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Authors

Jung, Su Yon
Mancuso, Nick
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et al.

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Research Article

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Genome-Wide Meta-analysis of Gene–Environmental Interaction for Insulin Resistance Phenotypes and Breast Cancer Risk in Postmenopausal Women

Su Yon Jung[1], Nick Mancuso[2], Herbert Yu[3], Jeanette Papp[4], Eric Sobel[4], and Zuo-Feng Zhang[5]

Abstract

Insulin resistance (IR)–related genetic variants are possibly associated with breast cancer, and the gene–phenotype–cancer association could be modified by lifestyle factors including obesity, physical inactivity, and high-fat diet. Using data from postmenopausal women, a population highly susceptible to obesity, IR, and increased risk of breast cancer, we implemented a genome-wide association study (GWAS) in two steps: (1) GWAS meta-analysis of gene–environmental (i.e., behavioral) interaction (G*E) for IR phenotypes (hyperglycemia, hyperinsulinemia, and homeostatic model assessment–insulin resistance) and (2) after the G*E GWAS meta-analysis, the identified SNPs were tested for their associations with breast cancer risk in overall or subgroup population, where the SNPs were identified at genome-wide significance. We found 58 loci (55 novel SNPs; 5 index SNPs and 6 SNPs, independent of each other) that are associated with IR phenotypes in women overall or women stratified by obesity, physical activity, and high-fat diet; among those 58 loci, 29 (26 new loci; 2 index SNPs and 2 SNPs, independently) were associated with postmenopausal breast cancer. Our study suggests that a number of newly identified SNPs may have their effects on glucose intolerance by interplaying with obesity and other lifestyle factors, and a substantial proportion of these SNPs' susceptibility can also interact with the lifestyle factors to ultimately influence breast cancer risk. These findings may contribute to improved prediction accuracy for cancer and suggest potential intervention strategies for those women carrying genetic risk that will reduce their breast cancer risk.

Introduction

Approximately 80% of new breast cancer cases and 90% of the cancer deaths occur in women ages 50 years and older (1, 2). Impaired glucose metabolism [i.e., insulin resistance (IR)]–related phenotypes, such as high blood level of homeostatic model assessment–insulin resistance (HOMA-IR), hyperglycemia, and compensatory hyperinsulinemia, have strong associations with breast cancer risk in postmenopausal women (3–5). In particular, high insulin levels have been associated with a 2-fold increase in postmenopausal breast cancer risk (4, 5), and HOMA-IR, reflecting high blood levels of insulin and glucose, is positively associated with breast cancer in postmenopausal women (3). Further, behavioral factors, including obesity, physical inactivity, and high-fat diet, may interact with the IR-related phenotypes, influencing breast cancer susceptibility (6–9).

Considering the relationships between IR phenotypes and breast cancer risk, IR-related genetic variants are possibly associated with increased risk of breast cancer. Further, previous reports have revealed obesity–IR-related gene signature–breast cancer pathways (10, 11) in *in vitro* studies and showed that IR-relevant SNPs have greater increases in IR traits among obese, inactive, and high-fat diet groups (12), implicating that obesity interacts with the associations between IR-genetic variants and IR phenotypes and jointly influences cancer susceptibility. Thus, the IR genotype–phenotype–cancer association could be

[1]Translational Sciences Section, Jonsson Comprehensive Cancer Center, School of Nursing, University of California, Los Angeles, Los Angeles, California. [2]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California. [3]Cancer Epidemiology Program, University of Hawaii Cancer Center, Honolulu, Hawaii. [4]Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California. [5]Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California.

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Corresponding Author: Su Yon Jung, University of California, Los Angeles, 700 Tiverton Ave, 3-264 Factor Building, Los Angeles, CA 90095. Phone: 310-825-2840; Fax: 310-267-0413; E-mail: sjung@sonnet.ucla.edu

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Table 1. Distributions of IR phenotypes in 6 genome-wide association studies (total $n = 11,794$)

Study	*n*	Phenotype		
		Fasting glucose	Fasting insulin	HOMA-IR
Circulating plasma level				
		mg/dL, median (range)	μIU/mL, median (range)	Median (range)
AS264	1,857	94.6 (72.0–140.0)	7.7 (1.6–37.6)	1.80 (0.34–10.02)
GARNET	2,201	94.0 (62.0–327.0)	7.7 (1.0–57.0)	1.83 (0.20–19.26)
GECCO-CYTO	1,353	94.1 (65.0–133.0)	7.1 (0.9–30.5)	1.64 (0.18–7.57)
GECCO-INIT	225	92.4 (73.0–191.0)	6.2 (0.9–45.6)	1.39 (0.18–21.51)
HIPFX	2,290	93.6 (68.0–257.0)	6.7 (1.3–47.9)	1.54 (0.30–12.67)
WHIMS	3,868	93.0 (39.0–296.0)	6.0 (0.3–104.1)	1.39 (0.07–24.68)
Binary analysis				
		<100 mg/dL/≥100 mg/dL[a] *n* (%)	≤8.6 μIU/mL/>8.6 μIU/mL[a] *n* (%)	<3.0/≥3.0[a] *n* (%)
AS264	1,857	1,693 (91.2)/164 (8.8)	1,141 (61.4)/716 (38.6)	1,708 (92.0)/149 (8.0)
GARNET	2,201	1,552 (70.5)/649 (29.5)	1,259 (57.2)/942 (42.8)	1,701 (77.3)/500 (22.7)
GECCO-CYTO	1,353	1,226 (90.6)/127 (9.4)	914 (67.6)/439 (32.4)	1,245 (92.0)/108 (8.0)
GECCO-INIT	225	204 (90.7)/21 (9.3)	163 (72.4)/62 (27.6)	215 (95.6)/10 (4.4)
HIPFX	2,290	2,108 (92.1)/182 (7.9)	1,704 (74.4)/586 (25.6)	2,177 (95.1)/113 (4.9)
WHIMS	3,868	2,960 (76.5)/908 (23.5)	2,795 (72.3)/1,073 (27.7)	3,405 (88.0)/463 (12.0)

NOTE: The HOMA-IR was estimated as glucose (mg/dL) × insulin (μIU/mL)/405 (21).
[a]Each of 3 phenotypes (glucose, insulin, and HOMA-IR) was categorized by using the corresponding cutoff values (100 mg/dL, 8.6 μIU/mL, and 3.0, respectively); blood levels higher than the threshold were considered to indicate glucose intolerance and/or IR status (21, 24–26).

modified by lifestyle factors including obesity, physical inactivity, and high-fat diet (Supplementary Fig. S1).

