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Genetic variation in TNF α PPAR γ and IRS-1 genes and association with breast cancer survival in the HEAL cohort

Catherine Duggan¹, Richard N. Baumgartner², Kathy B. Baumgartner², Leslie Bernstein³, Stephanie George⁴, Rachel Ballard⁴, Marian L. Neuhaus¹, and Anne McTiernan^{1,5}

¹Public Health Sciences, Fred Hutchinson Cancer Research Center

²Department of Epidemiology & Population Health, University of Louisville

³Department of Population Sciences, Beckman Research Institute, City of Hope

⁴Office of Disease Prevention, National Institutes of Health

⁵Department of Epidemiology, School of Public Health, University of Washington

Abstract

Tumor necrosis factor- α (TNF- α), peroxisome proliferator-activated receptor- (PPAR γ) 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. We investigated associations between TNF- α ⁻³⁰⁸G>A (rs1800629); PPAR γ Pro¹²Ala (rs1801282); and IRS-1 Gly⁹⁷²Arg (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 (HEAL) Study, a multiethnic, prospective cohort study of women diagnosed with stage I-IIIa breast cancer between 1995–1999 (median follow-up 14.7 years). Using Cox proportional hazards models adjusted for possible confounders, we evaluated associations between these polymorphisms and mortality. Carriers of the PPAR γ 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 polymorphism 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). Neither the PPAR γ nor the IRS-1 polymorphism was associated with mortality outcome. The TNF- α ⁻³⁰⁸G>A polymorphism was associated with reduced breast-cancer specific and all-cause mortality.

Contact Catherine Duggan, Epidemiology Program, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Telephone: (206) 667-2323, cduggan@fhcrc.org.

Ethical standards

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.

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

Keywords

PPAR γ IRS 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]

The peroxisome proliferator-activated receptor- γ (PPAR γ), 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 (Pro¹²Ala) single nucleotide polymorphism is a functional variant, with the Ala allele associated with reduced transactivation activity of PPAR γ [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 Pro¹²Ala 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 signalling.[21] IRS-1 plays a central role in insulin sensitivity, and association studies have shown that the IRS-1 Gly⁹⁷²Arg 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); PPAR γ Pro¹²Ala (rs1801282); and IRS-1 Gly⁹⁷²Arg (rs1801278) polymorphisms and risk 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-III 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] Briefly, 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 40–64 years; and in CA, 366 Black women, aged between 35–64 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 parent studies. Recruitment occurred from 1996–1999.

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 non-fatal 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 hours of collection, and stored at -80°C until analysis. DNA was extracted using Qiagen Midi-prep columns from buffy coat preparations.

SNP analysis—TNF α IRS-1 and PPARG γ 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; African Americans (AA). Serum measurements from fasting blood at 24 months post-diagnosis of glucose, insulin, adiponectin, and c-reactive protein (CRP) were measured as described previously.[1,25,3,26]

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 most recent. All-cause mortality was defined as time from the baseline interview to death from any cause, or end of follow-up (31 December 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 *vs.* GA + AA; PPAR γ Pro¹²Ala Pro/Pro *vs.* Pro/Ala + Ala/Ala; IRS-1 Gly⁹⁷²Arg Gly/Gly *vs.* Gly/Arg + Arg/Arg.

Differences in distribution of continuous variables between genotypes were estimated using the Kruskal-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 Chi-square test. Hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer-specific or all-cause mortality 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/m²; 18.5 and <25 kg/m²; 25 and <40 kg/m²; 40 kg/m²); 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 PPAR γ Pro¹²Ala, TNF- α ⁻³⁰⁸ G>A and IRS-1 Gly⁹⁷²Arg polymorphisms were 0.9%, 0.7% and 0.2% respectively (Table 1). Both TNF- α ⁻³⁰⁸ G>A and IRS-1 Gly⁹⁷²Arg polymorphisms were in Hardy-Weinberg (HW) equilibrium ($P>0.05$), but PPAR γ was not. However according to the dbSNP National Center for Biotechnology Information (NCBI) database, the PPAR γ Pro¹²Ala 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 PPAR γ Pro allele (96.1%) compared to other Hispanics (80.6%) and NHW (75.5%; $P<0.0001$, Tables 1 and 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/m²), tumor stage (local vs. regional) and presence or absence of type 2 diabetes. Carriers of the PPAR γ Ala allele were statistically significantly more likely to have a BMI ≥ 25 kg/m² (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%; $P=0.04$). PPAR γ , IRS-1 and TNF- α genotypes were 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 PPAR γ 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 g/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 the polymorphisms studied and biomarkers among African Americans.

