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Genome-wide Association Analysis of Proinflammatory Cytokines and Gene–lifestyle Interaction for Invasive Breast Cancer Risk: The WHI dbGaP Study

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ABSTRACT

Immune-related etiologic pathways to influence invasive breast cancer risk may interact with lifestyle factors, but the interrelated molecular genetic pathways are incompletely characterized. We used data from the Women's Health Initiative Database for Genotypes and Phenotypes Study including 16,088 postmenopausal women, a population highly susceptible to inflammation, obesity, and increased risk for breast cancer. With 21,784,812 common autosomal single-nucleotide polymorphisms (SNP), we conducted a genome-wide association (GWA) gene–environment interaction (G × E) analysis in six independent GWA Studies for proinflammatory cytokines [IL6 and C-reactive protein (CRP)] and their gene–lifestyle interactions. Subsequently, we tested for the association of the GWA SNPs with breast cancer risk. In women overall and stratified by obesity status (body mass index, waist circumference, and waist-to-hip ratio) and obesity-related lifestyle factors (exercise and high-fat diet), 88 GWA SNPs in 10 loci were associated with proinflammatory cytokines: 3 associated with IL6 (1 index SNP in *MAPK1* and 1 independent SNP in *DEC1*); 85 with CRP (3 index SNPs in *CRPP1, CRP, RP11–419N10.5, HNF1A-AS1, HNF1A*, and *C1q2orf43*; and two independent SNPs in *APOE* and *APOC1*). Of those, 27 in *HNF1A-AS1, HNF1A*, and *C1q2orf43* displayed significantly increased risk for breast cancer. We found a number of novel top markers for CRP and IL6, which interacted with obesity factors. A substantial proportion of those SNPs' susceptibility influenced breast cancer risk. Our findings may contribute to better understanding of genetic associations between pro-inflammation and cancer and suggest intervention strategies for women who carry the risk genotypes, reducing breast cancer risk.

Prevention Relevance: The top GWA-SNPs associated with pro-inflammatory biomarkers have implications for breast carcinogenesis by interacting with obesity factors. Our findings may suggest interventions for women who carry the inflammatory-risk genotypes to reduce breast cancer risk.

Introduction

Chronic inflammation may play an important role in the pathogenesis of noninflammatory diseases, including specific types of cancers such as colorectal, liver, and breast cancers, from tumor initiation through progression (1, 2). Activation of innate immunity involves a number of inflammatory cells, growth and transcription factors, chemokines, and proinflammatory mediators, creating a tissue microenvironment high in reactive oxygen and nitrogen species, leading to potential DNA alterations in nearby cells (3). The inflammatory response also elevates the circulating levels of cancer-promoting inflammatory cytokines such as C-reactive protein (CRP) and IL6 (2). These key biomarkers reflect different molecular pathways in the immune cascade in acute and chronic immune responses but may be inter-related in carcinogenesis. For example, IL6, upregulated by macrophages and adipose tissue, has promoted breast tumor initiation and progression (4). CRP, a major acute-phase reactant and a biomarker of chronic low-grade inflammation, partially induced by IL6, has been associated with increased risk of breast cancer (5). The molecular mechanisms of these markers in carcinogenesis have not been confirmed and are

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partially understood. IL6 regulates estrogen synthesis and aromatase activity responsible for estrogen production in adipose tissue, which is important in postmenopausal breast cancer (6). CRP levels are reduced when COX-2 action (promoting estrogen formation in adipose tissue) is inhibited (7). Thus, IL6 and CRP may provide a link between inflammatory pathways and breast cancer tumorigenesis.

The heritability of CRP and IL6 levels was 25%–40% (8, 9) and 60% (10), respectively, in Europeans on the basis of family and twin studies. Previous genome-wide association studies (GWAS) explain about 5% (11) and <2% (12) of interindividual variability of CRP and IL6 levels, respectively, suggesting that additional genetic contributions to CRP and IL6 concentrations remain largely undetermined. CRP and IL6 levels are also determined by obesity (overall and central; refs. 11–16), lipid metabolism (15), and obesity-related lifestyle factors such as exercise, high-fat diet, smoking, and alcohol (12, 17, 18). Thus, studying whether these lifestyle factors interact with genetic markers to influence CRP and IL6 concentrations may uncover the complicated genotype–phenotype pathway.

Few GWA gene–environment interaction (G × E) studies have examined those pleiotropic cytokines' gene–phenotype relationship with obesity factors. Particularly, genomic studies for CRP have investigated obesity-specific stratifications among GWA-based single-nucleotide polymorphisms (SNP) only (19, 20) or tested the G × E effect by introducing the interaction term into the gene–phenotype association (11). A full genome-wide scan within obesity strata at the initial stage throughout the GWA analysis may help to provide a more profound molecular basis for gene–phenotype pathways that are influenced by environment. In addition, a GWA analysis examining inflammatory markers as binary outcomes (e.g., normal range vs. chronic low-grade inflammation) rather than examining as a continuous variable, could address a nonlinearity issue that has frequently been violated in traditional linear regression and further identify SNPs whose effect reflects an allele-based risk magnitude of chronic low-grade inflammation.

Immune-related etiologic pathways that influence breast cancer may differ by menopausal status, probably owing to the role of sex hormones in mediating the innate and adaptive immune systems (21). Furthermore, in postmenopausal women, inflammatory cytokines and the genetic markers have displayed different associations with breast cancer according to obesity-related factors such as obesity status (overall and visceral; ref. 22), physical activity, and dyslipidemia (23, 24). Thus, our G × E study has focused on the risk for breast cancer among postmenopausal women, a population vulnerable to a high incidence of inflammation (25), obesity, and breast cancer (e.g., 80% of new cases occur in women age 50 years and older; ref. 26).

In this study, we hoped to identify SNPs explaining additional interindividual variability in cancer-promoting inflammatory biomarkers, including CRP and IL6. We conducted a GWA G × E study to characterize the genetic architecture of the biomarkers that interact with obesity factors. Next, we evaluated whether the identified SNPs in particular behavioral settings are associated with breast cancer risk in the identical behavioral setting. This may avoid bias derived from a different population structure by examining an identical genomic structure of the population for association with inflammatory cytokines and cancer risk simultaneously. We tested an empirical hypothesis that a substantial proportion of the susceptibility of GWA-based SNPs in CRP and IL6 affects breast cancer risk and that obesity lifestyle factors modify the relationship (Supplementary Fig. S1).

Materials and Methods

Study population

Our study included postmenopausal women who enrolled in the Women's Health Initiative (WHI) Harmonized and Imputed GWASs coordinated by the database of Genotypes and Phenotypes (dbGaP). These studies encompass the WHI Observational Studies and Clinical Trials to contribute a joint imputation and harmonization effort to the GWASs. Detailed rationale and study design have been discussed elsewhere (27, 28). The WHI study included women enrolled between 1993 and 1998 at 40 clinical centers across the United States. Eligible women were 50–79 years old, postmenopausal, expected to reside near the clinical centers for at least 3 years after enrollment, and able to provide written informed consent. The Harmonized and Imputed studies involved 6 GWASs (**Table 1**). Of the 16,088 who reported their race or ethnicity as non-Hispanic white (Supplementary Fig. S2), we excluded 2,714 who had been diagnosed with diabetes at or after enrollment. We also excluded 1,301 whose genetic information was found to be duplicated in the 6 GWASs and/or those with first- and second-degree relatives. In addition, we excluded 1,275 women whose genetic data did not pass the quality assurance (QA) test [outliers based on Principal Components (PCs)], leaving 10,798 (90% of the eligible 12,073) for our GWA G × E analysis. For the association with breast cancer, we next excluded 619 women who had been followed up for < 1 year and/or had been diagnosed with any type of cancer at enrollment, leaving a total of 10,179 women (94% of the 10,798 GWA participants). These women had been followed up through August 29, 2014, with a mean of 16 years follow-up, and 537 of them had developed invasive breast cancer. The Institutional Review Boards of each WHI participating clinical center and the University of California, Los Angeles, approved this study.

