementary Fig. S2A3).

In contrast, carriers of C2CD4B rs11071657 A allele in nonobese women had greater allele-dependent decreases in fasting insulin and HOMA-IR levels than in obese women (in waist circumference, P for interaction = 0.01 for insulin and <0.05 for HOMA-IR) (supplementary Fig. S3A2, B2). Likewise, these carriers were associated with greater allele-dependent decreases in fasting insulin and HOMA-IR levels in the low fat–diet group than in the high fat–diet group (supplementary Figs. S3A4, S3B4).

In addition, for the association with glycemic traits, allele A carriers of rs2191349 in DGKB/TMEM195 in women with BMI < 30 showed allele-dependent decreased levels, whereas the counterpart women had allele-dependent increases in fasting glucose (P for interaction = 0.01), insulin, and HOMA-IR levels. A similar pattern was shown for carriers of PROX1 rs340874 G allele: in nonobese (BMI <30), they were related to allele-dependent decreases in fasting glucose levels, but in obese women (BMI ≥30) they were associated with allele-dependent increased levels, although neither result reached statistical significance. Likewise, women with BMI ≥30 carrying G allele of rs11558471 in SLC30A8 were related to allele-dependent increases in glucose-related traits, differently from those with BMI <30 group ($P = 0.04$ for insulin and HOMA-IR levels) (Table 3).

A few SNP carriers in the physical-activity and fat-intake subgroups had different patterns of interactions with glycemic traits, than they had in the obesity-status subgroups. For fasting glucose levels, carriers of rs4506565 TCF7L2 T allele in physical-activity subgroups displayed patterns similar to

TABLE 1 Characteristics of participants, stratified by obesity (measured via BMI), in the Genomics and Randomized Trials Network Study of the Women's Health Initiative

	Nonobese group (BMI < 30.0) (n = 633)		Obese group (BMI ≥ 30.0) (n = 394)	
Characteristics	***n***	**(%)**	***n***	**(%)**
Age in years, median (range)	65	(50–79)	63	(50–79)[a]
Race				
White (not of Hispanic origin)	535	(84.5)	318	(80.7)[a]
Black	24	(3.8)	41	(10.4)
Other	74	(11.7)	35	(8.9)
Education				
≤ High school	254	(40.1)	179	(45.4)
> High school	379	(59.9)	215	(54.6)
Current marital status				
Not married	237	(37.4)	174	(44.2)[a]
Married	396	(62.6)	220	(55.8)
Family history of diabetes mellitus				
No	427	(67.5)	229	(58.1)[a]
Yes	206	(32.5)	165	(41.9)
Cancer ever				
No	613	(96.8)	386	(98.0)
Yes	20	(3.2)	8	(2.0)
Cardiovascular disease ever				
No	566	(89.4)	327	(83.0)[a]
Yes	67	(10.6)	67	(17.0)
Diabetes ever[b]				
No	627	(99.1)	377	(95.7)[a]
Yes	6	(0.9)	17	(4.3)
Hypertension ever				
No	465	(73.5)	237	(60.2)[a]
Yes	168	(26.5)	157	(39.8)
High cholesterol requiring pills ever				
No	563	(88.9)	354	(89.8)
Yes	70	(11.1)	40	(10.2)
Oral contraceptive use				
Never	388	(61.3)	231	(58.6)
Ever	245	(38.7)	163	(41.4)
Exogenous estrogen use				
No	400	(63.2)	248	(62.9)
Yes	233	(36.8)	146	(37.1)
Pregnancy history				
No	53	(8.4)	30	(7.6)
Yes	580	(91.6)	364	(92.4)
History of hysterectomy or oophorectomy				
No	359	(56.7)	190	(48.2)[a]
Yes	274	(43.3)	204	(51.8)
Age at menarche in years, median (range)	13	(≤ 9–≥ 17)	12	(≤ 9–≥ 17)[a]
Age at menopause in years, median (range)	49	(28–60)	48	(21–60)[a]

(*Continued*)