To address these hypotheses, we implemented a two-step approach: (1) a genome-wide association study (GWAS) meta-analysis of gene–environmental (i.e., behavioral) interaction (G*E) for IR phenotypes (hyperglycemia, hyperinsulinemia, and HOMA-IR) was conducted by incorporating obesity and other lifestyle factors and (2) after the G*E GWAS meta-analysis, the identified SNPs related to IR phenotypes were tested for their associations with breast cancer risk in overall or subgroup populations, where the SNPs were identified at genome-wide significance.

In the first step (GWAS meta-analysis of G*E interaction), we tested whether obesity and other lifestyle factors modify the association between genetic variants and IR phenotypes. More than 83 loci for one or more glycemic traits have been identified by GWA studies (13), and together they explain about 20% of the heritability of the traits being studied to a modest degree (14). The pathways between glycemic genetic factors and traits can be influenced by environmental/behavioral factors. Thus, incorporating key lifestyle factors in a gene–trait study may explain the remaining heritability. In addition, the functions of many of those identified genes are not yet known. Examining the interaction effect of lifestyle factors on the association between IR-related genetic variants and phenotypes may help elucidate those genes' role in impaired glucose homeostasis. Further, inclusion of such key lifestyle factors may reveal novel genetic susceptibility loci.

In the second step, we evaluated whether the SNPs that interact with lifestyle factors and thus were detected for their association with IR phenotypes in a particular behavioral setting (e.g., obesity/physical inactivity/high-fat diet) are associated with breast cancer risk in the identical behavioral setting. This may elucidate an empirical pathway where a significant proportion of the susceptibility of genes identified by GWAS interact with the lifestyle factors to influence the cancer risk (Supplementary Fig. S1). It can also help predict cancer risk more accurately and further the development of lifestyle interventions to improve prevention and treatment.

We conducted this study among postmenopausal women, a population highly susceptible to obesity, IR, and increased risk of breast cancer. We found 58 loci (including 55 novel ones) that were associated with IR phenotypes in women overall or women stratified by obesity, physical activity, and high-fat diet; among those 58 loci, 29 (including 26 novel ones) were associated with postmenopausal breast cancer risk.

Materials and Methods

Study population

The study included postmenopausal women enrolled in the Women's Health Initiative (WHI) Harmonized and Imputed GWASs, which contribute a joint imputation and harmonization effort for GWASs within the WHI Clinical Trials and Observational Studies. Detailed rationale and design of the studies have been described elsewhere (15, 16). WHI study participants were recruited from 40 clinical centers nationwide from October 1, 1993, to December 31, 1998; eligible women were 50 to 79 years old, postmenopausal, expected to live near the clinical centers for at least 3 years after enrollment, and able to provide written consent. The Harmonization and Imputation Studies involved 6 GWASs (MOPMAP[AS264]; GARNET; GECCO-CYTO; GECCO-INIT; HIPFX; and WHIMS; Table 1). Using those 6 GWASs, we initially included 16,088 women who reported their race or

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ethnicity as non-Hispanic white (Supplementary Fig. S2). In step 1, we excluded 2,714 who had diabetes mellitus at and/or after enrollment. In addition, we excluded 1,271 whose genetic information were duplicated and/or related to others in the dataset. Through genetic data quality cleaning (QC) process, we excluded additional 309 outliers based on principal components (PC), leaving 11,794 women (97% of the eligible 12,103) for the G*E GWAS meta-analysis of IR phenotypes. In step 2 (study of the association between identified SNPs in step 1 and breast cancer), we excluded 685 women who had been followed up for less than 1 year and/or had been diagnosed with any cancer at enrollment, resulting in a total of 11,109 women (94% of the eligible 11,794; 589 of them had developed breast cancer). The women had been followed up through August 29, 2014 (median, 16 years of follow-up). We obtained approval from the Institutional Review Boards of each participating clinical center of the WHI and the University of California, Los Angeles.

Data collection and cancer outcomes

The WHI coordinating center collected data using standardized written protocols with periodic visits for data quality assurance. At enrollment, participants completed self-administered questionnaires on demographic (age, education, family income, and family history of breast cancer) and lifestyle [depressive symptoms, smoking, physical activity, and diet (dietary alcohol in g/day and percentage of calories from saturated fatty acids (SFA)/day)] factors and on their reproductive histories [oral contraceptive and exogenous estrogen (E) use (E only or E + progestin (P) users), history of hysterectomy, and ages at menarche and menopause]. Anthropometric measurements such as height, weight, and waist and hip circumferences were measured at baseline by trained staff. The above 17 variables were initially identified from a literature review for their association with IR phenotypes and breast cancer, and after multicollinearity testing and univariate and stepwise regression analyses were selected for this study.

Cancer outcomes were breast cancer development and the time to develop breast cancer. The time between enrollment and cancer development, censoring, or study end-point was estimated as the number of days and then converted into years. The breast cancer outcomes were determined via a centralized review of medical charts. Cancer cases were coded according to the NCI's Surveillance, Epidemiology, and End-Results guidelines (17).

Genotyping and laboratory methods

The genotyped data were collected from the WHI Harmonized and Imputed 6 GWASs. These studies normalized the genotype calls to the reference panel GRCh37 and performed genotype imputation using 1,000 genomes reference panels (16). SNPs for harmonization were checked for pairwise concordance among all samples in the 6 GWASs. We compared the self-reported ethnicity with PCs; if any discrepancy or admixed participant was found, the secondary analysis was performed using follow-up demographic questionnaires (18). We included SNPs having a missing-call rate of <3% and a Hardy–Weinberg Equilibrium of $P \geq 10^{-4}$. In the secondary QC process, we selected SNPs with $\hat{R}^2 \geq 0.6$ imputation quality (19). We computed relatedness between samples using high-quality SNPs, including only HapMap3 SNPs with $\hat{R}^2 \geq 0.9$ (20). To minimize possible confounding due to shared environment, we excluded individuals with a kinship estimate > 0.25. We then computed 10 PCs using the same set of high-quality SNPs and excluded any outlier samples.