Data collection

The WHI coordinating center collected information by using standardized written protocols and conducted data QA with periodic visits. Participants had completed at enrollment self-administered questionnaires on the following information (listed for only those selected for our study): demographic and socioeconomic (age, education, race/ethnicity, family income, and family history of breast cancer), lifestyle (depressive symptom, smoking, and exercise), dietary [dietary alcohol intake in g/day and % calories from saturated fatty acids (SFA)/day] factors, and reproductive histories (history of hysterectomy, ages at menarche and menopause, oral contraceptive use, and

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Table 1. Distributions of proinflammatory phenotypes in six GWASs ($N = 10{,}798$).

Study[a]	N	Phenotype	
		IL6	CRP
Circulating plasma level		**pg/mL, mean (SD)**	**mg/L, mean (SD)**
AS264	1,603	4.478 (2.22)	3.883 (1.74)
GARNET	2,382	4.380 (3.16)	4.189 (7.50)
GECCO-CYTO	1,177	4.315 (3.04)	3.911 (3.81)
GECCO-INIT	216	3.221 (4.35)	4.323 (5.33)
HIPFX	1,909	4.548 (7.68)	3.752 (3.62)
WHIMS	3,511	4.472 (3.26)	3.360 (5.42)
Binary analysis		**<4.4 pg/mL/≥4.4 pg/mL[b] *n* (%)/*n* (%)**	**≤3.0 mg/L/>3.0 mg/L[b] *n* (%)/*n* (%)**
AS264	1,603	1,195 (74.5)/408 (25.5)	517 (32.3)/1,086 (67.7)
GARNET	2,382	1,748 (73.4)/634 (26.6)	1,446 (60.7)/936 (39.3)
GECCO-CYTO	1,177	901 (76.6)/276 (23.4)	497 (42.2)/680 (57.8)
GECCO-INIT	216	187 (86.6)/29 (13.4)	124 (57.4)/92 (42.6)
HIPFX	1,909	1,436 (75.2)/473 (24.8)	813 (42.6)/1,096 (57.4)
WHIMS	3,511	2,649 (75.4)/862 (24.6)	2,325 (66.2)/1,186 (33.8)

[a]Genotyping was run on several platforms: AS264 via Affymetrix Gene Titan, Axiom Genome-Wide Human CEU I Array Plate; GARNET via Illumina HumanOmni1-Quad v1–0 B; GECCO via Illumina 610 and Cytochip 370K; HIPFX via Illumina 550K and 610K; and WHIMS via HumanOmniExpress Exome-8v1_B.
[b]Each phenotype was categorized via the corresponding cut-off value (4.4 pg/mL for IL6 and 3.0 mg/L for CRP); higher blood level than the threshold to be considered either the fourth quartile of the participants (≥ 75%, 4.4 pg/mL for IL6) or high immune response and chronic low-grade inflammation (> 3.0 mg/L for CRP; refs. 39, 40).

2 types of exogenous estrogen use: unopposed estrogen-only and opposed estrogen plus progestin). Anthropometric data, including height, weight, and waist and hip circumferences, were measured at baseline by trained staff. Those 17 variables (**Table 2**) were initially selected on the basis of their association with inflammation and breast cancer among postmenopausal women through the literature review (23, 29, 30) and were confirmed by univariate, stepwise, and multicollinearity regression testing to be analyzed in our study.

Breast cancer development was our cancer outcome of interest. The time between enrollment and breast cancer development, censoring, or study endpoint was measured as the number of days and then converted into years. Breast cancer diagnosis was determined using a centralized review of medical charts by a committee of physicians on the basis of pathology or cytology reports. Cancer cases were coded according to the National Cancer Institute's Surveillance, Epidemiology, and End-Results guidelines (31).

Genotyping and laboratory methods

Genotyped data generated on several different platforms were extracted from the WHI dbGaP Harmonized and Imputed GWASs (**Table 1**). The genotypes were normalized to the reference panel GRCh37 and genotype imputation was performed via 1,000 genomes reference panels. (28) SNPs were checked for harmonization with pairwise concordance among all samples across the 6 GWASs. We crosschecked the self-reported race/ethnicity with PCs. If any discrepancy or admixed participants were detected, an additional analysis was performed with a follow-up demographic questionnaire (32). In the initial quality control (QC) process, SNPs were filtered with a missing-call rate of <2% and a Hardy–Weinberg Equilibrium of $P \geq$ 1E-04. In the secondary QC, we retained SNPs with $\hat{R}^2 \geq 0.6$ imputation quality (33). Within high-quality SNPs that included only HapMap3 SNPs with $\hat{R}^2 \geq 0.9$ (34), we estimated relatedness between samples. To reduce potential confounding due to shared environment, we excluded individuals with a kinship estimate >0.088 on the basis of the KING robust kinship estimator (35). We next computed 10 PCs in each GWAS using the same set of high-quality SNPs, but without relatedness, within the linkage disequilibrium (LD) and excluded outlier samples that fell outside of 6 SD on the basis of Mahalanobis distances. Finally, a total of 21,784,812 autosomal SNPs from 10,798 individuals were examined in this study.

Participants' fasting blood samples at enrollment were drawn by trained phlebotomists. Serum concentrations of IL6 and CRP were measured by using, respectively, the Quantitative Sandwich Enzyme Immunoassay technique (Quantikine HS Immunoassay Kit; R&D Systems, Inc.) and a high sensitivity immunoturbidimetric assay (Kimiya Biomedical Company) read with a Roche analyzer (Roche Diagnostics), with the median interassay coefficients of variation of 12.4% and 2.3%, respectively. About 30% of the phenotypes were replaced by imputation values using an unsupervised splitting of Random Survival Forest imputation (https://github.com/ehrlinger/randomForestSRC/blob/master/R/impute.rfsrc.R; ref. 36). In the overall and the obesity-specific strata of the GWA analysis, sensitivity testing was performed with and without imputed values for IL6 and CRP, producing regression estimates, genomic control inflation factors (λ_{GC}), Q-Q plots, and Manhattan plots; no significant differences were observed.

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Table 2. Participants' characteristics stratified by breast cancer.

Characteristics	Participants without breast cancer ($n = 9,642$) *n* (%)	Participants with breast cancer ($n = 537$) *n* (%)	*P*
Age in years, median (range)	67 (50–81)	67 (50–79)	0.085
Education			0.011
≤High school	3,476 (36.1)	164 (30.5)[a]	
>High school	6,166 (63.9)	373 (69.5)	
Family income			0.002
<$35,000	4,344 (46.1)	207 (39.2)[a]	
≥$35,000	5,088 (53.9)	321 (60.8)	
Family history of breast cancer			0.032
No	7,838 (81.3)	416 (77.5)[a]	
Yes	1,804 (18.7)	121 (22.5)	
BMI in kg/m^2, median (range)	26.84 (16.83–58.49)	28.23 (17.55–49.31)[a]	<0.001
BMI[b]			<0.001
<30.0 kg/m^2	6,859 (71.1)	320 (59.6)[a]	
≥30.0 kg/m^2	2,783 (28.9)	217 (40.4)	
WHR, median (range)	0.807 (0.444–1.393)	0.813 (0.640–1.263)[a]	0.013
WHR[b]			0.011
≤0.85	6,895 (71.5)	356 (66.3)[a]	
>0.85	2,747 (28.5)	181 (33.7)	
Waist in cm, median (range)	85.00 (62.00–125.00)	88.50 (63.50–125.00)[a]	<0.001
Waist[b]			<0.001
≤88 cm	5,756 (59.7)	268 (49.9)[a]	
>88 cm	3,886 (40.3)	269 (50.1)	
METs·hour/week[c]	7.25 (0.00–134.17)	6.75 (0.00–81.67)	0.487
METs·hour/week[c]			0.844
≥10.0	4,001 (41.5)	220 (41.0)	
<10.0	5,641 (58.5)	317 (59.0)	
How many cigarettes per day			<0.001
<15 cigarettes	5,432 (56.3)	250 (46.6)[a]	
≥15 cigarettes	4,210 (43.7)	287 (53.4)	
Depressive symptoms[d], median (range)	0.002 (0.0004–0.937)	0.002 (0.0005–0.880)	0.836
Dietary alcohol per day in g, median (range)	1.04 (0.00–183.76)	1.86 (0.00–127.15)[a]	0.001
% calories from SFA, median (range)	11.33 (2.22–32.39)	11.46 (3.73–21.50)	0.967
% calories from SFA[e]			0.297
<9.0%	2,174 (22.5)	132 (24.6)	
≥9.0%	7,468 (77.5)	405 (75.4)	
Age at menarche in years, median (range)	13 (≤ 9–≥ 17)	12 (≤9–≥17)[a]	0.002
Hysterectomy ever			0.004
No	6,143 (63.7)	376 (70.0)[a]	
Yes	3,499 (36.3)	161 (30.0)	
Age at menopause in years, median (range)	50 (20–60)	50 (21–63)	0.050
Oral contraceptive duration in years, median (range)	5.66 (0.08–47.00)	5.18 (0.08–21.00)[a]	<0.001
Exogenous estrogen use (E only use)			0.001
Never use	6,697 (69.5)	411 (76.5)[a]	
<5 years	1,361 (14.1)	51 (9.5)	
5 to <10 years	516 (5.4)	17 (3.2)	
10 + years	1,068 (11.1)	58 (10.8)	
Exogenous estrogen use (E + P use)			0.001
Never use	7,940 (82.3)	412 (76.7)[a]	
<5 years	927 (9.6)	64 (11.9)	
5 to <10 years	406 (4.2)	30 (5.6)	
10 + Years	369 (3.8)	31 (5.8)	

Abbreviations: E, estrogen; E+P, estrogen plus progestin.