TABLE 1 *(Continued)*

Characteristics	Nonobese group (BMI < 30.0) (n = 633)		Obese group (BMI ≥ 30.0) (n = 394)	
	n	(%)	*n*	(%)
METs·hour·week^{-1}				
<10	436	(68.9)	322	(81.7)[a]
≥10	197	(31.1)	72	(18.3)
Depressive symptom[c]				
< 0.06	587	(92.7)	359	(91.1)
≥ 0.06	46	(7.3)	35	(8.9)
Waist circumference in cm, median (range)	81.0	(64.0–106.0)	101.0	(69.6–125.0)[a]
Waist/hip ratio, median (range)	0.795	(0.574–1.128)	0.844	(0.621–1.238)[a]
Total calories in kcal, median (range)	1560.0	(605.9–4784.0)	1655.0	(603.5–4992.0)[a]
% calories from protein, median (range)	16.4	(5.2–33.0)	17.0	(9.2–32.0)
% calories from MFA, median (range)	12.7	(2.9–27.6)	13.7	(6.1–24.7)[a]
% calories from PFA, median (range)	6.4	(1.9–14.8)	6.9	(2.5–15.9)[a]
Dietary total sugars in g, median (range)	91.3	(21.5–525.9)	95.3	(16.9–264.0)
Glucose in mg/dl, median (range)	93.0	(67.0–362.0)	98.0	(71.0–316.0)[a]
Insulin in μIU/ml, median (range)[d]	8.3	(3.3–43.9)	13.2	(3.6–74.6)[a]
HOMA-IR, median (range)	1.9	(0.7–24.0)	3.3	(0.8–35.7)[a]

BMI, body mass index; HOMA-IR, homeostatic model assessment–insulin resistance; MET, metabolic equivalent; MFA, monounsaturated fatty acids; PFA, polyunsaturated fatty acids.

[a]$P < 0.05$, chi-square test or Wilcoxon's rank-sum test.

[b]A participant was considered to have diabetes if a doctor had ever said that she had diabetes when she was not pregnant.

[c]Depression scales were estimated by using a short form of the Center for Epidemiologic Studies Depression Scale and categorized with 0.06 as the cutoff to detect depressive disorders.

[d]Insulin levels were measured via either radioimmunoassay or automated ES300 method; the two methods gave comparable results at the insulin levels WHI participants were likely to have. When a participant had both results, the average levels were used in the analysis.

those in other obesity-status subgroups, but the allelic variant affected fasting insulin and HOMA-IR levels differently, with allele-dependent decreases in both at a greater magnitude in the active than in the inactive women (P for interaction = 0.02 in both levels) (supplementary Fig. S2B, C). Further, although carriers of rs35767 IGF1 A allele did not have a significant association with glucose-related traits in the obesity-status subgroups, the variant resulted in a greater allele-dependent decrease in fasting HOMA-IR levels in inactive women than in active women ($P = 0.04$; supplementary Table S3.3); additionally, women with high dietary fat intake had higher fasting glucose levels than women in the lower fat–diet group (P for interaction = 0.01; supplementary Table S3.4).

Cancer patients ($n = 28$) had no significant association with SNP-glucose variations. A sensitivity test using data including DM history ($n = 23$) compared with data excluding that characteristic revealed no apparent differences in both univariate and multivariate analyses.

4 | DISCUSSION

In this cross-sectional study of a large cohort of postmenopausal women, by using 15 glucose metabolism–related SNPs previously reported their association with glycemic metabolic traits (Chan et al., 2014; Dupuis et al., 2010; Manning et al., 2012), we investigated whether the genetic variants influence glucose homeostasis by interacting with obesity status or other relevant lifestyle factors. Limited previous studies have incorporated gene and lifestyle factors, and studying gene–environment interactions is very complex; the analytic results require careful interpretation (i.e., not focusing only on P values) and need to be ascertained with varied testing approaches (Jung, 2014). We used traditional but crucial methods for testing interactions such as stratification and graphic evaluations and found that most of the glycemic loci we studied interacted with obesity status, physical activity, and dietary fat intake.