Fasting blood samples were collected from each participant at enrollment by trained phlebotomists. Serum concentrations of glucose and insulin were measured by the hexokinase method on a Hitachi 747 instrument (Boehringer Mannheim Diagnostics) and by a radioimmunoassay method (Linco Research, Inc.), respectively, with average coefficients of variation of 1.28% and 10.93%, respectively. HOMA-IR was estimated as glucose (unit: mg/dL) × insulin (unit: μIU/mL)/405 (21). About 30% of phenotypes were replaced by imputed values using an unsupervised splitting of Random Survival Forest imputation (https://github.com/ehrlinger/randomForestSRC/blob/master/R/impute.rfsrc.R; ref. 22). Sensitivity test before and after imputation for each IR phenotype was performed in overall GWAS and in the G*E GWAS meta-analysis, producing estimates, *Q*–*Q* plot, and Manhattan plot; no apparently significant difference was observed.

Statistical analysis

Differences in baseline characteristics and allele frequencies by breast cancer were examined via unpaired two-sample *t* tests for continuous variables and χ^2 tests for categorical variables. If continuous variables were skewed or had outliers, Wilcoxon's rank-sum test was used. In step 1, GWA analysis was conducted via multiple logistic regression, adjusting for age and 10 PCs, to estimate ORs and 95% confidence intervals (CI) of IR phenotypes (as a binary quantitative variable; Table 1) with genotypes in additive, minor-allele–dominant and –recessive models. The combined findings based on the 6 GWASs were obtained from a meta-analysis assuming a fixed-effect model; heterogeneity among studies was tested using Cochran's Q statistics (23). For gene–environment interaction, two strategies were used: (1) G*E interaction term was included and tested in the GWA multiple regression and (2) GWAS analysis was performed in strata defined by body mass index (BMI), metabolic equivalents (MET)·hours/week, and % calories from SFA, with cut-off values of 30 kg/m^2, 10 MET, and 7%, respectively. Next, the results (either G*E or stratified GWAS analysis) from the 6 GWASs were combined in a meta-analysis assuming a fixed-effect

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Table 2. Characteristics of participants, stratified by breast cancer

Characteristic	Controls ($n = 10,520$) *n* (%)	Breast cancer cases ($n = 589$) *n* (%)
Age in years, median (range)	67 (50–81)	67 (50–79)
Education		
≤High school	3,761 (35.8)	179 (30.4)[a]
>High school	6,759 (64.2)	410 (69.6)
Annual family income		
<$35,000	4,674 (45.4)	217 (37.5)[a]
≥$35,000	5,630 (54.6)	361 (62.5)
Family history of breast cancer		
No	8,534 (81.1)	454 (77.1)[a]
Yes	1,986 (18.9)	135 (22.9)
METs·hour·week^{-1}[b]	7.50 (0–134.17)	7.00 (0–81.67)
METs·hour·week^{-1}[b]		
≥10.0	4,415 (42.0)	243 (41.3)
<10.0	6,105 (58.0)	346 (58.7)
Number of cigarettes smoked per day		
≤15	5,960 (56.7)	278 (47.2)[a]
>15	4,560 (43.3)	311 (52.8)
Depressive symptom[c], median (range)	0.002 (0–0.937)	0.002 (0.001–0.880)
Dietary alcohol per day in g, median (range)	1.06 (0–183.76)	1.88 (0–127.15)[a]
% calories from SFA, median (range)	11.29 (2.22–32.39)	11.49 (3.73–21.50)
% calories from SFA[d]		
<7.0%	960 (9.1)	50 (8.5)
≥7.0%	9,560 (90.9)	539 (91.5)
BMI in kg/m^2, median (range)	26.85 (15.42–58.49)	28.00 (17.55–49.31)[a]
BMI[e]		
<30.0 kg/m^2	7,505 (71.3)	357 (60.6)[a]
≥30.0 kg/m^2	3,015 (28.7)	232 (39.4)
Waist-to-hip ratio, median (range)	0.81 (0.44–1.39)	0.81 (0.64–1.26)
Waist-to-hip ratio		
≤0.85	7,514 (71.4)	398 (67.6)[a]
>0.85	3,006 (28.6)	191 (32.4)
Age at menarche in years, median (range)	13 (≤9–≥17)	12 (≤9–≥17)[a]
Hysterectomy ever		
No	6,739 (64.1)	414 (70.3)[a]
Yes	3,781 (35.9)	175 (29.7)
Age at menopause in years, median (range)	50 (20–60)	50 (21–63)
Oral contraceptive duration in years, median (range)	5.7 (0.1–47.0)	5.2 (0.1–21.0)[a]
Exogenous estrogen use (E-only use)		
Never	7,360 (70.0)	451 (76.6)[a]
<5 years	1,481 (14.1)	58 (9.8)
5 to < 10 years	546 (5.2)	18 (3.1)
10 + years	1,133 (10.8)	62 (10.5)
Exogenous estrogen use (E + P use)		
Never	8,681 (82.5)	454 (77.1)[a]
<5 years	1,010 (9.6)	73 (12.4)
5 to <10 years	434 (4.1)	30 (5.1)
10 to <15 years	244 (2.3)	21 (3.6)
≥15 years	151 (1.4)	11 (1.9)

[a]$P < 0.05$, χ^2 or Wilcoxon rank-sum test.
[b]Physical activity was estimated from recreational physical activity combining walking and mild, moderate, and strenuous physical activity. Each activity was assigned an 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. The total MET was stratified into 2 groups, with 10 METs as the cutoff according to current American College of Sports Medicine and American Heart Association recommendations (43).
[c]Depression scales were estimated using a short form of the Center for Epidemiologic Studies Depression Scale.
[d]Percentage of calories from SFA was stratified using 7% as the cutoff value according to the American Heart Association/American College of Cardiology dietary guidelines, which are aligned with the 2015–2020 Dietary Guidelines for Americans to help cardiovascular and metabolic disease reductions (44).
[e]BMI was categorized using the cutoff of 30 kg/m^2; BM ≥ 30.0 is considered obese (https://www.cdc.gov/obesity/adult/defining.html).

model. Linkage disequilibrium (LD) between identified SNPs at genome-wide significance was estimated, and the regional plot was created using LOCUSZOOM (http://locuszoom.org/).

In step 2, we conducted the multiple Cox proportional hazards regression in the 6 GWASs combined, with an assumption test via a Schoenfeld residual plot and rho and obtained HRs and 95% CIs for IR-related SNPs predicting breast cancer by adjusting for 17 covariates (Table 2). In step 1, multiple testing was corrected by adjusting *P* values to the genome-wide significance level ($P < 5E{-}08$). We did not consider multiple testing correction in step 2 for testing

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our hypothesis-driven questions (i.e., IR–SNPs in association with breast cancer in consistent environmental setting); a two-tailed P value < 0.05 was considered significant. PLINK2.0, Python2.7, and EIGENSTRAT were used for data cleaning and the QC process; PLINK1.9 (meta-analysis) and 2.0 (glm/interaction), for step 1; and R3.4.3, for phenotype imputation and step 2 (randomForestSRC, qqman, and survival packages).