[a]$P < 0.05$, χ^2 or Wilcoxon rank-sum test.

[b]BMI, WHR, and WST were categorized using 30 kg/m^2, 0.85, and 88 cm, respectively, where cut-off levels or higher fall within the overall or visceral obese range (https://www.cdc.gov/obesity/adult/defining.html).

[c]Physical activity was estimated from recreational physical activity combining walking and mild, moderate, and strenuous physical activity. Each activity was assigned a MET value corresponding to intensity; the total MET·hours/week 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 two groups, with 10 METs as the cutoff according to current American College of Sports Medicine and American Heart Association recommendations (63).

[d]Depression scales were estimated via a short form of the Center for Epidemiologic Studies Depression Scale.

[e]Percent calories from SFA was classified by 9%, addressing low sample power (i.e., containing a quarter in one side) and adherent to the American Heart Association and American College of Cardiology dietary guidelines, which are aligned with the 2015–2020 Dietary Guidelines for Americans to help cardiovascular and metabolic diseases reductions (64).

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Statistical analysis

Participants' baseline characteristics by breast cancer development were examined by unpaired two-sample *t* tests for continuous variables and χ^2 tests for categorical variables; if continuous variables were skewed or had outliers, Wilcoxon rank-sum test was used. A GWA analysis in each study was conducted via multiple linear and logistic regressions, adjusting for age and 10 PCs to produce effect sizes and ORs, respectively, and 95% confidence intervals (CI) of IL6 and CRP concentrations, with additive and minor allele–dominant and –recessive genetic models. In this study, we present the results from only the logistic regressions due to the high genomic control inflation factors ($\lambda_{GC} > 1.0$) from the linear regressions. Multiple testing was corrected by adjusting *P* values to the genome-wide significance level ($P < 5E\text{-}08$). The inverse variance-weighted fixed-effects meta-analysis was conducted to combine the findings across the 6 GWASs. Heterogeneity among studies was tested via Cochran Q statistic (37) at $P < 8E\text{-}03$ after Bonferroni corrections.

For all the SNPs at the initial stage of analysis, gene–obesity interactions were examined in each study (i) by introducing an interaction term into a regression model, adjusting for 10 PCs, and (ii) within the obesity strata defined by body mass index (BMI; cutoff, 30 kg/m^2), waist circumference (WST; cutoff, 88 cm), waist-to-hip ratio (WHR; cutoff, 0.85), metabolic equivalents (MET; cutoff, 10·hours/week), and % calories from SFA (cutoff, 9%). Meta-analysis was performed assuming fixed effects to combine the interaction results across the 6 GWASs. Multiple comparisons were adjusted by the Benjamini–Hochberg method (38). The LDs between the top GWA signals were computed, and regional plots were created using LOCUSZOOM (http://locuszoom.org/). Gene enrichment and functional annotation analyses were performed using DAVID v6.8 (https://david.ncifcrf.gov/).

With the top signals, we further conducted a multiple Cox proportional hazards regression in the combined 6 GWASs to obtain HRs and 95% CIs predicting a risk for breast cancer by adjusting for 17 confounding factors (**Table 2**). The proportional hazards assumptions were tested by a Schoenfeld residual plot and rho. Given that the SNP–cancer pathway was a hypothesis-driven question, the *P* value was not subject to multiple testing corrections; thus, a two-tailed $P < 0.05$ was considered statistically significant. During the data QC process, dose2plink script (http://genepi.qimr.edu.au/staff/sarahMe/dose2plink), PLINK1.9/2.0, KING kinship estimator, and R *stats* package on UCLA's Hoffman2 high performance computing cluster were used. For GWA, phenotype imputation, and SNP–cancer association tests, PLINK1.9/2.0 (glm/interaction/meta-analysis) and R3.5.1. (qqman/manhattan, randomForestSRC, and survival packages) were used.

Results

Distributions of the pro-inflammatory cytokines (CRP and IL6) in the 6 GWASs are displayed in **Table 1**. Blood-level thresholds were determined as reflecting either the chronic low-grade inflammation status (>3.0 mg/L for CRP) or the fourth quartile of the participants (≥4.4 pg/mL for IL6; refs. 39, 40). Allele frequencies in each study (Supplementary Table S1) were cross-checked with those in Europeans in the 1000 Genomes Project Ensembl GRCh37 (http://grch37.ensembl.org/Homo_sapiens/Info/Index). Participants' baseline characteristics by breast cancer development are shown in **Table 2**. Women who developed breast cancer were more likely to have greater incomes and a family history of breast cancer, be obese overall and viscerally, smoke ≥15 cigarettes/day, consume more dietary alcohol/day, have experienced menarche at earlier age, have undergone hysterectomy less frequently, and have shorter durations of oral contraceptive and E-only use but longer durations of estrogen + progestin use.

Of 21,784,812 common autosomal SNPs, 88 SNPs reached genome-wide significance (including 4 index SNPs and 3 SNPs, independent of each other) in 10 loci, 7 of which were novel, and 3 loci (SNPs near *CRP, HNF1A*, and *APOE*) that were previously observed. The genomic control inflation factor (λ_{GC}) was 1.0, indicating no evidence of type 1 error inflation. Those top genetic markers did not overlap between the two cytokine phenotypes. Identified loci explained 5.8% and 0.7% of interindividual variability of CPR and IL6, respectively.

CRP: GWAS G × E results and association with breast cancer

In the overall analysis, 82 top signals were detected in 7 loci of *CRPP1, CRP, HNF1A-AS1, HNF1A, C12orf43, APOE*, and *APOC1* (Supplementary Table S2; Supplementary Fig. S3A–S3H). Specifically, 8 SNPs near *CRPP1*, 1 near *CRPP1/CRP*, and 11 in intron, 3′-UTR, or 3′ flanking regions of *CRP* (index: rs2808628 with $r^2 > 0.7$, **Fig. 1A**) were associated with increased risk of chronic inflammation (approximately 20%). On the contrary, the following loci at 12q and 19q displayed about 20% of decreased risk: 23 in an intronic region of *HNF1A-AS1* and 31 in intronic or 3′-UTR regions of *HNF1A* (index: rs2243458 with $r^2 > 0.7$, **Fig. 1B**), 6 in intronic or 3′-UTR regions of *C12orf43* (index: rs1169311 with $r^2 > 0.9$; **Fig. 1C**), *APOE* rs429358, and *APOC1* rs5117.

When stratified by BMI (Supplementary Table S3), a number of SNPs near *HNF1A-AS1* and *HNF1A* (18 and 27 SNPs, respectively, with index: rs2243458) and one (rs1169311) in *C12orf43* were detected; these partially overlapped those identified from the overall analysis, with about 20% of decreased risk for chronic inflammation, only in the nonobese (BMI < 30) subgroup. In the obese subgroup (BMI ≥ 30), 3 novel SNPs in *CRP/RP11–419N10.5* in LD ($r^2 > 0.9$) were identified with 70% of increased risk for chronic inflammation. When stratified by WHR or WST, similar patterns were shown for SNPs on inflammation: SNPs near *CRP* and *CRP/RP11–419N10.5* with 35%–50% of increased risk in the viscerally obese subgroup only. SNPs near *HNF1A* were shown with decreased risk for inflammation, also only in the viscerally obese subgroup (Supplementary Table S3).