Compared to 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 post-diagnosis (HR=0.12 95% CI 0.02–0.86; HR=0.28 95% CI 0.10–0.77, respectively).

Neither the PPAR γ 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 PPAR γ Pro¹²Ala 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- κ B, mitogen-activated protein kinase (MAPK), c-Jun-terminal kinase (JNK), and cytotoxic cell-death via TNFR1 signaling.[29,30,28]. 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 vs. 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]

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] Obesity and insulin 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] PPAR γ also possesses anti-inflammatory properties. PPAR γ antagonizes the AP-1, STAT, and NF- κ B 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] PPAR γ also inhibits the expression of aromatase, an

enzyme involved in estrogen biosynthesis, which may support the hypothesis that PPAR γ may play a role in the etiology of breast cancer.[42] Finally, specific agonists for PPAR γ 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 PPAR γ variant Ala allele. The variant allele was also associated with statistically significant lower circulating levels of CRP. 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.[12,11,44]

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,[45] we found no associations between the Pro¹²Ala polymorphism and outcome. Some studies have investigated the association of PPAR γ Pro¹²Ala 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 PPAR γ Pro¹²Ala: 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 PPAR γ Pro¹²Ala 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 Gly⁹⁷²Arg variant is a risk factor for insulin resistance, particularly in obese patients; [22,23] Nevertheless, a meta-analysis and a large study of 9,000 individuals have reported conflicting results for the association between this variant and risk of type 2 diabetes.[46,47] Few studies exist that have examined the association between IRS-1 and breast cancer survival, 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 Gly⁹⁷²Arg 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 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 PPAR γ 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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References

1. Duggan C, Irwin ML, Xiao L, Henderson KD, Smith AW, Baumgartner RN, Baumgartner KB, Bernstein L, Ballard-Barbash R, McTiernan A. Associations of insulin resistance and adiponectin with mortality in women with breast cancer. *J Clin Oncol*. 2011; 29(1):32–39. [PubMed: 21115858]
2. Irwin ML, Duggan C, Smith AW, McTiernan A, Baumgartner RN, Baumgartner K, Bernstein L, Ballard-Barbash R. Fasting C-peptide levels and death due to all causes and breast cancer: The Health Eating Activity and Lifestyle (HEAL) Study. *J Clin Oncol*. 2011; 29(1):47–53. [PubMed: 21115859]
3. Pierce BL, Ballard-Barbash R, Bernstein L, Baumgartner RN, Neuhaus ML, Wener MH, Baumgartner KB, Gilliland FD, Sorensen BE, McTiernan A, Ulrich CM. Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. *J Clin Oncol*. 2009; 27(21):3437–3444. [PubMed: 19470939]
4. Purohit A, Newman SP, Reed MJ. The role of cytokines in regulating synthesis: implications for the etiology of breast cancer. *Breast Cancer Res*. 2002; 4:65–69. [PubMed: 11879566]
5. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer*. 2009; 9(5):361–371. [PubMed: 19343034]
6. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor- α in human obesity and insulin resistance. *J Clin Invest*. 1995; 95:2409–2415. [PubMed: 7738205]
7. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science*. 1993; 259(5091):87–91. [PubMed: 7678183]
8. Kroeger KM, Carville KS, Abraham LJ. The ⁻³⁰⁸ tumor necrosis factor- α promoter polymorphism effects transcription. *Mol Immunol*. 1997; 34(5):391–399. [PubMed: 9293772]
9. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. *Proc Natl Acad Sci U S A*. 1997; 94(7):3195–3199. [PubMed: 9096369]
10. Fajas L, Debril MB, Auwerx J. PPAR gamma: an essential role in metabolic control. *Nutr Metab Cardiovasc Dis*. 2001; 11(1):64–69.
11. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J. A Pro12Ala substitution in PPARGgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet*. 1998; 20(3):284–287. DOI: 10.1038/3099 [PubMed: 9806549]
12. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common PPARGgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet*. 2000; 26(1):76–80. DOI: 10.1038/79216 [PubMed: 10973253]
13. Tonjes A, Scholz M, Loeffler M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with Pre-diabetic phenotypes: meta-analysis of