Results

Distributions of IR phenotypes, including fasting glucose, insulin, and HOMA-IR levels, are presented in Table 1. Categorization of each phenotype was performed using the blood level threshold, where levels higher than the threshold are considered to be glucose intolerance or IR status (21, 24–26). Characteristics of study participants and their allele frequencies, by breast cancer, are shown in Table 2 and Supplementary Table S1. Women with breast cancer were more likely to have a family history of breast cancer, to smoke more cigarettes/day, to consume more dietary alcohol/day, to be obese, and to have shorter periods of oral contraceptive and E- only uses and longer periods of E + P use.

Step 1: G*E GWA meta-analysis

We conducted a meta-analysis of GWAS with 18,717,781 common autosomal SNPs, across 6 GWASs assuming a fixed-effect model, for IR phenotypes (fasting glucose, insulin, and HOMA-IR), adjusted for age and 10 genotyping PCs, in all the women and women stratified by BMI, physical activity, and % calories from SFA, accompanying an interaction test (G*E per allele). We found 58 loci (5 index SNPs and 6 SNPs, independent of each other) at genome-wide significance (P < 5E−08), 55 of which were novel and 3 (SNPs near *G6PC2*) that were previously described (27, 28). Overall, the SNPs did not overlap among the 3 IR phenotypes at genome-wide significance.

For *hyperglycemia* (Supplementary Table S2; Supplementary Fig. S3A–S3C), 5 SNPs in *G6PC2/MKLN1/NKX2-2* were detected: 3 near *G6PC2* with high LD (r^2 > 0.8; rs13431652 as index SNP; Fig. 1A) in the overall and high-fat diet (≥7% calories from SFA) groups; and rs117911989 in an intronic region of *MKLN1* and rs7273292 in an intergenic region of *NKX2-2* in the active (MET ≥ 10) group.

For *hyperinsulinemia* reflecting IR, 39 novel SNPs were found (Supplementary Table S3; Supplementary Fig. S3D–S3G). By interacting with BMI, 4 SNPs in an intergenic region of *NR5A2* reached genome-wide significance; 3 of them (rs10919774 as index SNP; Fig. 1B) were correlated with r^2 > 0.9 in an obese (BMI ≥ 30) group. Further, by interacting with physical activity, 34 SNPs were detected; in an inactive (MET < 10) group, those 34 SNPs, located in an intergenic region of *MTRR/LOC729506*, were correlated (r^2 > 0.7; rs13188458 as index SNP; Fig. 1C). In relation to interaction with a high-fat diet, 1 novel SNP (rs6683451) within 350 kb of *PLA2G4A*, a noncoding RNA in an intronic region of *LINC01036*, had genome-wide significance in the low-fat diet (<7% calories from SFA) group.

For *high level of HOMA-IR*, 14 novel SNPs had a genome-wide significant association (Supplementary Table S4; Supplementary Fig. S3H–S3K). Seven of those SNPs were correlated (r^2 > 0.8; rs77772624 as index SNP; Fig. 1D) in an intergenic region of *PABPC1P2* in the overall and high-fat diet groups. By interacting with SFA consumption, 5 SNPs (r^2 > 0.9; rs13277245 as index SNP; Fig. 1E) in an intergenic region of *MSC* and 1 SNP in an intronic region of *DOCK1* were further identified as having genome-wide associations with HOMA-IR in the low-fat diet group. Heterogeneity tests across the 6 GWASs revealed that none of the 58 SNPs were significant.

Step 2: After G*E GWAS meta-analysis, IR SNPs in association with breast cancer risk

Given the relationships between IR phenotypes and breast cancer risk, interacting with lifestyle factors, we carried forward all 58 loci from step 1 to evaluate their association with breast cancer risk in step 2, by pooling the 6 GWASs in the consistent behavioral settings, where the SNPs were identified at the genome-wide significance level. Of the 58 loci, 29 (2 index SNPs and 2 SNPs, independently) were associated with the risk of breast cancer (Tables 3–6): in the overall analysis, 3 SNPs (including the rs13431652 index SNP) in *G6PCs* (previously confirmed for association with breast cancer risk; ref. 29); in strata by BMI, 1 novel SNP (rs10919774 index SNP) in *NR5A2*; in strata by physical activity, 24 novel SNPs (including rs131885458 index SNP) in *MTRR/LOC729506*; and in strata by SFA consumption, 1 novel SNP in *DOCK1*.

In detail, women carrying 3 SNPs each in *G6PC2*, identified for their genome-wide significant association with hyperglycemia in the overall analysis, had an increased risk of breast cancer (Table 3). Particularly, carriers of rs573225-A and rs560887-C alleles had directional consistency with increased hyperglycemia and also greater risk for breast cancer. However, carriers of rs13431652-C allele, while they had a lower likelihood of hyperglycemia, had a higher risk of breast cancer.

In addition, women in the obese group (BMI ≥ 30) carrying *NR5A2* rs10919774 (index SNP)-A allele had a greater likelihood of hyperinsulinemia (Table 4), but the association with cancer was not significant in this obese group. However, in their counterparts (BMI < 30), those carriers had a substantially lower risk of breast cancer (dominant: HR, 0.29; 95% CI, 0.09–0.91; Table 4). Remarkably, in an inactive group (MET < 10), women carrying 24 *MTRR/LOC729506* SNPs in high LD (r^2 > 0.7;