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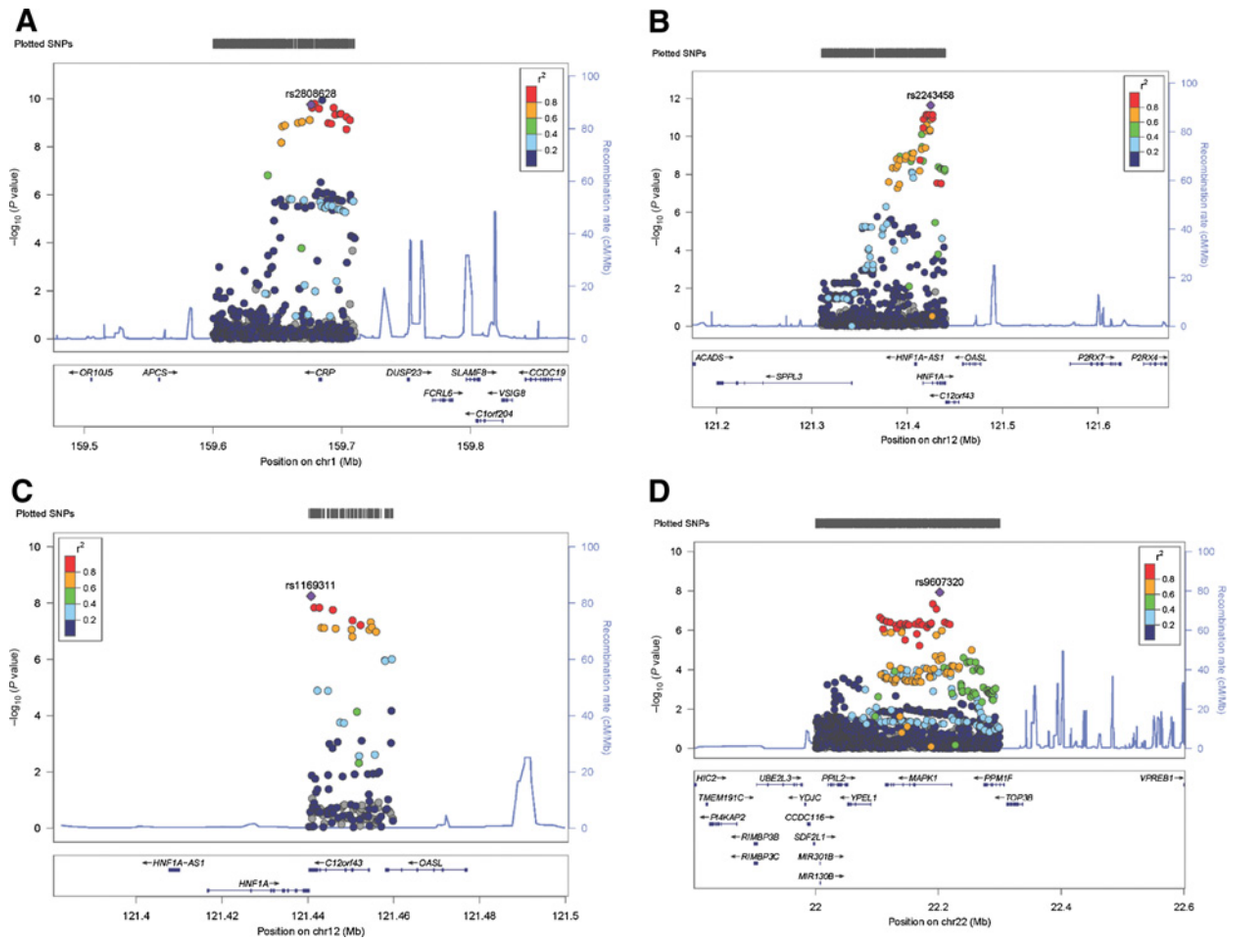

Figure 1.
Regional plots for the SNP association [Note: LD (r^2) shown by color intensity gradient]. **A,** Twenty SNPs nearby *CRP* and *CRPP1* ($r^2 > 0.7$) with CRP. **B,** Fifty-four SNPs nearby *HNF1A-AS1* and *HNF1A* ($r^2 > 0.7$) with CRP. **C,** Six SNPs nearby *C12orf43* ($r^2 > 0.9$) with CRP. **D,** Two SNPs nearby *MAPK1* ($r^2 > 0.8$) with IL6.

Table 3. Genome-wide associated SNPs with CRP and their associations with breast cancer risk in overall analysis.

SNP	Chr	Position[b]	Allele[a] (Ref/Alt)	OR[c]	*P*	Q	HR[d] (95% CI)	*P*
HNF1A rs2259852	12	121434833	A/G	0.84	4.95E-09	0.347	1.13 (1.00–1.27)	0.058
HNF1A rs2464195	12	121435475	A/G	0.84	4.96E-09	0.358	1.13 (1.00–1.27)	0.057
HNF1A rs2259816	12	121435587	T/G	0.84	5.16E-09	0.360	1.13 (1.00–1.28)	0.054
HNF1A rs1169306	12	121438311	T/C	0.84	5.13E-09	0.371	1.13 (1.00–1.27)	0.058
HNF1A rs735396	12	121438844	C/T	0.84	5.25E-09	0.375	1.13 (1.00–1.27)	0.058
HNF1A rs1169309	12	121439192	T/G	0.84	6.61E-09	0.356	1.13 (1.00–1.27)	0.057
HNF1A rs1169310	12	121439433	A/G	0.84	5.28E-09	0.376	1.13 (1.00–1.27)	0.058
C12orf43 rs1169311	12	121440731	T/C	0.84	5.64E-09	0.375	1.13 (1.00–1.27)	0.057

Note: Only SNPs significant on genome-wide level included ($N = 10{,}179$).
Abbreviations: Alt, alternative; Chr, chromosome; CI, confidence interval; Q, Cochran Q; Ref, reference.
[a]Additive genetic model regressed.
[b]GRCh 37 coordinated.
[c]GWA analysis for CRP adjusted for age and 10 PCs.
[d]HR adjusted by age, education, annual family income, family history of breast cancer, BMI, waist-to-hip ratio, physical activity, depressive symptoms, How many cigarettes per day, dietary alcohol in g/day, % calories from SFA/day, hysterectomy, ages at menarche and menopause, oral contraceptive use, and exogenous estrogen only-use and plus progestin use.

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Table 4. Genome-wide associated SNPs with CRP and their relationships with breast cancer risk in obesity-stratified (BMI and WST) analysis.

