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BRIEF REPORT

Genetic variation in TNFα, PPARγ, and IRS-1 genes, and their association with breast-cancer survival in the HEAL cohort

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Abstract

Purpose Tumor necrosis factor-α (TNF-α), peroxisome proliferator-activated receptor-γ (PPARγ), and insulin receptor substrate-1 (IRS-1) are associated with obesity, insulin resistance, and inflammation. Few data exist on associations between polymorphisms in these genes and mortality in breast cancer survivors.

Methods We investigated associations between TNF-α $^{-308}$G > A (rs1800629); PPARγ Pro^{12}Ala (rs1801282); and IRS-1 Gly^{972}Arg (rs1801278) polymorphisms and anthropometric variables, circulating levels of previously measured biomarkers, and tumor characteristics in 553 women enrolled in the Health, Eating, Activity, and Lifestyle Study, a multiethnic, prospective cohort study of women diagnosed with stage I–IIIA breast cancer between 1995 and 1999 (median follow-up 14.7 years). Using Cox proportional hazards models adjusted for possible confounders, we evaluated associations between these polymorphisms and mortality.

Results Carriers of the PPARγ variant allele had statistically significantly lower rates of type 2 diabetes ($P = 0.04$), lower BMI ($P = 0.01$), and HOMA scores [$P = 0.004$; non-Hispanic White (NHWs) only]; carriers of the TNF-α variant A allele had higher serum glucose ($P = 0.004$, NHW only); and the IRS-1 variant was associated with higher leptin levels ($P = 0.003$, Hispanics only). There were no associations between any of the polymorphisms and tumor characteristics. Among 141 deaths, 62 were due to breast cancer. Carriers of the TNF-α-variant A allele had a decreased risk of breast-cancer-specific mortality [hazard ratio (HR) 0.30; 95% confidence interval (CI) 0.10–0.83] and all-cause mortality (HR 0.51; 95% CI 0.28–0.91).

Conclusions Neither the PPARγ nor the IRS-1 polymorphism was associated with mortality outcome. The TNF-α $^{-308}$ G > A polymorphism was associated with reduced breast-cancer-specific and all-cause mortality.

Keywords PPARγ IRS-1 TNF-α breast-cancer-specific mortality · All-cause mortality

Introduction

Overweight and obesity are associated with a state of chronic systemic inflammation associated with poor prognosis in breast cancer [1–3].

Tumor Necrosis Factor alpha (TNF-α) is a cytokine which, when chronically produced, can act as a tumor promoter in breast cancer via a number of mechanisms including increased activity of aromatase [4], enhanced angiogenesis, and altered cytokine production that creates a more pro-inflammatory state [5]. It is upregulated in people with overweight/obesity, and plays a role in the development of insulin resistance [6, 7]. Some studies have reported that the − 308 G > A polymorphism, located in the 3′ UTR, can alter TNF-α expression levels, with the A allele associated with high constitutive and inducible levels of TNF-α. [8, 9].

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The peroxisome proliferator-activated receptor-gamma (PPARγ), a heterodimer, is a candidate tumor suppressor gene, and a member of the nuclear hormone receptor superfamily that plays a critical role in a variety of biological processes, including regulating adipocyte differentiation, glucose and lipid homeostasis, intracellular insulin-signaling events, and insulin sensitization [10]. The proline to alanine ($Pro^{12}Ala$) single-nucleotide polymorphism is a functional variant, with the Ala allele associated with reduced transactivation activity of PPARγ [11] The variant allele is associated with lower BMI, reduced risk of type 2 diabetes, lower fasting serum glucose levels. [11–13], and lower rates of telomere shortening compared with the Pro/Pro genotype [14]. Results from studies investigating the association between the $Pro^{12}Ala$ polymorphism and breast cancer risk have been conflicting: some studies reported a marginally increased risk associated with the Ala genotype [15, 16], although others did not [17, 18]. To our knowledge, the only study examining the association between this polymorphism and mortality was a large cohort of healthy men and women, which found no association with any cancer-associated mortality [19].

Insulin receptor substrate (IRS)-1 is one of multiple proteins that mediate signal transduction of the activated insulin receptor [20]. It is activated by numerous growth factor receptors, and TNF-α can both decrease the expression of IRS-1 and increase phosphorylation, impairing its ability to bind to the insulin receptor and initiate downstream signaling [21]. IRS-1 plays a central role in insulin sensitivity, and association studies have shown that the IRS-1 $Gly^{972}Arg$ variant is a risk factor for insulin resistance, particularly in obese patients [22, 23]. To our knowledge, no studies have investigated the association between IRS-1 polymorphisms and mortality in breast-cancer survivors.