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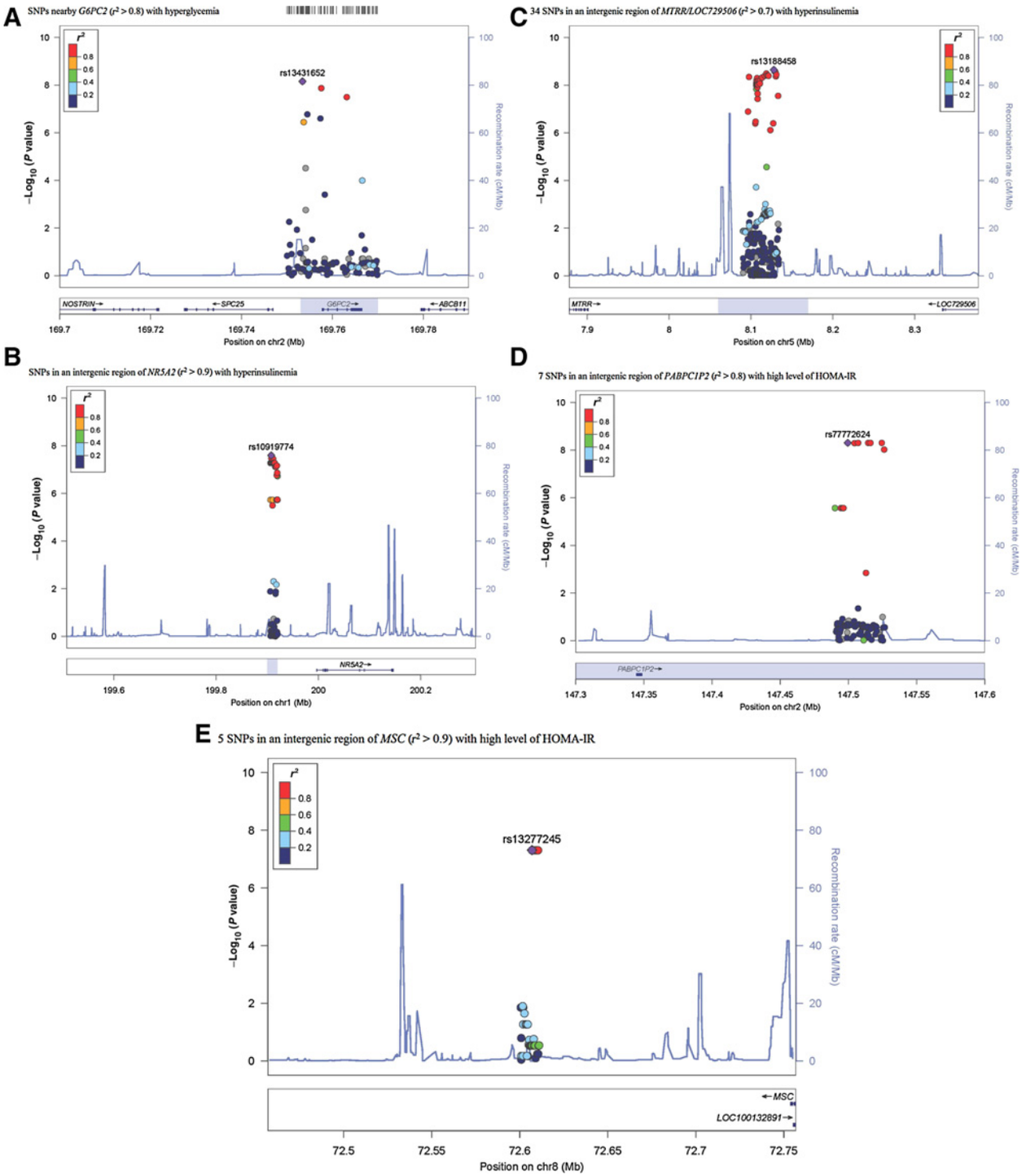

Figure 1.

Regional SNP association plots. (Note: LD (r^2) shown by color intensity gradient). **A,** 3 SNPs nearby *G6PC2* (r^2 > 0.8) with hyperglycemia. **B,** SNPs in an intergenic region of *NR5A2* (r^2 > 0.9) with hyperinsulinemia. **C,** 34 SNPs in an intergenic region of *MTRR/LOC729506* (r^2 > 0.7) with hyperinsulinemia. **D,** 7 SNPs in an intergenic region of *PABPC1P2* (r^2 > 0.8) with high level of HOMA-IR. **E,** 5 SNPs in an intergenic region of *MSC* (r^2 > 0.9) with high level of HOMA-IR.

index SNP: rs13188458) had a greater likelihood of hyperinsulinemia and also a greater risk of breast cancer (Table 5).

It is interesting to note that women who carried *DOCK1* rs11384760-T allele had a 5 times greater chance to develop breast cancer when they consumed a greater percentage

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Table 3. Genome-wide meta-analysis of overall test and/or interaction test with the stratified analysis for the association with hyperglycemia and multiple Cox regression for the genotypes of *G6PC2* rs13431652, rs573225, and rs560887 for predicting breast cancer risk

SNP	Genetic model	Allele[a] (Ref/Alt)	OR[b]	P[b]	Q[b]	HR[c] (95% CI)	P
***G6PC2* rs13431652**	**Allelic**	**T/C**	**0.79**	**6.99E−0.9**	**0.706**	**1.13 (1.00–1.28)**	**0.047**
***G6PC2* rs573225**	**Allelic**	**G/A**	**1.25**	**1.34E−08**	**0.607**	**1.17 (1.03–1.32)**	**0.013**
	Genotypic	**GG**				Referent	
		GA				1.11 (0.94–1.32)	0.22
		AA				**1.41 (1.09–1.84)**	**0.009**
	Recessive	**GG + GA/AA**				**1.34 (1.05–1.71)**	**0.019**
***G6PC2* rs560887**	**Allelic**	**T/C**	**1.25**	**3.17E−08**	**0.612**	**1.19 (1.05–1.34)**	**0.007**
	Genotypic	**TT**				Referent	
		TC				1.12 (0.95–1.34)	0.181
		CC				**1.47 (1.13–1.92)**	**0.005**
	Recessive	**TT + TC/CC**				**1.39 (1.08–1.80)**	**0.011**

NOTE: Only SNPs that are significantly genome-wide associated with hyperglycemia in overall/interaction (G*E or subgroup) analysis and breast cancer were included. Numbers in bold face are statistically significant.

Abbreviations: Alt, alternative allele; *Q*, Cochran's Q; Ref, reference allele.

[a]Additive genetic model regressed in genome-wide meta-analysis.

[b]Results from genome-wide meta-analysis of overall test for the association with hyperglycemia.

[c]HR adjusted by age, education, annual family income, family history of breast cancer, depressive symptom, smoking, physical activity, dietary alcohol in g/day, % calories from SFAs/day, BMI, waist-to-hip ratio, hysterectomy ever, ages at menarche and menopause, oral contraceptive use, exogenous estrogen–only use, and exogenous estrogen plus progestin use.

of calories from SFA (≥7%; Table 6), but those carriers had greater likelihood of IR (Table 6), not in this high-fat diet group, but in the counterpart group (<7% calories from SFA).