- 57 studies on nondiabetic individuals. *Diabetes Care*. 2006; 29(11):2489–2497. DOI: 10.2337/dc06-0513 [PubMed: 17065690]
14. Garcia-Calzon S, Martinez-Gonzalez MA, Razquin C, Corella D, Salas-Salvado J, Martinez JA, Zalba G, Marti A. Pro12Ala polymorphism of the PPARGgamma2 gene interacts with a mediterranean diet to prevent telomere shortening in the PREDIMED-NAVARRA randomized trial. *Circ Cardiovasc Genetics*. 2015; 8(1):91–99. DOI: 10.1161/circgenetics.114.000635
 15. Vogel U, Christensen J, Nexø BA, Wallin H, Friis S, Tjønneland A. Peroxisome proliferator-activated receptor-gamma2 Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes. *Carcinogenesis*. 2007; 28(2):427–434. DOI: 10.1093/carcin/bgl1170 [PubMed: 16959787]
 16. Wang Y, McCullough ML, Stevens VL, Rodriguez C, Jacobs EJ, Teras LR, Pavluck AL, Thun MJ, Calle EE. Nested case-control study of energy regulation candidate gene single nucleotide polymorphisms and breast cancer. *Anticancer Res*. 2007; 27(1B):589–593. [PubMed: 17348446]
 17. Memisoglu A, Hankinson SE, Manson JE, Colditz GA, Hunter DJ. Lack of association of the codon 12 polymorphism of the peroxisome proliferator-activated receptor gamma gene with breast cancer and body mass. *Pharmacogenetics*. 2002; 12(8):597–603. [PubMed: 12439219]
 18. Gallicchio L, McSorley MA, Newschaffer CJ, Huang HY, Thuita LW, Hoffman SC, Helzlsouer KJ. Body mass, polymorphisms in obesity-related genes, and the risk of developing breast cancer among women with benign breast disease. *Cancer Detect Prev*. 2007; 31(2):95–101. DOI: 10.1016/j.cdp.2007.02.004 [PubMed: 17428620]
 19. Gallicchio L, Chang HH, Christo DK, Thuita L, Huang HY, Strickland P, Ruczinski I, Clipp S, Helzlsouer KJ. Single nucleotide polymorphisms in obesity-related genes and all-cause and cause-specific mortality: a prospective cohort study. *BMC medical genetics*. 2009; 10:103.doi: 10.1186/1471-2350-10-103 [PubMed: 19818126]
 20. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, Cahill DA, Goldstein BJ, White MF. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature*. 1991; 352(6330):73–77. DOI: 10.1038/352073a0 [PubMed: 1648180]
 21. Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, Dunaif A, White MF. Insulin/IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J Clin Invest*. 2001; 107(2):181–189. DOI: 10.1172/jci10934 [PubMed: 11160134]
 22. Baroni MG, Arca M, Sentinelli F, Buzzetti R, Capici F, Lovari S, Vitale M, Romeo S, Di Mario U. The G972R variant of the insulin receptor substrate-1 (IRS-1) gene, body fat distribution and insulin-resistance. *Diabetologia*. 2001; 44(3):367–372. DOI: 10.1007/s001250051628 [PubMed: 11317670]
 23. Clausen JO, Hansen T, Bjorbaek C, Echwald SM, Urhammer SA, Rasmussen S, Andersen CB, Hansen L, Almind K, Winther K, et al. Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. *Lancet*. 1995; 346(8972):397–402. [PubMed: 7623569]
 24. McTiernan A, Rajan KB, Tworoger SS, Irwin M, Bernstein L, Baumgartner R, Gilliland F, Stanczyk FZ, Yasui Y, Ballard-Barbash R. Adiposity and sex hormones in postmenopausal breast cancer survivors. *J Clin Oncol*. 2003; 21(10):1961–1966. [PubMed: 12743149]
 25. Irwin ML, McTiernan A, Bernstein L, Gilliland FD, Baumgartner R, Baumgartner K, Ballard-Barbash R. Relationship of obesity and physical activity with C-peptide, leptin, and insulin-like growth factors in breast cancer survivors. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(12):2881–2888. [PubMed: 16365005]
 26. Duggan C, Risques RA, Alfano CM, Prunkard D, Imayama I, Holte S, Baumgartner RN, Baumgartner KB, Bernstein L, Ballard-Barbash R, Rabinovitch PS, McTiernan A. Change in peripheral blood leukocyte telomere length and mortality in breast cancer survivors. *JNCI*. 2014; 106(4)doi: 10.1093/jnci/dju035
 27. Cox DR. Regression models and life tables. *J Royal Stat Soc*. 1972; 34:187–220.
 28. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nature reviews Rheumatology*. 2016; 12(1):49–62. DOI: 10.1038/nrrheum.2015.169 [PubMed: 26656660]

29. Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants - past, present and future. *Cytokine Growth Factor Rev.* 2014; 25(4):453–472. DOI: 10.1016/j.cytogfr.2014.07.016 [PubMed: 25169849]
30. Tartaglia LA, Rothe M, Hu YF, Goeddel DV. Tumor necrosis factor's cytotoxic activity is signaled by the p55 TNF receptor. *Cell.* 1993; 73(2):213–216. [PubMed: 8386591]
31. Gaudet MM, Egan KM, Lissowska J, Newcomb PA, Brinton LA, Titus-Ernstoff L, Yeager M, Chanock S, Welch R, Peplonska B, Trentham-Dietz A, Garcia-Closas M. Genetic variation in tumor necrosis factor and lymphotoxin-alpha (TNF-LTA) and breast cancer risk. *Hum Genet.* 2007; 121(3–4):483–490. [PubMed: 17216494]
32. Mestiri S, Bouaouina N, Ahmed SB, Khedhaier A, Jrad BB, Remadi S, Chouchane L. Genetic variation in the tumor necrosis factor-alpha promoter region and in the stress protein hsp70-2: susceptibility and prognostic implications in breast carcinoma. *Cancer.* 2001; 91(4):672–678. [PubMed: 11241233]
33. Park KS, Mok JW, Ko HE, Tokunaga K, Lee MH. Polymorphisms of tumour necrosis factors A and B in breast cancer. *Eur J Immunogenet.* 2002; 29(1):7–10. [PubMed: 11841482]
34. Smith KC, Bateman AC, Fussell HM, Howell WM. Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. *Eur J Immunogenet.* 2004; 31(4):167–173. [PubMed: 15265021]
35. Kohaar I, Tiwari P, Kumar R, Nasare V, Thakur N, Das BC, Bharadwaj M. Association of single nucleotide polymorphisms (SNPs) in TNF-LTA locus with breast cancer risk in Indian population. *Breast Cancer Res Treat.* 2009; 114(2):347–355. [PubMed: 18409070]
36. Fang F, Yao L, Yu X, Yu L, Wu Q, Yu L. TNFα 308 G/A polymorphism is associated with breast cancer risk: a meta-analysis involving 10,184 cases and 12,911 controls. *Breast Cancer Res Treat.* 2009
37. Shen C, Sun H, Sun D, Xu L, Zhang X, Liu A, Jia X, Bai J, Chen F, Yu Y, Jin Y, Yu J, Fu S. Polymorphisms of tumor necrosis factor-alpha and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat.* 2011; 126(3):763–770. DOI: 10.1007/s10549-010-1184-5 [PubMed: 20882404]
38. DeMichele A, Martin AM, Mick R, Gor P, Wray L, Klein-Cabral M, Athanasiadis G, Colligan T, Stadtmauer E, Weber B. Interleukin-6 -174G-->C polymorphism is associated with improved outcome in high-risk breast cancer. *Cancer Res.* 2003; 63(22):8051–8056. [PubMed: 14633738]
39. Sookoian SC, Gonzalez C, Pirola CJ. Meta-analysis on the G-308A tumor necrosis factor alpha gene variant and phenotypes associated with the metabolic syndrome. *Obesity Res.* 2005; 13(12): 2122–2131.
40. Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK. PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. *Proc Natl Acad Sci U S A.* 2003; 100(11):6712–6717. DOI: 10.1073/pnas.1031789100 [PubMed: 12740443]
41. Delerive P, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors in inflammation control. *J Endocrinol.* 2001; 169(3):453–459. [PubMed: 11375115]
42. Rubin GL, Zhao Y, Kalus AM, Simpson ER. Peroxisome proliferator-activated receptor gamma ligands inhibit estrogen biosynthesis in human breast adipose tissue: possible implications for breast cancer therapy. *Cancer Res.* 2000; 60(6):1604–1608. [PubMed: 10749129]
43. Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, Zhang M, Fletcher C, Singer S, Spiegelman BM. Terminal differentiation of human breast cancer through PPAR gamma. *Molecular cell.* 1998; 1(3):465–470. [PubMed: 9660931]
44. Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. *Am J Epidemiol.* 2010; 171(6):645–655. DOI: 10.1093/aje/kwp450 [PubMed: 20179158]
45. Duggan C, Irwin ML, Xiao L, Henderson KD, Smith AW, Baumgartner RN, Baumgartner KB, Bernstein L, Ballard-Barbash R, McTiernan A. Associations of insulin resistance and adiponectin with mortality in women with breast cancer. *J Clin Oncol.* 2011; 29(1):32–39. DOI: 10.1200/jco.2009.26.4473 [PubMed: 21115858]