Here, we evaluate the association between TNF-α $^{-308}$ G > A (rs1800629); PPARγ $Pro^{12}Ala$ (rs1801282); and IRS-1 $Gly^{972}Arg$ (rs1801278) polymorphisms and risks of both breast-cancer-specific and all-cause mortality, in a cohort of 553 women enrolled in the Health, Eating, Activity, and Lifestyle (HEAL) Study, a multiethnic, prospective cohort study of women diagnosed with stage I–IIIA breast cancer.

Materials and methods

Study setting, participants, and recruitment

The Health, Eating, Activity, and Lifestyle (HEAL) Study is a population-based, multicenter, multiethnic prospective cohort study that has enrolled 1,183 women diagnosed with breast cancer to evaluate whether diet, weight, physical activity, or other exposures affect breast-cancer prognosis. The aims, study design and recruitment procedures have been published previously.[24]. In brief, women diagnosed with Stage 0 (in situ) to Stage IIIA breast cancer were recruited into the HEAL study through surveillance, epidemiology, and end results (SEER) registries in New Mexico (NM), Los Angeles County (CA), and western Washington (WA). Baseline surveys were conducted on average 6 months after diagnosis. In NM, we recruited 615 women, 18 years or older; in WA, 202 women, aged between 40 and 64 years; and in CA, 366 Black women, aged between 35 and 64 years, who had participated in the Los Angeles portion of the Women's Contraceptive and Reproductive Experiences Study, or in a study of in situ breast cancer. Recruitment was restricted in WA to women aged 40–64 years because of competing studies, and in CA to women aged 35–64 at diagnosis because of design of the parentstudies. Recruitment occurred from 1996 to 1999.

The HEAL study was performed with the approval of Institutional Review Boards of participating centers, in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each participant. Seven hundred fifty-three women had been genotyped. We excluded 175 women with a diagnosis of Stage 0 (in situ) disease given the low likelihood of mortality in these women, and 25 with nonfatal breast-cancer events < 9 months before their 30-month interview to avoid potential confounding from possible recent treatment. The final sample size is 553. During a median follow-up period of 14.7 years, 141 deaths occurred, of which 62 were due to breast cancer.

Data collection and covariates

Specimens

A 30-mL fasting blood sample collected from patients at their baseline and 24-month interviews was processed within 3 h of collection, and stored at – 80 °C until analysis. DNA was extracted using Qiagen Midi-prep columns from buffy coat preparations.

SNP analysis

TNFα IRS-1 and PPARGγ were genotyped using the Taqman allelic discrimination method with the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). We included 10% replicate samples and genotype concordance was 100%.

Covariates

Standardized information, including medical history, anthropometrics, demographic and lifestyle information,

was collected during interviews at baseline and 24 months. Disease stage, estrogen (ER) receptor status, and adjuvant therapy details were abstracted from medical records. A 4-category race/ethnicity/study-site variable was created as race/ethnicity and study-site were highly correlated: Non-Hispanic whites in NM; non-Hispanic whites in WA; Hispanics; and African Americans (AA). Serum measurements from fasting blood at 24 months postdiagnosis of glucose, insulin, adiponectin, and C-reactive protein (CRP) were measured as described previously [1, 3, 25, 26].

Stage of disease and cancer treatment

Participants were classified as having Stage 0 (in situ), Stage I (localized), or Stage II–IIIA (regional) breast cancer based on AJCC stage of disease classification contained within SEER registry records. This analysis includes only women having Stage I–IIIa diagnoses. Estrogen receptor (ER) status of tumors was categorized as positive, negative, or unknown/borderline; HER2/neu status was unavailable for the majority of participants. Clinical data were obtained from medical record reviews. Treatment was categorized into three groups: surgery only, surgery plus radiation only, or surgery with any chemotherapy with or without radiation.

Outcome assessment

Information on vital status was obtained from SEER records. Cause-of-death codes were acquired from linkages with relevant SEER databases, which obtain data from state and the National Death Index. If alive, individuals were followed through their last follow-up assessment or SEER vital status update, whichever was the most recent. All-cause mortality was defined as time from the baseline interview to death from any cause, or end of follow-up (December 31, 2012). Breast-cancer-specific mortality was defined as death from breast cancer or end of follow-up, with the same intervals as for all-cause mortality.