Discussion

Population-based epidemiologic studies for gene–environment interactions at the genome-wide level have been focus of a growing number of studies. This study, to our best knowledge, is the first to examine the IR genotype–phenotype–breast cancer association by incorporating lifestyle factors at the genome-wide level. We found a number of novel genome-wide significant SNPs in relation to IR phenotypes by analyzing the interaction with several lifestyle factors; these associations would have been missed without the incorporation of the lifestyle factors. Further, in the consistent environmental settings, we found that many of the IR SNPs were significantly associated with postmenopausal breast cancer risk.

Several SNPs in *G6PC2*, *PLA2G4A*, *PABPC1P2*, *DOCK1*, and *MSC*, in association with IR phenotypes, interacted with higher SFA consumption. Fatty acids are considered signaling molecules; through cellular sensing mechanisms with transcription factors, they activate or inactivate cellular processes and metabolisms (30). A number of SNPs for IR phenotypes are related to the genes encoding transcription factors, so dietary fat intake may influence the expressions or activities of those genes through an allele-specific manner where different SNPs may exert distinct biological effects on the glucose homeostasis–related phenotypes.

In our overall analysis, we replicated other investigators' previous findings (27, 28) of 3 SNPs in *G6PC2* in relation to hyperglycemia. *G6PC2* is in the glucose-6-phosphatase catalytic subunit family, which regulates glucose metabolism and insulin secretion in pancreatic beta cells (27, 31). Particularly, *G6PC2* rs560886 explains around 1% of the total variance in fasting glucose levels (31). We found that

Table 4. Genome-wide meta-analysis of overall test and/or interaction test with the stratified analysis for the association with hyperinsulinemia and multiple Cox regression for the genotypes of *NR5A2* rs10919774 for predicting breast cancer risk, stratified by BMI

			Interaction test for BMI		BMI < 30.0 kg/m² (*n* = 7,862)					BMI ≥ 30.0 kg/m² (*n* = 3,247)				
SNP	Genetic model	Allele[a] (Ref/Alt)	P	Q	OR[b]	P[b]	Q[b]	HR[c] (95% CI)	P	OR[b]	P[b]	Q[b]	HR[c] (95% CI)	P
***NR5A2* rs10919774**	**Genotypic**	**GG**	1.45E−06	0.707	0.93	0.421	0.319	Referent		**1.98**	**2.53E−08**	**0.726**	Referent	
		GA						**0.31 (0.09–0.99)**	**0.049**				0.48 (0.06–3.65)	0.478
		AA						**0.29 (0.09–0.90)**	**0.033**				0.69 (0.10–4.99)	0.716
	Dominant	**GG/GA + AA**						**0.29 (0.09–0.91)**	**0.033**				0.67 (0.09–4.84)	0.695

NOTE: Only SNPs that are significantly genome-wide associated with hyperinsulinemia in overall/interaction (G*E or subgroup) analysis and breast cancer were included. Numbers in bold face are statistically significant.

Abbreviations: Alt, alternative allele; *Q*, Cochran's *Q*; Ref, reference allele.

[a]Additive genetic model regressed in genome-wide meta-analysis.

[b]Results from genome-wide meta-analysis of interaction test for the association with hyperinsulinemia.

[c]HR adjusted by age, education, annual family income, family history of breast cancer, depressive symptom, smoking, physical activity, dietary alcohol in g/day, % calories from SFAs/day, waist-to-hip ratio, hysterectomy ever, ages at menarche and menopause, oral contraceptive use, exogenous estrogen–only use, and exogenous estrogen plus progestin use.

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Table 5. Genome-wide meta-analysis of overall test and/or interaction test with the stratified analysis for the association with hyperinsulinemia and multiple Cox regression for the genotypes of SNPs in *MTRR* and *LOC729506* genes for predicting breast cancer risk, stratified by physical activity