SNP	Chr	Position[a]	Allele[b] (Ref/Alt)	Non-obese group					Obese group				
				OR[c]	*P*[d]	Q	HR[e] (95% CI)	*P*	OR[c]	*P*[d]	Q	HR[e] (95% CI)	*P*
				Non overall-obese group, BMI < 30 (n = 7,179)					<Overall obese group, BMI ≥ 30 (n = 3,000)				
HNF1A-AS1 **rs11065365**	**12**	**121392040**	**G/A**	**0.79**	**1.42E-08**	**0.209**	0.92 (0.76–1.10)	0.360	0.76	2.75E-05	0.724	**1.27 (1.02–1.58)**	**0.031**
HNF1A-AS1 **rs7953249**	**12**	**121403724**	**G/A**	**0.81**	**6.45E-09**	**0.154**	0.94 (0.80–1.10)	0.432	0.78	7.58E-05	0.746	**1.26 (1.04–1.53)**	**0.017**
HNF1A-AS1 **rs7135337**	**12**	**121404155**	**A/C**	**0.80**	**3.19E-09**	**0.150**	0.95 (0.81–1.11)	0.505	0.77	4.75E-05	0.790	**1.28 (1.06–1.56)**	**0.012**
HNF1A-AS1 **rs1920792**	**12**	**121404584**	**C/T**	**1.22**	**1.56E-08**	**0.062**	1.06 (0.91–1.24)	0.451	1.28	0.0001	0.456	**0.75 (0.62–0.91)**	**0.004**
HNF1A-AS1 **rs10774579**	**12**	**121405210**	**C/T**	**1.22**	**1.72E-08**	**0.062**	1.06 (0.91–1.24)	0.457	1.28	0.0001	0.456	**0.75 (0.62–0.91)**	**0.004**
HNF1A-AS1 **rs2393792**	**12**	**121406293**	**A/G**	**1.22**	**2.96E-08**	**0.081**	1.04 (0.89–1.22)	0.605	1.27	0.0002	0.349	**0.73 (0.60–0.89)**	**0.002**
HNF1A-AS1 **rs142632970**	**12**	**121413345**	**G/A**	**0.80**	**8.81E-09**	**0.805**	0.96 (0.81–1.14)	0.633	0.80	0.001	0.219	**1.24 (1.01–1.51)**	**0.041**
HNF1A-AS1 **rs7139079**	**12**	**121415293**	**G/A**	**0.80**	**1.37E-09**	**0.140**	0.95 (0.81–1.12)	0.566	0.75	5.26E-06	0.762	**1.26 (1.04–1.53)**	**0.018**
HNF1A-AS1 **rs2464190**	**12**	**121415390**	**C/T**	**0.80**	**3.22E-09**	**0.163**	0.94 (0.80–1.10)	0.441	0.75	1.02E-05	0.615	**1.26 (1.03–1.53)**	**0.023**
HNF1A **rs1169289**	**12**	**121416622**	**G/C**	**0.81**	**1.68E-08**	**0.156**	0.96 (0.81–1.12)	0.580	0.77	3.62E-05	0.703	**1.25 (1.03–1.52)**	**0.027**
HNF1A **rs7979473**	**12**	**121420260**	**A/G**	**0.80**	**7.62E-10**	**0.123**	1.01 (0.86–1.18)	0.944	0.74	3.95E-06	0.896	**1.24 (1.02–1.51)**	**0.032**
HNF1A **rs7979478**	**12**	**121420263**	**A/G**	**0.79**	**1.17E-10**	**0.167**	0.99 (0.84–1.16)	0.864	0.75	1.15E-05	0.937	**1.24 (1.01–1.51)**	**0.036**
HNF1A **rs7970695**	**12**	**121423376**	**G/A**	**0.79**	**5.40E-10**	**0.139**	1.01 (0.86–1.18)	0.928	0.75	4.96E-06	0.911	**1.24 (1.02–1.50)**	**0.031**
HNF1A **rs9738226**	**12**	**121423659**	**A/G**	**0.79**	**5.94E-10**	**0.130**	1.01 (0.86–1.19)	0.902	0.75	4.96E-06	0.912	**1.24 (1.02–1.51)**	**0.031**
HNF1A **rs2393791**	**12**	**121423956**	**C/T**	**0.79**	**5.16E-10**	**0.139**	1.01 (0.86–1.18)	0.916	0.75	4.80E-06	0.911	**1.24 (1.02–1.50)**	**0.031**
HNF1A **rs2393776**	**12**	**121424406**	**G/A**	**0.79**	**3.23E-10**	**0.218**	1.01 (0.86–1.18)	0.918	0.74	2.31E-06	0.731	**1.23 (1.01–1.49)**	**0.037**
HNF1A **rs2393775**	**12**	**121424574**	**G/A**	**0.79**	**3.25E-10**	**0.225**	1.01 (0.86–1.18)	0.915	0.74	2.30E-06	0.732	**1.23 (1.01–1.49)**	**0.037**
HNF1A **rs7310409**	**12**	**121424861**	**A/G**	**0.79**	**3.30E-10**	**0.225**	1.01 (0.86–1.18)	0.915	0.74	2.31E-06	0.731	**1.23 (1.01–1.49)**	**0.037**
HNF1A **rs2264782**	**12**	**121432603**	**T/C**	**0.82**	**4.64E-08**	**0.241**	1.06 (0.90–1.24)	0.512	0.76	1.50E-05	0.616	**1.24 (1.02–1.51)**	**0.030**
HNF1A **rs2259852**	**12**	**121434833**	**A/G**	**0.81**	**2.91E-08**	**0.273**	1.05 (0.90–1.24)	0.519	0.76	2.45E-05	0.643	**1.25 (1.03–1.51)**	**0.027**
HNF1A **rs2464195**	**12**	**121435475**	**A/G**	**0.81**	**2.82E-08**	**0.283**	1.05 (0.90–1.24)	0.517	0.76	2.51E-05	0.641	**1.25 (1.03–1.51)**	**0.027**
HNF1A **rs2259816**	**12**	**121435587**	**T/G**	**0.81**	**3.05E-08**	**0.281**	1.06 (0.90–1.24)	0.498	0.76	2.54E-05	0.642	**1.25 (1.03–1.51)**	**0.027**
HNF1A **rs1169306**	**12**	**121438311**	**T/C**	**0.81**	**2.81E-08**	**0.305**	1.05 (0.90–1.24)	0.522	0.76	2.54E-05	0.637	**1.25 (1.03–1.51)**	**0.027**
HNF1A **rs735396**	**12**	**121438844**	**C/T**	**0.81**	**2.83E-08**	**0.309**	1.05 (0.90–1.24)	0.520	0.76	2.54E-05	0.637	**1.24 (1.02–1.51)**	**0.027**
HNF1A **rs1169309**	**12**	**121439192**	**T/G**	**0.81**	**3.11E-08**	**0.294**	1.05 (0.90–1.24)	0.513	0.77	2.97E-05	0.626	**1.25 (1.03–1.51)**	**0.027**
HNF1A **rs1169310**	**12**	**121439433**	**A/G**	**0.81**	**2.85E-08**	**0.311**	1.05 (0.90–1.24)	0.520	0.76	2.54E-05	0.637	**1.24 (1.02–1.51)**	**0.027**
C12orf43 **rs1169311**	**12**	**121440731**	**T/C**	**0.81**	**2.98E-08**	**0.312**	1.05 (0.90–1.24)	0.514	0.76	2.60E-05	0.637	**1.24 (1.02–1.51)**	**0.027**
				Non visceral-obese group, WST ≤ 88 (n = 6,024)					**Visceral obese group, WST > 88 (n = 4,155)**				
HNF1A **rs2393776**	**12**	**121424406**	**G/A**	0.82	1.25E-06	0.168	0.97 (0.81–1.15)	0.708	**0.75**	**3.47E-08**	**0.530**	**1.23 (1.04–1.46)**	**0.017**
HNF1A **rs2393775**	**12**	**121424574**	**G/A**	0.82	1.27E-06	0.173	0.97 (0.81–1.15)	0.711	**0.75**	**3.42E-08**	**0.528**	**1.23 (1.04–1.46)**	**0.017**
HNF1A **rs7310409**	**12**	**121424861**	**A/G**	0.82	1.28E-06	0.172	0.97 (0.81–1.15)	0.711	**0.75**	**3.47E-08**	**0.530**	**1.23 (1.04–1.47)**	**0.017**

Note: Only SNPs significant on genome-wide level included. Numbers in bold face are statistically significant.

Abbreviations: Alt, alternative; Chr, chromosome; CI, confidence interval; Q, Cochran Q; Ref, reference.

[a]GRCh 37 coordinated.

[b]Additive genetic model regressed.

[c]GWA analysis for CRP adjusted for age and 10 PCs.

[d]*P* values were adjusted to correct for multiple testing via the Benjamini–Hochberg approach.

[e]HR adjusted by age, education, annual family income, family history of breast cancer, body mass index (not in BMI-stratified), WHR (not in WST-stratified), physical activity, depressive symptoms, how many cigarettes per day, dietary alcohol in g/day, % calories from SFA/day, hysterectomy, ages at menarche and menopause, oral contraceptive use, and exogenous estrogen only-use and plus progestin use.

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In relation to breast cancer risk with the top signals, the loci at 12q, including *HNF1A-AS1, HNF1A*, and *C12orf43* were associated, while the loci at 1q (*CRPP1, CRP*, and *RP11–419N10.5*) were not. In particular, CRP-decreasing SNPs near *HNF1A* in the overall analysis were associated with 13% increased risk for breast cancer in the identical overall group (**Table 3**). CRP-decreasing SNPs near *HNF1A-AS1, HNF1A*, and *C12orf43* in the nonobese (BMI < 30) subgroup displayed a slightly decreased risk without reaching statistical significance, but in the counterpart obese subgroup (BMI ≥ 30), approximately 25% increased risk for breast cancer was observed (**Table 4**). In addition, CRP-decreasing SNPs near *HNF1A* in the viscerally obese subgroup showed a 23% increased risk for breast cancer in the same viscerally obese subgroup (**Table 4**).