Statistical analysis

Due to the low frequency of the homozygous variant genotypes for all three polymorphisms, heterozygous and homozygous variants were combined for comparison with the homozygous wild-type (WT; recessive model): TNF-α − 308 GG versus GA + AA; PPARγ Pro^{12}Ala Pro/Pro versus Pro/Ala + Ala/Ala; and IRS-1 Gly^{972}Arg Gly/Gly versus Gly/Arg + Arg/Arg.

Differences in distribution of continuous variables between genotypes were estimated using the Kruskall–Wallis test, stratified by race/ethnicity. To apply Bonferroni correction for multiple testing for each genotype, statistical significance was set at 0.05/7 = 0.007. Differences in categorical variables were compared using the χ^2 test. Hazard ratios (HR) and 95% confidence intervals (CI) for breast-cancer-specific or all-cause mortality rates were based on the partial likelihood for Cox's proportional hazards model [27]. The proportional hazard assumption was tested using Schoenfeld residuals, and no violation of the proportionality assumption was found. Age in days was used as the underlying time variable, with entry and exit time defined as the participant's age at the baseline interview, and age at death from either breast cancer or any cause, or end of follow-up, respectively.

We based variable inclusion in the Cox models on a log-likelihood ratio test, with the following covariates: race/ethnicity/study-site; BMI (categorical < 18.5 kg/m^2; ≥ 18.5 and < 25 kg/m^2; ≥ 25 and < 40 kg/m^2; ≥ 40 kg/m^2); summary tumor stage (local vs. regional), and treatment received at diagnosis (surgery; surgery + radiotherapy; chemotherapy). We included the race/ethnicity/study-site variable to adjust for different distributions of race/ethnicity by study site. Covariates considered but not included in the final model (as they did not significantly change the likelihood ratio score) were menopausal status, education, smoking status, tamoxifen use, ER status, and HOMA scores.

The Wald statistic was used to test for trend across levels. All *P* values are two-sided. Analyses were performed using STATA 11 (Statacorp, TX USA).

Results

Allele frequencies for the variant alleles of PPARγ Pro^{12}Ala, TNF-α $^{-308}$ G > A, and IRS-1 Gly^{972}Arg polymorphisms were 0.9, 0.7, and 0.2%, respectively (Table 1). Both TNF-α $^{-308}$ G > A and IRS-1 Gly^{972}Arg polymorphisms were in Hardy–Weinberg (HW) equilibrium ($P > 0.05$), but PPARγ was not. However, according to the dbSNP National Center for Biotechnology Information (NCBI) database, the PPARγ Pro^{12}Ala SNP was not in HW equilibrium in individuals of Hispanic heritage; when we excluded Hispanics from our group, the SNP was in equilibrium ($P > 0.05$).

African Americans were more likely to be carriers of the PPARγ Pro allele (96.1%) compared with other Hispanics (80.6%) and NHW (75.5%; $P < 0.0001$, Tables 1, 2). IRS-1 and TNF-α genotypes were unrelated to racial/ethnic group.

We compared the distribution of genotypes by estrogen receptor status (ER), BMI (</≥ 25 kg/m^2), tumor stage (local vs. regional), and the presence or absence of type 2 diabetes. Carriers of the PPARγ Ala allele were statistically significantly more likely to have a BMI ≤ 25 kg/m^2 (Pro/Pro 33.6% vs. Pro/Ala 46.9%; $P = 0.01$; Table 2), and were less likely to have type 2 diabetes (Pro/Pro, 10.8%, vs. Pro/Ala, 4.1%; $P = 0.04$). PPARγ, IRS-1, and TNF-α genotypes were

Table 1 Characteristics of the health, eating, activity, and lifestyle (HEAL) cohort ($N = 553$)