Most carriers of the genetic variants among obese, inactive, or high dietary fat–intake groups had greater allele-dependent increases in glucose-intolerance traits, compared with the respective counterpart groups. Our results are consistent with those of other studies (Chan et al., 2014; Clayton et al., 2011; Dupuis et al., 2010; Manning et al., 2012; Nettleton, Hivert, & Lemaitre, 2013; Walford et al., 2012) in that carriers of common variants in GCKR and GLIS3 are associated with glucose intolerance. Moreover, these carriers show greater allele-dependent increases in fasting

TABLE 2 Allele frequencies of 15 glucose metabolism–relevant SNPs, stratified by obesity (measured via BMI) among 1,027 participants in the Genomics and Randomized Trials Network Study of the Women's Health Initiative

				Effect allele frequency	
				Nonobese group (BMI < 30.0)	Obese group (BMI ≥ 30.0)
SNP	Chromosome	Nearest gene	Risk allele (effect/other)	(n = 633)	(n = 394)
rs340874	1	*PROX1*	G/A	52.2	49.1
rs780094	2	*GCKR*	G/A	60.8	63.6
rs560887	2	*G6PC2*	G/A	76.1	74.9
rs11920090	3	*SLC2A2*	A/T	14.7	13.7
rs2191349	7	*DGKB/TMEM195*	A/C	53.7	53.8
rs4607517	7	*GCK*	A/G	16.4	16.6
rs11558471	8	*SLC30A8*	G/A	31.4	29.2
rs7034200	9	*GLIS3*	A/C	52.0	51.9
rs10885122	10	*ADRA2A*	C/A	84.4	83.9
rs4506565	10	*TCF7L2*	T/A	31.1	31.5
rs11605924	11	*CRY2*	A/C	49.5	52.3
rs174550	11	*FADS1*	G/A	32.8	33.0
rs10830963	11	*MTNR1B*	G/C	28.1	24.4
rs11071657	15	*C2CD4B*	A/G	63.2	65.9
rs35767	12	*IGF1*	A/G	15.7	19.9

BMI, body mass index; SNP, single-nucleotide polymorphism.

glucose levels among obese and high fat–diet women than among their counterparts. In particular, carriers of the TCF7L2 variant demonstrated an inverse relationship with insulin and HOMA-IR levels, but this relationship interacted with physical activity; i.e., active women had greater allele-dependent decreases in both fasting insulin and HOMA-IR levels than inactive women.

Interestingly, two SNPs carriers in DGKB/TMEM195 and SLC30A8 had a positive predisposition to glucose intolerance only among obese women, in contrast with their negative association among nonobese women. This suggests a robust gene–obesity interaction with glucose metabolism. Most previous investigators have reported only a positive relationship of these variants with glucose-relevant traits, without accounting for the effect modification of the obesity status (Boesgaard et al., 2010; Chan et al., 2014; Dupuis et al., 2010; Walford et al., 2012).

Although heterogeneity of interaction effects between obesity subgroups was observed for G6PC2 and FADS1, our findings overall are consistent with those of previous meta-analysis and epidemiologic studies, and their gene functions have been established. A TCF7L2 variant that has been related to T2DM (Nettleton, McKeown, & Kanoni, 2010; Palmer, McDonough, & Hicks, 2012; Saxena, Elbers, & Guo, 2012; Scott, Chu, & Grarup, 2012; Zeggini et al., 2008) indicates involvement in beta-cell malfunction; thus, in this and other studies (Dupuis et al., 2010; Kirchhoff et al., 2008; Nettleton et al., 2013; Walford et al., 2012), the common variant in this gene is inversely related to insulin levels with increased glucose levels. Moreover, we found a more profound effect of this variant among obese or inactive women, implying a gene–obesity interaction that may contribute to the heterogeneity of T2DM etiology. In addition, GCKR and IGF1 variants were related to IR; both are highly expressed in the liver, contributing to hepatic IR (Chan et al., 2014). GCKR encodes a glucokinase regulatory protein, which inhibits glucokinase, a key protein in glucose metabolism, leading to increased hepatic glucose production (Chan et al., 2014; Walford, Green, & Neale, 2012). This supports the biological plausibility of our findings, indicating that this variant is related to increased glucose and insulin levels. Further, our finding that these carriers, among women with visceral obesity or high-fat diet, are associated with greater allele-dependent increases in glucose and insulin levels suggests adiposity's strong role in modulating the GCKR variant's effect on glucose homeostasis. IGF1 encodes insulin-like growth factor 1 and the null effect of this gene is abnormal glucose homeostasis, but the role of the IGF1 variant in insulin sensitivity and glucose tolerance is not well understood (Chan et al., 2014; Dupuis et al., 2010). Our data for this variant revealed heterogeneous results (increased fasting glucose but decreased HOMA-IR levels), warranting further study of this variant's function and potential interaction with lifestyle factors.