		Interaction test for PA		Active group (MET ≥ 10) (n = 4,658)					Inactive group (MET < 10) (n = 6,451)				
SNP[a]	Allele (Ref/Alt)[b]	*P*	*Q*	OR[c]	*P*[c]	*Q*[c]	HR[d] (95% CI)	*P*	OR[c]	*P*[c]	*Q*[c]	HR[d] (95% CI)	*P*
***MTRR* rs13182814**	**CC + CT/TT**	2.18E−05	0.644	0.95	0.312	0.456	0.93 (0.72–1.19)	0.559	**1.27**	**9.90E−09**	**0.547**	**1.25 (1.00–1.55)**	**0.047**
***MTRR* rs13163063**	**AA + AC/CC**	2.93E−05	0.637	0.95	0.359	0.449	0.93 (0.72–1.19)	0.556	**1.27**	**8.83E−09**	**0.555**	**1.25 (1.00–1.55)**	**0.047**
***MTRR* rs35009176**	**GG + GA/AA**	2.85E−05	0.650	0.95	0.363	0.452	0.93 (0.72–1.20)	0.564	**1.27**	**7.82E−09**	**0.562**	**1.25 (1.01–1.55)**	**0.044**
***MTRR* rs34411024**	**AA + AG/GG**	2.44E−05	0.637	0.95	0.357	0.439	0.93 (0.72–1.19)	0.562	**1.27**	**6.14E−09**	**0.553**	**1.25 (1.01–1.55)**	**0.044**
***MTRR* rs6555516**	**TT + TA/AA**	2.92E−05	0.634	0.95	0.400	0.455	0.92 (0.72–1.19)	0.541	**1.27**	**4.99E−09**	**0.555**	**1.25 (1.00–1.55)**	**0.045**
***MTRR* rs6555517**	**AA + AG/GG**	2.74E−05	0.650	0.95	0.357	0.452	0.93 (0.72–1.19)	0.561	**1.27**	**7.82E−09**	**0.562**	**1.25 (1.01–1.55)**	**0.044**
***MTRR* rs6555518**	**TT + TC/CC**	2.77E−05	0.638	0.95	0.346	0.484	0.93 (0.73–1.20)	0.601	**1.27**	**8.26E−09**	**0.527**	**1.24 (1.00–1.54)**	**0.049**
***MTRR* rs7447098**	**AA + AG/GG**	2.77E−05	0.638	0.95	0.346	0.484	0.93 (0.73–1.20)	0.601	**1.27**	**8.26E−09**	**0.527**	**1.24 (1.00–1.54)**	**0.049**
***MTRR* rs6555519**	**AA + AT/TT**	4.02E−05	0.676	0.95	0.379	0.475	0.93 (0.72–1.20)	0.585	**1.27**	**1.23E−08**	**0.546**	**1.25 (1.01–1.56)**	**0.041**
***MTRR* rs7447152**	**AA + AG/GG**	3.05E−05	0.602	0.95	0.345	0.458	0.93 (0.72–1.19)	0.552	**1.27**	**9.55E−09**	**0.528**	**1.25 (1.00–1.55)**	**0.048**
***MTRR* rs7444691**	**GG + GT/TT**	3.05E−05	0.602	0.95	0.345	0.458	0.93 (0.72–1.19)	0.552	**1.27**	**9.55E−09**	**0.528**	**1.25 (1.00–1.55)**	**0.048**
***MTRR* rs17131**	**CC + CT/TT**	2.34E−05	0.657	0.95	0.343	0.473	0.93 (0.72–1.19)	0.554	**1.27**	**6.64E−09**	**0.565**	**1.25 (1.01–1.55)**	**0.045**
***MTRR* rs13169903**	**AA + AT/TT**	2.94E−05	0.648	0.95	0.344	0.483	0.92 (0.72–1.19)	0.536	**1.27**	**9.47E−09**	**0.521**	**1.24 (1.00–1.54)**	**0.049**
***MTRR* rs1847915**	**CC + CT/TT**	2.60E−05	0.656	0.95	0.364	0.469	0.93 (0.72–1.20)	0.564	**1.27**	**6.32E−09**	**0.554**	**1.25 (1.01–1.55)**	**0.043**
***MTRR* rs6555520**	**CC + CA/AA**	2.69E−05	0.649	0.95	0.364	0.469	0.93 (0.72–1.20)	0.564	**1.27**	**6.96E−09**	**0.549**	**1.25 (1.01–1.55)**	**0.043**
***MTRR* rs6555521**	**CC + CA/AA**	2.42E−05	0.631	0.95	0.323	0.436	0.92 (0.72–1.19)	0.545	**1.27**	**8.58E−09**	**0.512**	**1.24 (1.00–1.54)**	**0.049**
***LOC729506* rs2123640**	**TT + TC/CC**	1.19E−05	0.633	0.94	0.285	0.384	1.00 (0.78–1.29)	0.978	**1.28**	**3.20E−09**	**0.586**	**1.25 (1.00–1.55)**	**0.047**
***LOC729506* rs7716902**	**CC + CA/AA**	1.21E−05	0.618	0.94	0.289	0.365	1.00 (0.78–1.29)	0.997	**1.28**	**3.84E−09**	**0.578**	**1.24 (1.00–1.55)**	**0.049**
***LOC729506* rs13188458**	**GG + GT/TT**	9.81E−06	0.641	0.94	0.281	0.395	0.98 (0.76–1.27)	0.900	**1.29**	**2.26E−09**	**0.490**	**1.25 (1.01–1.56)**	**0.043**
***LOC729506* rs13188952**	**GG + GA/AA**	1.06E−05	0.661	0.94	0.286	0.437	0.98 (0.76–1.26)	0.880	**1.28**	**2.50E−09**	**0.487**	**1.25 (1.01–1.56)**	**0.043**
***LOC729506* rs10512942**	**CC + CA/AA**	9.45E−06	0.675	0.94	0.281	0.452	0.96 (0.74–1.24)	0.750	**1.28**	**2.88E−09**	**0.477**	**1.25 (1.00–1.56)**	**0.045**
***LOC729506* rs34799743**	**CC + CG/GG**	9.02E−06	0.659	0.94	0.257	0.454	0.96 (0.74–1.24)	0.752	**1.28**	**4.24E−09**	**0.463**	**1.25 (1.01–1.56)**	**0.044**
***LOC729506* rs13166872**	**GG + GT/TT**	1.04E−05	0.669	0.94	0.282	0.472	0.96 (0.74–1.24)	0.745	**1.28**	**3.62E−09**	**0.464**	**1.25 (1.00–1.56)**	**0.045**
***LOC729506* rs17198862**	**GG + GC/CC**	3.43E−05	0.672	0.95	0.324	0.408	1.00 (0.78–1.29)	0.983	**1.26**	**2.79E−08**	**0.572**	**1.28 (1.03–1.59)**	**0.028**

NOTE: Only SNPs that are significantly genome-wide associated with hyperinsulinemia in overall/interaction (G*E or subgroup) analysis and breast cancer were included. Numbers in bold face are statistically significant.
Abbreviations: Alt, alternative allele; PA, physical activity; Ref, reference allele.

[a]Although SNPs were located between MTRR/LOC729506, the genes closest to the SNPs were selected.

[b]All genetic models are recessive. Although additive models' results are presented in genome-wide meta-analysis, the recessive model for each SNP had a similar effect size and *P* value.

[c]Results from genome-wide meta-analysis of interaction test for the association with HOMA-IR.

[d]HR adjusted by age, education, annual family income, family history of breast cancer, depressive symptom, smoking, dietary alcohol in g/day, % calories from SFAs/day, BMI, waist-to-hip ratio, hysterectomy ever, ages at menarche and menopause, oral contraceptive use, exogenous estrogen–only use, and exogenous estrogen plus progestin use.

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Table 6. Genome-wide meta-analysis of overall test and/or interaction test with the stratified analysis for the association with HOMA-IR and multiple Cox regression for the genotypes of *DOCK1* rs113847670 for predicting breast cancer risk, stratified by percentage of calories from SFA

			Interaction test for SFA		% Calories from SFA < 7.0 % (n = 1,010)					% Calories from SFA ≥ 7.0 % (n = 10,099)				
SNP	Genetic model	Allele[a] (Ref/Alt)	*P*	*Q*	OR[b]	*P*[b]	*Q*[b]	HR[c] (95% CI)	*P*	OR[b]	*P*[b]	*Q*[b]	HR[c] (95% CI)	*P*
DOCK1 **rs113847670**	**Genotypic**	**CC**	1.03E−05	1.000	**9.18**	**2.85E−08**	**0.571**	Reference		0.99	0.987	0.325	Reference	
		CT						0.50 (0.12–2.08)	0.341				1.22 (0.89–1.67)	0.209
		TT						N/A	N/A				**5.37 (1.33–21.63)**	**0.018**
	Recessive	**CC + CT/TT**						N/A	N/A				**5.28 (1.31–21.30)**	**0.019**

NOTE: Only SNPs that are significantly genome-wide associated with HOMA-IR in overall/interaction (G*E or subgroup) analysis and breast cancer were included. Numbers in bold face are statistically significant.