	All N^a (%)	Non-hispanic white N (%)	African American N (%)	Hispanic N (%)
	553	318	156	62
Study site				
Western Washington	103 (18.6%)	87 (27.4%)	0	2 (3.2%)
New Mexico	294 (53.2%)	231 (72.6%)	0	60 (96.8%)
Los Angeles	156 (28.2%)	0	156 (100.0%)	0
Body mass index (kg/m^2)				
Mean (SD)	27.9 (6.5)	26.5 (5.7)	30.8 (7.5)	26.9 (4.6)
Age (years)				
Mean (SD)	57.5 (10.7)	60.3 (11.0)	52.3 (7.8)	56.3 (11.3)
Menopausal status at blood draw				
Premenopausal	86 (15.6%)	44 (13.8%)	28 (17.9%)	13 (21.0%)
Postmenopausal	428 (77.3%)	261(82.1%)	112 (71.8%)	43 (69.4%)
Unknown	39 (7.1%)	13 (4.1%)	16 (10.3%)	6 (9.6%)
Estrogen receptor status				
Negative	112 (20.3%)	44 (13.8%)	53 (34.0%)	14 (22.6%)
Positive	389 (70.3%)	247 (77.7%)	91 (58.3%)	37 (59.7%)
Unknown	52 (9.4%)	27 (8.5%)	12 (7.7%)	11 (17.7%)
SEER summary stage				
Local	393 (71.1%)	247 (77.7%)	88 (56.4%)	47 (75.8%)
Regional	160 (28.9%)	71 (22.3%)	68 (43.6%)	15 (24.2%)
Treatment at diagnosis				
Surgery	129 (23.3%)	70 (22.0%)	39 (25.0%)	18 (29.0%)
Surgery and radiotherapy	207 (37.4%)	141 (44.3%)	37 (23.7%)	22 (35.5%)
Any chemotherapy	217 (39.2%)	107 (33.7%)	80 (51.3%)	22 (35.5%)
TNFα $^{-308}$ G > A				
Wildtype (GG)	469 (84.8%)	274 (86.2%)	124 (79.5%)	54 (87.1%)
Heterozygous (GA)	80 (14.5%)	41 (12.9%)	31 (19.8%)	8 (12.9%)
Homozygous (AA)	4 (0.7%)	3 (0.9%)	1 (0.6%)	0
PPARγ Pro^{12} Ala				
Wildtype (Pro/Pro)	455 (82.3%)	240 (75.5%)	150 (96.2%)	50 (80.7%)
Heterozygous (Pro/Ala)	93 (16.8%)	73 (23.0%)	6 (3.8%)	12 (19.3%)
Homozygous (Ala/Ala)	5 (0.9%)	5 (1.5%)	0	0
Insulin receptor substrate 1 Gly (G)^{972}Arg (R)				
Wildtype (GG)	487 (88.1%)	277 (87.1%)	149 (89.2%)	57 (91.9)
Heterozygous (GR)	65 (11.7%)	41 (12.9%)	17 (10.2%)	5 (8.1%)
Homozygous (RR)	1 (0.2%)	0	1 (0.6%)	0

SEER surveillance, epidemiology and end results

[a] 17 patients were described as 'other race'; this accounts for the differences in numbers between racial/ethnic subgroups and the overall total

unrelated to tumor stage or ER status at diagnosis, across any racial or ethnic group (data not shown).

We next examined associations between genotypes and serum levels of inflammatory biomarkers (CRP and SAA); insulin and glucose; the adipokines adiponectin and leptin measured at 30-months post diagnosis, and baseline BMI (continuous). After Bonferroni correction, NHW carriers of the PPARγ Ala allele had statistically significantly lower HOMA scores (1.5 vs. 2.1, $P = 0.004$), (Table 3). NHW carriers of the TNF-α variant A allele had statistically significantly higher serum concentrations of glucose (100.7 vs. 91.8 mg/dL; $P = 0.004$). Finally, Hispanic participants with the IRS Arg variant allele had higher circulating levels of leptin (37.4 vs. 20.1 μg/mL, $P = 0.003$). While results did not reach statistical significance, we observed higher serum levels of insulin and higher HOMA scores among NHW carriers of the variant IRS Arg allele. There were no associations between any of

Table 2 Associations between single-nucleotide polymorphisms (SNPs) and baseline participant characteristics