Glucose intolerance, representing impaired beta-cell function and/or deregulation of the insulin signaling pathway,

TABLE 3 Results from linear regression for 15 glucose metabolism–relevant SNPs predicting glucose, insulin, and HOMA-IR levels, stratified by obesity (measured by BMI) among 1,027 participants in the Genomics and Randomized Trials Network Study of the Women's Health Initiative

	Nonobese group (BMI < 30.0) (n = 633)						Obese group (BMI ≥ 30.0) (n = 394)					
	Glucose		Insulin[b]		HOMA-IR[b]		Glucose		Insulin[b]		HOMA-IR[b]	
SNP name	Effect size[c] (95% CI)		Effect size[c] (95% CI)		Effect size[c] (95% CI)		Effect size[c] (95% CI)		Effect size[c] (95% CI)		Effect size[c] (95% CI)	
rs340874	−1.05	(−2.98–0.89)	−0.02	(−0.07–0.02)	−0.03	(−0.08–0.02)	2.60	(−0.35–5.55)	0.04	(−0.20–0.10)	0.06	(−0.01 - 0.13)
rs780094	0.87	(−1.01–2.76)	0.03	(−0.02–0.08)	0.04	(−0.02–0.09)	0.66	(−2.61–3.94)	0.04	(−0.02 - 0.11)	0.05	(−0.03 - 0.13)
rs560887	**3.71**	**(1.49–5.93)** [a]	−0.01	(−0.07–0.04)	0.02	(−0.04–0.08)	1.12	(−2.59–4.83)	0.06	(−0.02–0.13)	0.07	(−0.02 - 0.16)
rs11920090	0.08	(−2.52–2.68)	−0.03	(−0.09–0.03)	−0.04	(−0.11–0.03)	−1.67	(−6.18–2.84)	−0.02	(−0.11–0.07)	−0.03	(−0.14 - 0.08)
rs2191349	−0.34	(−2.25–1.58)	**−0.05**	**(−0.09–−0.001)**	−0.05	(−0.10–0.002)	0.88	(−2.35–4.10)	0.01	(−0.06–0.07)	0.01	(−0.07 - 0.09)
rs4607517	0.38	(−2.32–3.09)	−0.02	(−0.08–0.05)	−0.01	(−0.09–0.06)	3.50	(−0.81–7.80)	−0.02	(−0.11–0.06)	−0.001	(−0.10 - 0.10)
rs11558471	**−2.50**	**(−4.55–−0.45)**	−0.03	(−0.08–0.02)	−0.05	(−0.10–0.01)	1.58	(−2.00–5.15)	**0.08**	**(0.01–0.15)**	**0.09**	**(0.01 - 0.17)**
rs7034200	0.99	(−0.94–2.92)	0.01	(−0.03–0.06)	0.02	(−0.03–0.08)	2.06	(−1.11–5.22)	−0.002	(−0.07–0.06)	0.02	(−0.06 - 0.09)
rs10885122	0.24	(−2.34–2.83)	0.0001	(−0.06–0.06)	0.003	(−0.07–0.07)	2.84	(−1.39–7.07)	0.01	(−0.07–0.10)	0.04	(−0.06 - 0.14)
rs4506565	0.52	(−1.49–2.53)	−0.04	(−0.09–0.01)	−0.03	(−0.09–0.02)	**3.28**	**(0.03–6.52)**	0.02	(−0.05–0.08)	0.04	(−0.04 - 0.11)
rs11605924	0.52	(−1.46–2.51)	−0.03	(−0.08–0.02)	−0.03	(−0.08–0.02)	2.14	(−0.89–5.18)	0.02	(−0.04–0.08)	0.03	(−0.04 - 0.11)
rs174550	1.95	(−0.11–4.00)	0.03	(−0.02–0.08)	0.05	(−0.01–0.10)	2.09	(−1.08–5.27)	0.06	(0.00006–0.13)	**0.08**	**(0.005 - 0.16)**
rs10830963	1.44	(−0.72–3.60)	−0.03	(−0.09–0.02)	−0.02	(−0.08–0.04)	1.57	(−2.03–5.17)	0.02	(−0.05–0.10)	0.04	(−0.05 - 0.13)
rs11071657	−0.99	(−3.01–1.03)	**−0.07**	**(−0.12–−0.02)**	**−0.08**	**(−0.13–−0.02)**	−0.52	(−3.79–2.75)	−0.01	(−0.07–0.06)	−0.01	(−0.09 - 0.07)
rs35767	−0.41	(−3.00–2.17)	−0.03	(−0.09–0.04)	−0.03	(−0.10–0.04)	0.46	(−3.49–4.41)	−0.05	(−0.13–0.03)	−0.05	(−0.15 - 0.04)

BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model assessment–insulin resistance; SNP, single-nucleotide polymorphism

Numbers in **bold** face are statistically significant ($P < 0.05$).

[a]Bonferroni correction for multiple testing was applied and statistically significant effect-size is indicated.

[b]Number of participants in analysis with insulin or HOMA-IR levels as an outcome of interest was 1,012 (621, nonobese group; 391, obese group).

[c]Effect sizes were estimated from an additive model of linear regression (i.e. regressed against effect allele) adjusting for age, race, education, marital status, family history of diabetes, cancer ever, cardiovascular disease ever, diabetes ever, hypertension ever, high cholesterol requiring pills ever, oral contraceptive use, exogenous estrogen use, pregnancy history, history of hysterectomy or oophorectomy, age at menarche, age at menopause, physical activity, depression, waist/hip ratio, and selected diet-related variables (total calories, % calories from protein, % calories from monounsaturated fatty acids, % calories from polyunsaturated fatty acids, and dietary total sugars).

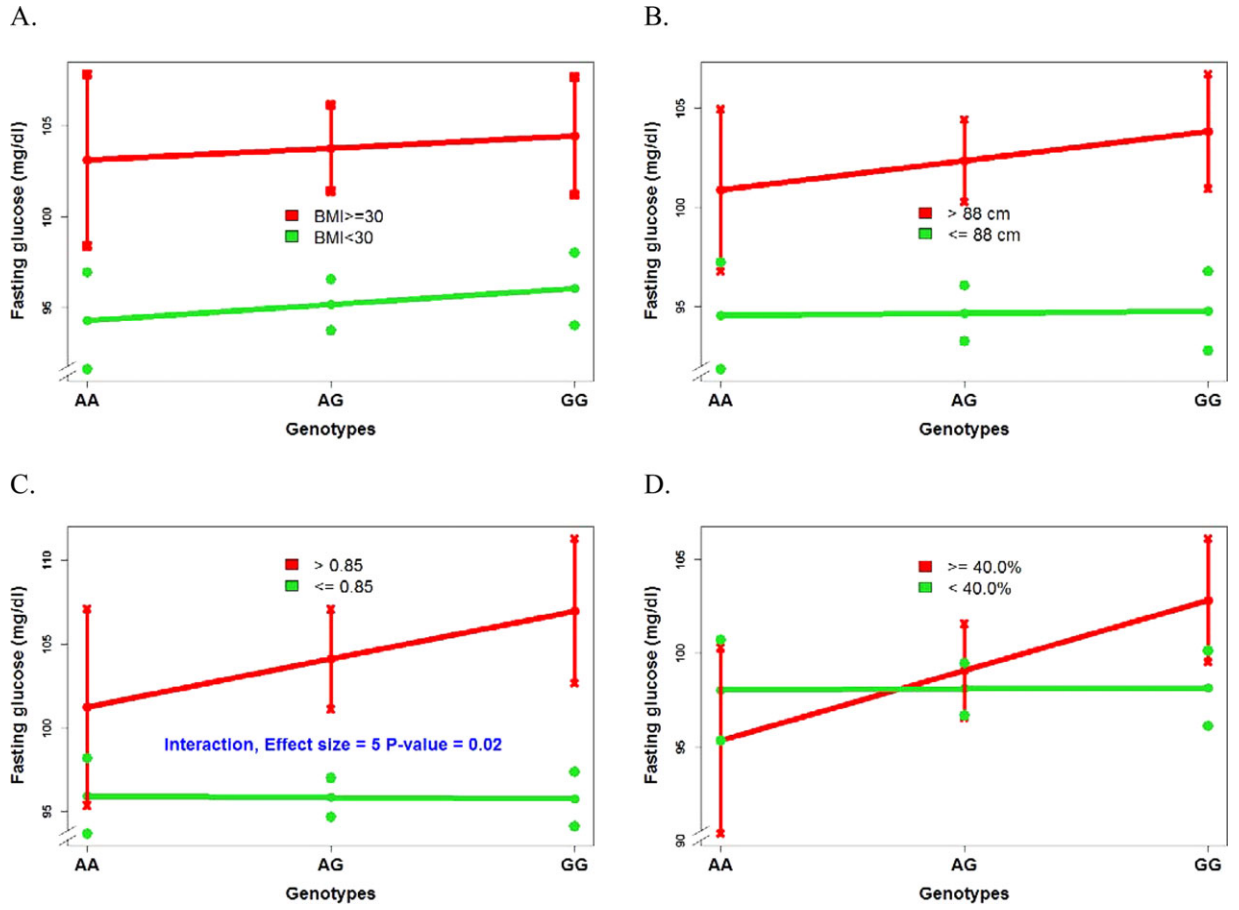

FIGURE 1 Graphs present distributions of adjusted mean levels of fasting glucose by genotypes of rs780094 in GCKR in 1,027 participants in the Genomics and Randomized Trials Network Study of the Women's Health Initiative, stratified by ***A*** body mass index, ***B*** waist circumference, ***C*** waist/hip ratio, and ***D*** high fat diet intake

is key to the development of chronic diseases such as T2DM, metabolic syndrome, and CVD (Ainsworth et al., 2000; Arcidiacono et al., 2012; Belkina & Denis, 2010; McCarthy, 2010). Particularly, defects in the insulin-secretion system and abnormal glucose homeostasis leading to IR are crucial determinants of many obesity-relevant cancer types (Boesgaard et al., 2010; Bookman et al., 2013; Clayton et al., 2011), suggesting that the adiposity-related carcinogenetic pathways intermingle with the glucose-intolerance system (Boesgaard et al., 2010; Clayton et al., 2011; Zeggini, Scott, & Saxena, 2008). Obesity potentiates the effect of glucose metabolism–relevant genetic variants on glucose homeostatic traits (Arcidiacono et al., 2012). Our results reflect the joint effect of genetic predisposition and relevant lifestyle modifiers, including