Similar genetic associations for the CRP phenotype were observed when stratified by physical activity and % calories from SFA (**Table 5**). SNPs near *HNF1A-AS1* and *HNF1A* (index: rs2393776 with $r^2 > 0.7$) were associated with 35% decreased risk for chronic inflammation (except for few SNPs *HNF1A-AS1* with increased risk) in the less-fat diet subgroup, whereas SNPs near *CRPP1* and *CRP* (index: rs2808628) were associated with 20% increased risk for chronic inflammation in the physically inactive and high-fat diet subgroups. No associations of those SNPs with breast cancer were detected (Supplementary Table S4).

IL6: GWAS G × E results and association with breast cancer

Three of the most significant signals were detected in two loci of *DEC1* and *MAPK1* (**Table 6**; Supplementary Fig. S3I–S3L). In all participants and in the nonviscerally obese and high-fat diet subgroups, rs149109490 near *DEC1* was strongly associated with increased IL6 levels, reflecting high risk for chronic low-grade inflammatory status (OR = 3.61; $P = 3.42E-08$; Q = 0.078). Two *MAPK1* SNPs (rs56398890 and rs9607320) in LD ($r^2 > 0.9$) displayed 25% decreased risk in the physically active subgroup only. None of the top SNPs were associated with breast cancer risk (Supplementary Table S5).

Gene–ontology annotation analyses (Supplementary Tables S6 and S7) indicated *CRP* involvement in the acute-phase inflammation response; *HNF1A, APOC1*, and *APOE* in glucose, lipoprotein, triglyceride, and cholesterol metabolisms; and *DEC1* and *MAPK1* in the regulation of mitogen-activated protein kinase/PI3K/ERK 1/2 cascades, apoptotic process, cellular response to DNA damage stimulus, and cell differentiation and migration.

Discussion

A growing number of population-based genomic studies have emphasized the role of environmental factors in modifying gene–phenotype pathways. To our knowledge, this is the first study to characterize genetic determinants of inflammatory cytokines and subsequently their genomic associations with breast cancer development in an identical postmenopausal population, at the genome-wide level, by incorporating obesity factors as an effect modifier. A number of novel top-GWA signals have been detected in relation to chronic low-grade inflammation on the basis of CRP and IL6 concentrations. Some of the top loci for their associations with chronic inflammation and breast cancer would not have been detected without the incorporation of the obesity factors.

Many genes annotated to the CRP-related variants mainly clustered in the innate and adaptive immune functions (*CRP*) or the glucose and lipid metabolisms (*HNF1A* and *APOC1*; refs. 11, 13). In particular, the *CRP* gene encodes a member protein of the pentaxin family involving host defense functions during the acute-phase response to tissue infection to promote phagocytosis and the complement system by interacting with the DNA in circulating cells (41). Several *CRP* and *CRPP1* SNPs detected in our study are located at 1q21 to 25 region, which contains genes encoding proteins with immune- and inflammation-associated functions (42). Furthermore, the CRP-related SNPs were mainly clustered in the 3′-UTL or 3′ flaking region (e.g., rs1205) of the *CRP* gene involved in the post-transcriptional process by regulating mRNA stability, localization, and translational efficiency; thus, those SNPs in this region may affect CRP production (19). The *CRP* gene is a single-copy gene. Within about 16-kb upstream and downstream from the *CRP* gene, only one other sequence is *CRPP1*, a pseudogene, with 50%–80% homology to *CRP* (43). CRP-related SNPs near *CRPP1* have thus far been reported only in African Americans (14). We found a different set of *CRPP1* SNPs that were novel in non-Hispanic whites.

A positive reciprocal feedback role between CRP and adiposity pathophysiology has been suggested (15, 20), and CRP-related genes were correlated with BMI/WST/WHR-related genes (13). Consistent with previous findings (19, 44), we found that *CRP/CRPP1* SNPs were related to elevated CRP levels (i.e., greater risk for chronic inflammation) among those who were obese or had obesity lifestyles, indicating that obesity factors play a role in regulating the effect of *CRP/CRPP1* genes on CRP levels. Of note, the *CRP/CRPP1* gene–phenotype relationships were more profound in women with central obesity, suggesting an important role of adipose tissue distribution in CRP gene–phenotype pathways. Furthermore, the novel SNPs we found near *CRP/RP11–419N10.5* were associated with up to 70% elevated risk for chronic inflammation only in women with overall and central obesity, but the biological implications of those SNPs on *RP11–419N10.5*, a processed pseudogene, are unclear.

Hepatic nuclear factor 1 alpha (HNF1A) is a key regulator of CRP protein. It binds to promotor regions of the *CRP* gene to regulate CRP synthesis in liver cells (45). Thus, HNF1A is required for *CRP* gene expression (46). This implicates the functional role of *HNF1A* SNPs in altering the expression of its target gene, *CRP*; *HNF1A* mutations led to loss of function in reducing CRP levels (47). This provides a biologically plausible basis for our findings that SNPs in *HNF1A/HNF1A-AS1* were

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Table 5. GWA analysis for the association with CRP, stratified by obesity-related lifestyle factors (physical activity and fat diet).