	TNF-α G $^{-308}$ A		PPARγ Pro^{12}Ala		IRS-1 Gly^{972}Arg	
	GG $N = 469$ (%)	GA/AA $N = 84$ (%)	Pro/Pro $N = 455$ (%)	Pro/Ala Ala/Ala $N = 98$ (%)	GG $N = 487$ (%)	GR/RR $N = 66$ (%)
Race/ethnicity[a]						
NHW	274 (86.2)	44 (13.8)	240 (75.5)	78 (24.5)	277 (87.1)	41 (12.9)
Hispanic	54 (87.1)	8 (12.9)	50 (80.6)	12 (19.4)	57 (91.9)	5 (8.1)
African American	124 (79.5)	32 (20.5)	150 (96.1)	6 (3.9)	138 (88.5)	18 (11.5)
	$P = 0.14$		$P = <0.0001$		$P = 0.55$	
Body mass index (kg/m^2)						
< 25	173 (36.9)	26 (31.0)	153 (33.6)	46 (46.9)	179 (36.8)	20 (30.3)
> 25	296 (63.1)	58 (69.0)	302 (66.4)	52 (53.1)	308 (63.2)	46 (6.7)
	$P = 0.29$		$P = 0.01$		$P = 0.31$	
Type 2 diabetes[b]						
No	419 (89.3)	77 (91.7)	403 (88.6)	93 (94.9)	436 (89.5)	60 (90.9)
Yes	47 (10.0)	6 (7.1)	49 (10.8)	4 (4.1)	47 (9.7)	6 (9.1)
	$P = 0.41$		$P = 0.04$		$P = 0.87$	

Chi squared test

[a] 17 participants were described as 'other race'; they were excluded from analysis

[b] Diabetic status of 4 participants is unknown; they were excluded from analyses

the polymorphisms studied and biomarkers among African Americans.

Compared with TNF-α WT GG homozygotes, carriers of the A variant allele had a statistically significant reduced risk of breast-cancer-specific (HR 0.30 95% CI 0.10–0.83) and all-cause mortality (HR 0.51 95% CI 0.28–0.91) in a model adjusted for tumor stage, treatment at diagnosis, race/ethnicity, and BMI (Table 4). Similar results were observed when data were censored at 10 years postdiagnosis (HR 0.12 95% CI 0.02–0.86; HR 0.28 95% CI 0.10–0.77, respectively).

Neither the PPARγ Ala allele nor the IRS-1 polymorphism was associated with all-cause or breast-cancer-specific mortality.

Discussion

Here, we report that breast-cancer survivors who were carriers of the TNF-α variant A allele had a statistically significantly reduced risk of both breast-cancer-specific mortality and all-cause mortality. Neither the PPARγ Pro^{12}Ala polymorphism nor the IRS-1 polymorphism was associated with outcome.

The TNF-α − 308 G > A polymorphism, located in the 3′ UTR, alters TNF-α expression levels; [8, 9] while the A allele is associated with high constitutive and inducible levels of TNF-α [9]. TNF-α has diverse roles in homeostasis and disease pathogenesis [28], and its role in cancer etiology is complex. In its soluble or membrane-bound form, it binds to two transmembrane receptor molecules—TNFR1 (a death domain containing protein) and TNFR2. Ligand–receptor interactions trigger a variety of signaling pathways including NF-κB, mitogen-activated protein kinase (MAPK), c-Jun-terminal kinase (JNK), and cytotoxic cell-death via TNFR1 signaling [28–30]. Thus, while the inverse association between the variant allele and mortality is surprising, the complex downstream signaling effects of TNF-α receptor binding make it difficult to predict its effects in breast-cancer survivors.

Some studies, although not all [31], have reported associations with breast-cancer risk and the − 308 polymorphism; [32–35] furthermore, a meta-analysis based on 11 studies with 10,184 cases and 12,911 controls, demonstrated a statistically significant increased breast-cancer risk for GG vs. GA + AA (recessive model; odds ratio (OR) 1.10 95% CI 1.04–1.17) [36]. The study reported that while risk was not statistically significantly associated with any genotype in combined worldwide populations, stratification by race/ethnicity demonstrated that the TNF-α $^{-308}$ A allele was associated with a decreased risk of breast cancer versus the G allele in Caucasian individuals (OR 0.93, 95% CI 0.88–0.98). However, in women of African descent only, the TNF-α $^{-308}$ AA genotype was statistically significantly associated with increased risk, compared with the GG or GA genotype (A/A vs. G/G, OR 4.09 95% CI 1.46–11.43; A/A vs. G/A OR 4.86 95% CI 1.75–13.53; A/A vs. G/A + G/G OR 4.25 95% CI 1.55–11.63) [37].