obesity status, physical activity, and high dietary–fat intake. This could be due to tissue-specific responses to an obesogenic environment by producing hormones and proinflammatory cytokines that are involved in glucose metabolic–signaling cascades and may interact with genetic variants determining glucose homeostasis (Ainsworth et al., 2000; Arcidiacono et al., 2012; Belkina & Denis, 2010); but further experimental confirmation for the related mechanisms is required. Moreover, in addition to beta-cell function–related variants, further studies incorporating variants associated with the insulin signaling pathway may explain these complicated mechanisms more comprehensively.

We chose not to consider a multiple-comparison adjustment in the data analysis for testing our hypothesis-driven questions. On the basis of prior findings of 15 loci significantly associated with glucose metabolism, we hypothesized their interactions with lifestyle modifiers and explored their modulation with genetic variants that influence glucose homeostasis. We acknowledge that with many analyses, we might have a few false positive results, and the results should be interpreted with care, especially when *P*-values are close to the assumed level of significance. We evaluated glucose traits at one time point, which prevented us from assessing changes over time in those circulating levels. Additionally, our study was conducted among postmenopausal women, which limits the generalizability to other populations. However, our sample was drawn from a well-characterized cohort with varied covariates, which could reduce potential confounding. Finally, all of our data underwent rigid data QA processes, which may lessen information bias, including misclassification.

In conclusion, our findings support the important role of obesity in modifying glucose homeostasis in response to glucose metabolism–relevant genetic variants. Our study may provide a better understanding of the identification of gene–lifestyle interactions and will inform research on the role of glucose homeostasis in the etiology of chronic disease including obesity-related cancer and development of intervention strategies to reduce disease risk in postmenopausal women.

CONFLICT OF INTERESTS

There are no financial disclosures and conflicts of interest.

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