SNP	Chr	Position[a]	Allele[b] (Ref/Alt)	Interaction test			Active/less-fat diet group			Inactive/high-fat diet group		
				OR[c]	*P*	Q	OR[c]	*P*[d]	Q	OR[c]	*P*[d]	Q
				Interaction test for PA			MET ≥ 10 (n = 4,221)			MET < 10 (n = 5,958)		
***CRPP1* rs2808628**	**1**	**159676011**	**G/A**	0.97	0.354	0.401	1.16	0.002	0.203	**1.26**	**9.13E-09**	**2.00E-04**
***CRPP1* rs2808629**	**1**	**159676796**	**G/A**	0.97	0.368	0.352	1.16	0.002	0.210	**1.26**	**1.28E-08**	**2.00E-04**
***CRPP1/CRP* rs2794520**	**1**	**159678816**	**C/T**	0.97	0.361	0.350	1.16	0.002	0.244	**1.26**	**9.62E-09**	**2.00E-04**
***CRP* rs1205**	**1**	**159682233**	**C/T**	0.97	0.335	0.306	1.16	0.002	0.246	**1.26**	**1.03E-08**	**2.00E-04**
***CRP* rs3091244**	**1**	**159684665**	**G/A**	1.03	0.354	0.037	0.85	0.001	0.078	**0.80**	**1.01E-08**	**<8E-03**
***CRP* rs1341665**	**1**	**159691559**	**G/A**	0.97	0.377	0.528	1.15	0.003	0.258	**1.25**	**4.85E-08**	**9.00E-04**
***CRP* rs2211320**	**1**	**159693605**	**G/A**	0.98	0.450	0.361	1.17	0.001	0.273	**1.26**	**2.24E-08**	**3.00E-04**
***CRP* rs7551731**	**1**	**159694779**	**T/C**	0.97	0.322	0.381	1.15	0.003	0.292	**1.26**	**1.57E-08**	**6.00E-04**
***CRP* rs7553007**	**1**	**159698549**	**G/A**	0.97	0.350	0.414	1.16	0.003	0.296	**1.26**	**1.86E-08**	**9.00E-04**
***CRP* rs4546916**	**1**	**159699249**	**G/T**	0.97	0.354	0.412	1.16	0.003	0.303	**1.26**	**1.87E-08**	**8.00E-04**
***CRP* rs4287174**	**1**	**159703442**	**T/A**	0.97	0.344	0.431	1.16	0.003	0.315	**1.26**	**2.06E-08**	**9.00E-04**
***CRP* rs12037186**	**1**	**159706230**	**A/G**	0.97	0.332	0.342	1.15	0.004	0.264	**1.26**	**2.01E-08**	**5.00E-04**
				Interaction test for SFA			% cal. from SFA < 9.0 (n = 2,306)			% cal. from SFA ≥ 9.0 (n = 7,873)		
***CRPP1* rs2592887**	**1**	**159652939**	**C/T**	0.99	0.725	0.053	1.15	0.033	0.008	**1.21**	**1.27E-08**	**0.007**
***CRPP1* rs1470515**	**1**	**159653599**	**C/T**	0.99	0.787	0.023	1.18	0.015	0.002	**1.22**	**4.70E-09**	**0.010**
***CRPP1* rs2592902**	**1**	**159655726**	**G/T**	0.99	0.773	0.022	1.18	0.015	0.002	**1.22**	**4.59E-09**	**0.008**
***CRPP1* rs2808624**	**1**	**159665921**	**C/G**	0.99	0.741	0.028	1.17	0.017	0.002	**1.22**	**3.53E-09**	**0.010**
***CRPP1* rs11265257**	**1**	**159668984**	**C/T**	0.99	0.730	0.029	1.17	0.018	0.002	**1.22**	**3.17E-09**	**0.010**
***CRPP1* rs876537**	**1**	**159674933**	**C/T**	0.99	0.730	0.032	1.17	0.017	0.002	**1.22**	**2.73E-09**	**0.011**
***CRPP1* rs2808628**	**1**	**159676011**	**G/A**	1.01	0.854	0.037	1.24	0.002	0.010	**1.23**	**4.53E-09**	**7.00E-04**
***CRPP1* rs2808629**	**1**	**159676796**	**G/A**	1.01	0.766	0.041	1.24	0.002	0.010	**1.22**	**7.62E-09**	**0.001**
***CRPP1/CRP* rs2794520**	**1**	**159678816**	**C/T**	1.01	0.732	0.033	1.24	0.002	0.009	**1.22**	**6.36E-09**	**0.001**
***CRP* rs1205**	**1**	**159682233**	**C/T**	1.01	0.736	0.049	1.24	0.002	0.013	**1.22**	**9.46E-09**	**0.002**
***CRP* rs3091244**[e]	**1**	**159684665**	**G/A**	0.99	0.826	0.266	0.81	0.002	0.152	**0.83**	**3.12E-08**	**9.00E-04**
***CRP* rs2027471**[e]	**1**	**159689388**	**T/A**	1.01	0.758	0.018	1.23	0.003	0.010	**1.21**	**2.74E-08**	**0.001**
***CRP* rs1341665**[e]	**1**	**159691559**	**G/A**	1.01	0.797	0.018	1.22	0.004	0.010	**1.21**	**2.49E-08**	**0.002**
***CRP* rs2211320**	**1**	**159693605**	**G/A**	1.01	0.677	0.027	1.26	0.001	0.009	**1.23**	**9.94E-09**	**0.002**
***CRP* rs7551731**[e]	**1**	**159694779**	**T/C**	1.01	0.659	0.031	1.25	0.002	0.009	**1.22**	**1.82E-08**	**0.003**
***CRP* rs7553007**[e]	**1**	**159698549**	**G/A**	1.02	0.636	0.029	1.25	0.001	0.009	**1.22**	**2.04E-08**	**0.005**
***CRP* rs4546916**[e]	**1**	**159699249**	**G/T**	1.02	0.639	0.028	1.25	0.001	0.009	**1.22**	**1.99E-08**	**0.005**
***CRP* rs4287174**[e]	**1**	**159703442**	**T/A**	1.01	0.662	0.026	1.25	0.002	0.009	**1.22**	**2.30E-08**	**0.004**
***CRP* rs4428887**[e]	**1**	**159703462**	**A/G**	1.01	0.753	0.014	1.23	0.003	0.010	**1.21**	**4.50E-08**	**0.003**
***CRP* rs12037186**[e]	**1**	**159706230**	**A/G**	1.02	0.581	0.017	1.25	0.002	0.007	**1.22**	**3.00E-08**	**0.002**
***HNF1A-AS1* rs11065365**	**12**	**121392040**	**G/A**	1.13	0.0003	0.701	**0.64**	**5.91E-10**	**0.461**	0.87	0.0002	0.460
***HNF1A-AS1* rs7953249**	**12**	**121403724**	**G/A**	1.10	0.002	0.766	**0.68**	**7.19E-09**	**0.444**	0.88	0.0001	0.304
***HNF1A-AS1* rs7135337**	**12**	**121404155**	**A/C**	1.10	0.002	0.763	**0.67**	**5.16E-09**	**0.431**	0.87	6.09E-05	0.319
***HNF1A-AS1* rs1920792**	**12**	**121404584**	**C/T**[f]	0.91	0.002	0.544	**1.45**	**7.64E-09**	**0.208**	1.12	0.0004	0.440
***HNF1A-AS1* rs10774579**	**12**	**121405210**	**C/T**	0.91	0.002	0.546	**1.43**	**2.92E-08**	**0.075**	1.12	0.0004	0.435
***HNF1A-AS1* rs2393792**[e]	**12**	**121406293**	**A/G**	0.91	0.002	0.568	**1.43**	**4.67E-08**	**0.061**	1.12	0.001	0.572
***HNF1A-AS1* rs2255531**	**12**	**121414915**	**A/G**	1.11	0.002	0.977	**0.67**	**1.05E-08**	**0.913**	0.86	2.73E-05	0.133
***HNF1A-AS1* rs7139079**	**12**	**121415293**	**G/A**	1.10	0.001	0.745	**0.66**	**8.91E-10**	**0.631**	0.87	2.04E-05	0.145
***HNF1A-AS1* rs2464190**	**12**	**121415390**	**C/T**	1.11	0.001	0.459	**0.66**	**8.24E-10**	**0.526**	0.87	5.72E-05	0.082
***HNF1A* rs1169289**	**12**	**121416622**	**G/C**	1.09	0.006	0.239	**0.68**	**1.69E-08**	**0.338**	0.87	6.50E-05	0.085

(Continued on the following page)

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Table 5. GWA analysis for the association with CRP, stratified by obesity-related lifestyle factors (physical activity and fat diet). (Cont'd)

SNP	Chr	Position[a]	Allele[b] (Ref/Alt)	Interaction test			Active/less-fat diet group			Inactive/high-fat diet group		
				OR[c]	*P*	Q	OR[c]	*P*[d]	Q	OR[c]	*P*[d]	Q
***HNF1A* rs1169288**	**12**	**121416650**	**C/A**	1.10	0.005	0.907	**0.66**	**2.90E-08**	**0.793**	0.84	1.94E-06	0.160
***HNF1A* rs2244608**	**12**	**121416988**	**G/A**	1.09	0.006	0.944	**0.67**	**2.07E-08**	**0.796**	0.84	9.16E-07	0.291
***HNF1A* rs1169284**	**12**	**121419926**	**C/T**	1.10	0.003	0.941	**0.66**	**6.49E-09**	**0.849**	0.84	1.05E-06	0.242
***HNF1A* rs7979473**	**12**	**121420260**	**A/G**[f]	1.11	0.001	0.696	**0.65**	**2.29E-10**	**0.683**	0.86	1.50E-05	0.158
***HNF1A* rs7979478**	**12**	**121420263**	**A/G**[f]	1.11	0.001	0.656	**0.65**	**1.88E-10**	**0.639**	0.86	9.31E-06	0.299
***HNF1A* rs1183910**	**12**	**121420807**	**A/G**	1.10	0.003	0.904	**0.65**	**3.58E-09**	**0.900**	0.84	1.33E-06	0.236
***HNF1A* rs11065384**	**12**	**121423285**	**T/C**	1.10	0.003	0.908	**0.65**	**4.11E-09**	**0.904**	0.84	1.24E-06	0.226
***HNF1A* rs7970695**	**12**	**121423376**	**G/A**[f]	1.10	0.001	0.570	**0.65**	**3.60E-10**	**0.626**	0.86	1.09E-05	0.149
***HNF1A* rs11065385**	**12**	**121423386**	**A/G**	1.10	0.003	0.902	**0.65**	**4.01E-09**	**0.906**	0.84	1.13E-06	0.228
***HNF1A* rs9738226**	**12**	**121423659**	**A/G**[f]	1.10	0.002	0.616	**0.65**	**4.49E-10**	**0.649**	0.86	1.11E-05	0.146
***HNF1A* rs2393791**	**12**	**121423956**	**C/T**[f]	1.10	0.001	0.543	**0.65**	**3.13E-10**	**0.626**	0.86	1.16E-05	0.141
***HNF1A* rs2393776**	**12**	**121424406**	**G/A**[f]	1.11	0.001	0.669	**0.65**	**1.80E-10**	**0.765**	0.86	8.07E-06	0.218
***HNF1A* rs2243458**	**12**	**121424490**	**T/A**[f]	1.11	0.002	0.824	**0.65**	**1.58E-09**	**0.937**	0.84	1.09E-06	0.300
***HNF1A* rs2393775**	**12**	**121424574**	**G/A**[f]	1.11	0.001	0.666	**0.65**	**1.84E-10**	**0.767**	0.86	8.16E-06	0.223
***HNF1A* rs7310409**	**12**	**121424861**	**A/G**[f]	1.11	0.001	0.665	**0.65**	**1.84E-10**	**0.768**	0.86	8.23E-06	0.222
***HNF1A* rs1169292**	**12**	**121426478**	**T/C**	1.10	0.004	0.732	**0.66**	**1.08E-08**	**0.928**	0.84	1.74E-06	0.295
***HNF1A* rs1169294**	**12**	**121426594**	**A/G**	1.10	0.004	0.734	**0.66**	**1.16E-08**	**0.921**	0.84	1.20E-06	0.284