Table 3 Associations between polymorphisms in TNF-α, PPARγ, IRS-1, and serum biomarker levels and body mass index (BMI), stratified by race/ethnicity

Non-hispanic white	TNFα G $^{-308}$A		P^{a}	PPARγ Pro^{12}Ala		P^{a}	IRS-1 Gly^{972}Arg		P^{a}
	GG $N = 274$	GA/AA $N = 44$		Pro/Pro $N = 240$	Pro/Ala Ala/Ala $N = 78$		GG $N = 277$	GR/RR $N = 41$	
	Mean analyte (SD)	Mean analyte (SD)		Mean analyte (SD)	Mean analyte (SD)		Mean analyte (SD)	Mean analyte (SD)	
Insulin μIU/mL$^{N = 315}$	8.2 (7.9)	8.0 (5.8)	0.85	8.6 (8.3)	6.9 (5.0)	0.02	7.9 (7.5)	10.1 (8.1)	0.02
Glucose mg/dL$^{N = 311}$	91.8 (24.3)	100.7 (21.7)	0.004	93.9 (23.4)	90.1 (26.2)	0.10	92.3 (22.1)	97.7 (34.5)	0.52
HOMA score$^{b\ N = 308}$	1.98 (2.8)	2.1 (1.6)	0.24	2.1 (3.0)	1.5 (1.2)	0.004	1.9 (2.7)	2.6 (2.7)	0.03
Adiponectin μg/mL$^{N = 317}$	19.2 (10.4)	17.9 (8.3)	0.63	18.8 (9.8)	19.8 (11.1)	0.76	19.1 (10.1)	18.2 (9.9)	0.56
Leptin μg/mL$^{N = 318}$	20.9 (15.0)	23.5 (16.8)	0.53	21.8 (15.6)	19.5 (13.9)	0.32	21.1 (15.2)	22.1 (15.9)	0.87
CRP^{c}μg/mL$^{N = 312}$	3.1 (3.9)	3.2 (4.3)	0.71	3.2 (3.8)	2.9 (4.5)	0.08	3.1 (4.0)	2.9 (3.6)	0.98
BMI kg/m$^{2\ N = 318}$	25.8 (5.3)	26.5 (6.5)	0.93	26.1 (5.7)	25.4 (4.6)	0.67	26.4 (5.5)	27.4 (6.7)	0.62
Hispanic	TNFα G $^{-308}$A		P^{a}	PPARγ Pro^{12}Ala		P^{a}	IRS-1 Gly^{972}Arg		P^{a}
	GG $N = 54$	GA/AA $N = 8$		Pro/Pro $N = 50$	Pro/Ala Ala/Ala $N = 12$		GG $N = 57$	GR/RR $N = 5$	
Insulin μIU/mL$^{N = 61}$	10.1 (10.9)	6.4 (2.6)	0.23	10.2 (11.3)	7.4 (3.0)	0.54	9.7 (10.7)	8.7 (4.3)	0.81
Glucose mg/dL$^{N = 60}$	90.5 (24.6)	79.2 (13.5)	0.22	89.8 (26.0)	86.3 (10.7)	0.68	89.3 (24.7)	85.8 (1.8)	0.74
HOMA score$^{b\ N = 60}$	2.5 (3.4)	1.3 (0.5)	0.23	2.5 (3.6)	1.6 (0.8)	0.75	2.3 (3.4)	1.8 (0.9)	0.71
Adiponectin μg/mL$^{N = 62}$	14.1 (6.9)	15.7 (7.8)	0.56	14.1 (7.3)	15.2 (5.9)	0.61	14.1 (7.1)	16.5 (6.0)	0.50
Leptin μg/mL$^{N = 62}$	22.3 (11.0)	16.5 (7.6)	0.26	21.4 (10.9)	22.0 (10.8)	0.76	20.1 (9.8)	37.4 (9.9)	0.003
CRP^{c}μg/mL$^{N = 61}$	4.2 (4.9)	5.0 (9.3)	0.30	4.3 (5.1)	4.4 (7.7)	0.29	4.3 (5.8)	4.5 (3.1)	0.31
BMI kg/m$^{2\ N = 62}$	26.6 (5.0)	26.2 (5.0)	0.67	26.1 (4.5)	28.5 (6.3)	0.17	26.5 (4.5)	31.9 (2.7)	0.02
African American	TNFα G $^{-308}$A		P^{a}	PPARγ Pro^{12}Ala		P^{a}	IRS-1 Gly^{972}Arg		P^{a}
	GG $N = 124$	GA/AA $N = 32$		Pro/Pro $N = 150$	Pro/Ala Ala/Ala $N = 6$		GG $N = 138$	GR/RR $N = 18$	
Insulin μIU/mL$^{N = 155}$	16.9 (18.5)	12.0 (7.2)	0.29	14.9 (13.7)	39.9 (50.2)	0.02	16.0 (17.3)	15.0 (13.8)	0.54
Glucose mg/dL$^{N = 153}$	140.1 (79.3)	121.2 (35.0)	0.32	136.8 (74.2)	121.7 (17.3)	0.97	133.8 (69.3)	154.8 (95.8)	0.84
HOMA score$^{b\ N = 152}$	6.1 (7.8)	3.8 (2.9)	0.29	5.4 (6.4)	12.8 (16.9)	0.07	5.4 (6.4)	7.6 (11.4)	0.74
Adiponectin μg/mL$^{N = 155}$	12.9 (10.8)	8.8 (5.4)	0.10	12.3 (10.2)	8.3 (5.2)	0.44	11.9 (10.4)	14.0 (7.6)	0.11
Leptin μg/mL$^{N = 156}$	33.3 (22.8)	33.4 (19.9)	0.67	33.1 (22.3)	39.0 (21.3)	0.37	34.0 (22.2)	27.7 (22.2)	0.12
CRP^{c}μg/mL$^{N = 156}$	7.0 (15.5)	5.5 (7.2)	0.54	6.6 (14.4)	8.7 (8.9)	0.69	6.8 (14.9)	5.7 (6.7)	0.95
BMI kg/m$^{2\ N = 156}$	30.7 (7.9)	30.9 (6.0)	0.66	30.7 (7.3)	31.4 (12.4)	0.85	31.0 (7.5)	28.8 (7.0)	0.13