Abbreviations: Alt, alternative allele; N/A, not available; *Q*, Cochran's *Q*; Ref, reference allele.

[a]Additive genetic model regressed in genome-wide meta-analysis.

[b]Results from genome-wide meta-analysis of interaction test for the association with HOMA-IR.

[c]HR adjusted by age, education, annual family income, family history of breast cancer, depressive symptom, smoking, physical activity, dietary alcohol in g/day, BMI, waist-to-hip ratio, hysterectomy ever, ages at menarche and menopause, oral contraceptive use, exogenous estrogen–only use, and exogenous estrogen plus progestin use.

those SNPs were associated with an increased risk of breast cancer, suggesting that impaired glucose homeostasis, by itself and/or by interrelating with other insulin-related pathways, influences the carcinogenesis of the breast.

For many of genes linked by GWASs to metabolic traits, including IR, the mechanism by which the encoded proteins affect disease risk is unknown. Some intergenic or intronic SNPs may affect the function of transcriptional control structures, including enhancers and silencers (31). In this GWA study, we found 55 novel loci associated with one of the IR phenotypes, 29 of which were associated with breast cancer. However, most of these SNPs' underlying mechanisms have not been revealed in relation to glucose intolerance and breast cancer.

NKX2-2 is a homeodomain transcription factor that is crucial for pancreatic cell growth; *NKX2-2*–repressed mice exhibited reduced expression of the insulin gene, impaired insulin secretion, and ultimately, glucose intolerance and diabetes (32); in humans, *NKX2-2* repression is involved in neonatal diabetes (33). This may explain our finding of one SNP (rs7273292) being associated with hyperglycemia in an active group.

The *NR5A2/LRH-1* gene encodes nuclear receptor subfamily 5 group A member 2, a transcription factor that is critical in the adult pancreas for the regulation of exocrine function to maintain homeostasis (34). In our study, 3 SNPs were associated with hyperinsulinemia in the BMI $\geq$ 30 group. In addition, *NR5A2/LRH-1* is a key regulator of the estrogen response in breast cancer cells, promoting breast cancer cell proliferation, motility, and invasion. It also contributes to breast cancer cells' progression in postmenopausal women (35). In our study, one index SNP (rs10919774) showed a substantially reduced risk of breast cancer in the nonobese group, implying that the effect of the SNP/gene on cancer may be exerted only in the setting of adiposity.

One previous study reported an association between an *MTRR* SNP and type 2 diabetes (T2DM) in adipocyte tissues (36). The mechanism whereby such an SNP interacts with obesity in T2DM is not clear, but hyperhomocysteinemia, caused by mutations in *MTRR*, can induce IR in adipose tissue by provoking endoplasmic reticular stress, resulting in inhibited insulin signaling. In our study, several *MTRR* SNPs were associated with hyperinsulinemia in the physically inactive group. We further found these SNPs were associated with a higher risk of breast cancer in the same group of women. However, previous studies evaluating a relationship between *MTRR* SNPs and cancer showed an association only in lung and colorectal cancers (37, 38); in breast cancer, there was no significant association (39), which may be explained by neglecting the consideration of interactions with obesity-related factors.

In relation to the association with a high level of HOMA-IR, we found several SNPs near *MSC*, a gene that is a downstream target of the beta cell–receptor signal-transduction pathway. One GWAS meta-analysis (40) showed that an SNP related to MSC had a greater association with abdominal obesity, and pathway analysis showed that the SNP was related to higher triglyceride, fasting insulin, and T2DM traits. In our study, the relationship between *MSC* SNPs and IR had genome-wide significance only after group stratification by SFA consumption, supporting the hypothesis that *MSC* genetic variants may influence IR by interacting with fatty acids.

DOCK1, in insulin cellular signaling, acts as a substrate and is recruited to provide specific docking sites for other downstream signaling proteins, leading to activation of both Ras-to-MAP kinases and PI3K-to-AKT signaling cascades (41). Thus, mutation of the *DOCK1* gene can alter the insulin signaling pathway,

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influencing glucose metabolism. In our study, 1 SNP near *DOCK1* was associated with IR, but only in the low-fat diet group, which warrants further biological study. In addition, *DOCK1* in breast cancer cells mediates Rac activation, promoting breast cancer cell progression and metastasis (42). We found that 1 SNP in *DOCK1* had a 5 times greater likelihood of developing breast cancer in the counterpart (high-fat diet) group.

Despite our noteworthy findings, due to the constraints of available data, our study was confined to non-Hispanic white postmenopausal women, and therefore the generalizability of our results to other populations is limited. Also, owing to insufficient statistical power, we did not conduct any subtype analyses of breast cancer cases.

Our results suggest that a number of newly identified IR SNPs may produce their effects on glucose intolerance by interacting with obesity and other lifestyle factors and that a substantial proportion of those SNPs' susceptibility interacts with those lifestyle factors to ultimately influence breast cancer risk. Our findings may contribute to improved accuracy in predicting cancer and suggest intervention strategies for those women who carry the genetic risk to reduce their risk for breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.Y. Jung, J. Papp
Development of methodology: S.Y. Jung, J. Papp
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.Y. Jung, N. Mancuso, H. Yu, J. Papp, E. Sobel, Z.-F. Zhang
Writing, review, and/or revision of the manuscript: S.Y. Jung, N. Mancuso, H. Yu, J. Papp, E. Sobel, Z.-F. Zhang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.Y. Jung
Study supervision: S.Y. Jung

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Investigators and Academic Centers: Brigham and Women's Hospital, Harvard Medical School, Boston, MA: JoAnn E. Manson; MedStar Health Research Institute/Howard University, Washington, DC: Barbara V. Howard; Stanford Prevention Research Center, Stanford, CA: Marcia L. Stefanick; The Ohio State University, Columbus, OH: Rebecca Jackson; University of Arizona, Tucson/Phoenix, AZ: Cynthia A. Thomson; University at Buffalo, Buffalo, NY: Jean Wactawski-Wende; University of Florida, Gainesville/Jacksonville, FL: Marian Limacher; University of Iowa, Iowa City/Davenport, IA: Robert Wallace; University of Pittsburgh, Pittsburgh, PA: Lewis Kuller; Wake Forest University School of Medicine, Winston-Salem, NC: Sally Shumaker.

Women's Health Initiative Memory Study: Wake Forest University School of Medicine, Winston-Salem, NC: Sally Shumaker.

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