Note: Only genome-wide significant SNPs included. Numbers in bold face are statistically significant.
Abbreviations: Alt, alternative; Chr, chromosome; PA, physical activity; Q, Cochran Q; Ref, reference.
[a]GRCh 37 coordinated.
[b]Additive genetic model regressed.
[c]GWA analysis for CRP adjusted for age and 10 PCs.
[d]*P* values were adjusted to correct for multiple testing via the Benjamini–Hochberg approach.
[e]SNPs did not reach the genome-wide significant level after multiple testing was corrected.
[f]The results from the additive model are presented; the dominant model had similar effect sizes and *P* values.

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Table 6. GWA analysis for the association with IL6.

SNP	Chr	Position[a]	Allele[b] (Ref/Alt)	Overall analysis/Interaction test OR[c]	P	Q	Nonobese/less-fat diet/Active group OR[c]	P[d]	Q	Obese/high-fat diet/Inactive group OR[c]	P[d]	Q
				Overall analysis								
***DEC1* rs149109490**	**9**	**118330052**	**T/C**	**2.81**	**3.77E-08**	**0.36**						
				Interaction test for WHR			**Non-visceral-obese group (WHR ≤ 0.85, *n* = 7,251)**			**Visceral obese group (WHR > 0.85, *n* = 2,928)**		
***DEC1* rs149109490**	**9**	**118330052**	**T/C**	0.70	0.056	0.169	**3.61**	**3.42E-08**	**0.078**	1.74	0.105	0.770
				Interaction test for SFA			**Less-fat diet group (% cal. from SFA < 9.0, *n* = 2,306)**			**High-fat diet group (% cal. from SFA ≥ 9.0, *n* = 7,873)**		
***DEC1* rs149109490**	**9**	**118330052**	**T/C**	1.14	0.607	0.891	2.01	0.199	0.907	**3.19**	**4.21E-08**	**0.144**
				Interaction test for PA			**Active group (MET ≥ 10, *n* = 4,221)**			**Inactive group (MET < 10, *n* = 5,958)**		
***MAPK1* rs56398890**	**22**	**22190785**	**A/T**	1.16	5.29E-06	0.533	**0.74**	**4.54E-08**	**0.978**	1.00	0.909	0.335
***MAPK1* rs9607320**	**22**	**22202164**	**T/C**	1.17	3.73E-06	0.718	**0.73**	**1.18E-08**	**0.938**	0.99	0.897	0.292

Note: Only genome-wide significant SNPs included in overall and interaction tests (G × E or stratified analysis). Numbers in bold face are statistically significant.
Abbreviations: Alt, alternative; Chr, chromosome; PA, physical activity; Q, Cochran Q; Ref, reference.
[a]GRCh 37 coordinated.
[b]Additive genetic model regressed.
[c]GWA analysis for IL6 adjusted for age and 10 PCs.
[d]*P* values were adjusted to correct for multiple testing via the Benjamini–Hochberg approach.

associated with reduced risk for chronic inflammation (i.e., decreased CRP levels). Furthermore, similar to previous literature (48, 49), we found that the decreased effects of the *HNF1A* SNPs on CRP levels were more profound in the low-fat diet subgroup, conferring the regulating role of CRP on expression of proprotein convertase subtilisin/kexin type-9, a key regulator of low-density lipoprotein (LDL) metabolism, via the p38MAPK–HNF1A pathway in hepatic cells, as recently reported by *in vivo* studies (50, 51). Also, we found novel SNPs at *C12orf43*, which is located downstream of *HNF1A* in a tail-to-tail manner (52), that were associated with a reduced risk for chronic inflammation (i.e., decreased CRP levels); this finding warrants further biological mechanistic study of a link between the particular gene, its mutation, and CPR phenotype.

For many genes discovered by GWASs for metabolic traits, such as chronic inflammation, the mechanism by which the encoded proteins affect disease risk is controversial. Some intergenic or intronic SNPs may affect the function of transcriptional control structures, including enhancers and silencers (53). Of the 88 top markers in our study, 27 in *HNF1A/HNF1A-AS1/C12orf43* displayed the increased risk for breast cancer. Their functional significance and the molecular mechanisms of the target genes that mediated breast carcinogenesis remain unclear. The *HNF1A* gene regulates tissue-specific expression of multiple genes in the liver, pancreas (45), proximal tubule of the kidney (54), and epithelial cells of the intestine (55), and its mutation has been associated with maturity-onset diabetes of the young type 3 (56), hepatocellular adenoma (HCC; ref. 57), and endometrial (58) and pancreatic carcinomas (59). Whether the *HNF1A* gene and its mutation act as a tumor suppressor (59) or an oncogene (55, 60) on the specific types of tumor (HCC and pancreatic cancer) has not been determined. For example, one study (61) reported that *HNF1A* silencing in HCC cells led to overexpression of several genes encoding growth factor receptors, components of translational machinery, cell cycle, and angiogenesis regulators, which promote cell proliferation and suppress apoptosis. Another study (60) presented high *HNF1A* gene expression in pancreatic cancer stem cells and ductal adenocarcinoma. Our study is the first to show that SNPs near *HNF1A/HNF1A-AS1* in overall and viscerally obese subgroups were associated with increased risk for breast cancer, calling for study of the biological molecular mechanisms in the gene–breast cancer pathways.

In relation to increased risk for chronic low-grade inflammation on the basis of IL6, we found one novel SNP near *DEC1*, whose expression was suppressed in esophageal cancer (62), suggestive of a candidate tumor suppressor. *DEC1* mutations' functional implications in other tumor cells have not been determined. In our study, the SNP near *DEC1* had a substantial suppressive effect on breast cancer development, although that effect did not reach statistical significance.

Because of the restrictions of the available data, our study focused on two key inflammatory biomarkers (CRP and IL6) and examined obesity factors at screening in cross-section fashion. In addition, our study was confined to non-Hispanic white postmenopausal women, so the generalizability of our results to other populations is limited. All identified loci contributed to 6.3% of interindividual variability in proinflammatory cytokines. Future DNA methylation studies may address this low heritability. in addition,

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the chronic inflammation pathway involved in breast cancer among postmenopausal women may differ by the cancer's molecular subtype and the exogenous estrogen intake; our study could not delineate such differences due to insufficient statistical power. Finally, some meta-analysis results indicated pleiotropic effects of inflammatory cytokines.

In conclusion, our study suggests that a number of newly identified top signals may exert their effect on chronic low-grade inflammation by interacting with obesity factors and may have implications for breast carcinogenesis in postmenopausal women. Our findings may contribute to better understanding of the molecular genetic associations between proinflammation and cancer and suggest potential intervention strategies, such as body weight control, for women who carry the inflammatory-risk genotypes, thus reducing their risk for breast cancer.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

S.Y. Jung: Conceptualization, resources, data curation, formal analysis, funding acquisition, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **P.A. Scott:** Data curation, software, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. **J.C. Papp:** Conceptualization, resources, software, supervision, validation, writing-original draft, writing-review and editing. **E.M. Sobel:** Conceptualization, resources, data curation, supervision, investigation, writing-original draft, writing-review and editing. **M. Pellegrini:** Conceptualization, resources, supervision, writing-original draft, writing-review and editing. **H. Yu:** Conceptualization, resources, supervision, investigation, writing-original draft, writing-review and editing. **S. Han:** Formal analysis, visualization, methodology, writing-original draft, writing-review and editing. **Z.-F. Zhang:** Conceptualization, resources, supervision, validation, visualization, methodology, writing-original draft, writing- review and editing.

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Cancer Prevention Research

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