[a]Kruskall–Wallis test

[b]*HOMA* homeostatic model assessment

[c]*CRP* C-reactive protein

Table 4 Associations between polymorphisms in TNF-α, PPARγ, and IRS-1 and breast-cancer-specific and all-cause mortality

Genotype	Events/*N* total	Unadjusted		Adjusted[a]	
		Hazard ratio	95% confidence intervals	Hazard ratio	95% confidence intervals
Breast-cancer mortality					
TNF-α G $^{-308}$A					
GG (wildtype)	58/469	1.00	Ref.	1.00	Ref.
GA/AA	4/84	0.36	0.13–0.99	0.30	0.10–0.83
P^b		0.05		0.02	
PPAR γ Pro^{12}Ala					
Pro/Pro	53/455	1.00	Ref.	1.00	Ref.
Pro/Ala Ala/Ala	9/98	0.83	0.41–1.70	1.23	0.58–2.61
P^b		0.63		0.58	
IRS-1 Gly^{972}Arg					
Gly/Gly	57/487	1.00	Ref.	1.00	Ref.
Gly/Arg Arg/Arg	5/66	0.66	0.27–1.66	0.69	0.27–1.73
P^b		0.38		0.44	
All cause mortality					
TNF-α G $^{-308}$A					
GG (Wildtype)	128/469	1.00	Ref.	1.00	Ref.
GA/AA	13/84	0.64	0.36–1.14	0.51	0.28–0.91
P^b		0.13		0.03	
PPAR γ Pro^{12}Ala					
Pro/Pro	115/455	1.00	Ref.	1.00	Ref.
Pro/Ala Ala/Ala	26/98	1.07	0.69–1.64	1.42	0.91–2.23
P^b		0.75		0.12	
IRS-1 Gly^{972}Arg					
Gly/Gly	128/487	1.00	Ref.	1.00	Ref.
Gly/Arg Arg/Arg	13/66	0.79	0.45–1.41	0.83	0.46–1.79
P^b		0.43		0.54	

[a]Adjusted for race/ethnicity/study-site; BMI (categorical < 18.5 kg/m^2; ≥ 18.5 and < 25 kg/m^2; ≥ 25 and < 40 kg/m^2; ≥ 40 kg/m^2); SEER (Surveillance, Epidemiology, and End Results Program) summary tumor stage (local vs. regional) and treatment received at diagnosis (surgery; surgery + radiotherapy; chemotherapy)

[b]Wald test for trend

Few studies have been conducted on the association between the TNF-α polymorphism and breast-cancer prognosis. In contrast to our findings, a Tunisian case–control study of 243 cases and 174 controls found that the AA genotype was associated with reduced disease-free survival (relative risk (RR) 2.75; $P = 0.01$) and shorter overall survival (RR 4.08; $P = 0.01$) [32]. In a study of 80 patients with breast cancer, the polymorphism was not associated with either outcome [38]. Finally, obesity and circulating insulin levels were statistically significantly associated with the TNF-α $^{-308}$ polymorphism in a meta-analysis, with the carriers of the rare allele more likely to have obesity and higher HOMA scores [39]. In contrast, we found no such associations.

In addition to its role in regulating adipocyte differentiation, glucose and lipid homeostasis, intracellular insulin-signaling events, and insulin sensitization [10], PPARγ also possesses anti-inflammatory properties. PPARγ antagonizes the AP-1, STAT, and NF-κB pathways, thereby inhibiting initiation of the inflammatory response [40, 41]. This is especially relevant to breast-cancer survivors, in whom increased inflammatory biomarker levels have been associated with poor survival [3]. PPARγ also inhibits the expression of aromatase, an enzyme involved in estrogen biosynthesis, which may support the hypothesis that PPARγ may play a role in the etiology of breast cancer [42]. Finally, specific agonists for PPARγ induce differentiation and suppress markers of malignancy in breast-cancer cells in vitro [43]. Similar to studies in healthy individuals [11–13], we report lower mean BMI, lower mean concentrations of serum glucose and leptin, and higher mean concentrations of adiponectin in breast-cancer survivors who are carriers

of the PPARγ variant Ala allele. Our findings of lower rates of type 2 diabetes, lower BMI, and improved insulin sensitivity among carriers of the variant Ala allele are consistent with other studies, again, in individuals without a history of cancer [11, 12, 44].

Despite its statistically significant association with HOMA scores which we have previously demonstrated are associated with breast-cancer-specific and all- cause mortality in this cohort [1], we found no associations between the Pro^{12}Ala polymorphism and outcome. Some studies have investigated the association of PPARγ Pro^{12}Ala polymorphism with breast-cancer risk: and some, although not all [17, 18], have found a marginally increased risk of breast cancer associated with the Ala genotype [15, 16]. In another study, the Ala allele was associated with lower risk of breast cancer, but the authors attributed this to high alcohol consumption and an interaction between alcohol and PPARγ Pro^{12}Ala: alcohol intake was associated with 21% increased risk of breast cancer among homozygous carriers of the wild-type Pro-allele [15]. In contrast, a case–control study nested within the prospective Nurses' Health Study of 725 incident cases of breast cancer and 953 matched controls, found no significant association between the PPARγ Pro^{12}Ala polymorphism and either risk of incident breast cancer, plasma hormones, plasma cholesterol, BMI, weight gain since age 18 years, or waist-to-hip ratio [17].

IRS-1 plays a central role in insulin sensitivity, and association studies have shown that the IRS-1 Gly^{972}Arg variant is a risk factor for insulin resistance, particularly in obese patients; [22, 23] Nevertheless, a meta-analysis and a large study of 9000 individuals have reported conflicting results for the association between this variant and risk of type 2 diabetes [45, 46]. Few studies exist that have examined the association between IRS-1 and risk of breast-cancer, and to our knowledge no studies have investigated the association between mortality and IRS-1 polymorphisms in breast-cancer survivors. We report that among Hispanic women, the variant allele was associated with higher circulating levels of leptin. We found no association between the IRS Gly^{972}Arg polymorphism and either breast-cancer-specific or all-cause mortality.

Our study has important limitations. The cohort was established before some current treatments such as aromatase inhibitors and trastuzumab were available, and therefore we cannot estimate what effect these variants might have on survival in women using these treatments. Finally, our relatively the small number of deaths does not allow accurate assessment of risk in specific subcategories such as premenopausal women or women from specific race/ethnic groups.

In summary, while PPARγ Pro12Ala polymorphism was not associated with breast-cancer mortality, carriers of the variant allele had statistically significantly lower rates of type 2 diabetes, and lower HOMA scores, although the latter was only observed among NHW. The TNF-α $^{-308}$ variant allele was associated with both higher serum glucose levels among NHW, and with reduced risk of mortality in this cohort of women diagnosed with breast cancer. Given the unexpected directions of these associations, confirmation in larger cohorts of breast-cancer survivors with greater numbers of outcomes is needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All research described in this manuscript complies with laws governing Protection of Human Subjects of Biomedical and Behavioral Research. The research was performed with the approval of Institutional Review Boards of participating centers, in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services.

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