46. Jellema A, Zeegers MP, Feskens EJ, Dagnelie PC, Mensink RP. Gly972Arg variant in the insulin receptor substrate-1 gene and association with Type 2 diabetes: a meta-analysis of 27 studies. *Diabetologia*. 2003; 46(7):990–995. DOI: 10.1007/s00125-003-1126-4 [PubMed: 12819898]
47. Florez JC, Sjogren M, Burt N, Orho-Melander M, Schayer S, Sun M, Almgren P, Lindblad U, Tuomi T, Gaudet D, Hudson TJ, Daly MJ, Ardlie KG, Hirschhorn JN, Altshuler D, Groop L. Association testing in 9,000 people fails to confirm the association of the insulin receptor substrate-1 G972R polymorphism with type 2 diabetes. *Diabetes*. 2004; 53(12):3313–3318. [PubMed: 15561965]

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Table 1

Characteristics of the Health, Eating, Activity, and Lifestyle (HEAL) cohort (N=553)

	All ^a N (%)	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/m²)				
Mean (s.d.)	27.9 (6.5)	26.5 (5.7)	30.8 (7.5)	26.9 (4.6)
Age (years)				
Mean (s.d.)	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^b 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 Pro¹² 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				

	All ^a N (%)	Non-Hispanic White N (%)	African American N (%)	Hispanic N (%)
	553	318	156	62
Gly (G)⁹⁷²Arg (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

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

^b Surveillance, Epidemiology and End Results (SEER)

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

	TNF- α G ⁻³⁰⁸ A		PPAR γ 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 ^b						
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 ^c						
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	

^aChi squared test;^b17 participants were described as 'other race'; they were excluded from analysis;^cDiabetic status of 4 participants is unknown; they were excluded from analyses

Table 3

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

Non-Hispanic White	TNF α G ⁻³⁰⁸ A			p ^a	PPAR γ Pro ¹² Ala			p ^a	IRS-1 Gly ⁹⁷² Arg			p ^a
	GG N=274	GA/AA N=44	Mean analyte (sd)		Pro/Pro N=240	Pro/Ala Ala/Ala N=78	Mean analyte (sd)		GG N=277	GR/RR N=41	Mean analyte (sd)	
Insulin IU/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 g/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 g/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 g/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	TNF α G ⁻³⁰⁸ A			p ^a	PPAR γ Pro ¹² Ala			p ^a	IRS-1 Gly ⁹⁷² Arg			p ^a
	GG N=54	GA/AA N=8	Mean analyte (sd)		Pro/Pro N=50	Pro/Ala Ala/Ala N=12	GG N=57		GR/RR N=5	Mean analyte (sd)		
Insulin IU/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 g/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 g/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 g/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			

Non-Hispanic White	TNF α G-308A		p ^a	PPAR γ 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)	
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	TNF α G-308A		p ^a	PPAR γ 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	
	Mean analyte (sd)	Mean analyte (sd)		Mean analyte (sd)	Mean analyte (sd)		Mean analyte (sd)	Mean analyte (sd)	
Insulin IU/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 g/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 g/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 g/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

^aKruskal Wallis test.^bHOMA: Homeostatic Model Assessment^cCRP: C-reactive protein

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

Genotype	Events/N total	Unadjusted		Adjusted <i>a</i>	
		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	53/455	1.00	Ref.	1.00	Ref.
Pro/Pro	9/98	0.83	0.41–1.70	1.23	0.58–2.61
Pro/Ala Ala/Ala					
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)	128/46	1.00	Ref.	1.00	Ref.
GA/AA	9	0.64	0.36–1.14	0.51	0.28–0.91
p^b	13/84		0.13		0.03
PPAR γ					

Genotype	Events/N total	Unadjusted		Adjusted ^a	
		Hazard Ratio	95% Confidence Intervals	Hazard Ratio	95% Confidence Intervals
Pro¹²Ala	115/45	1.00	Ref.	1.00	Ref.
Pro/Pro	5	1.07	0.69–1.64	1.42	0.91–2.23
Pro/Ala Ala/Ala	26/98				
P^b			0.75		0.12
IRS-1 Gly⁹⁷²Arg					
Gly/Gly	128/48	1.00	Ref.	1.00	Ref.
Gly/Arg Arg/Arg	7	0.79	0.45–1.41	0.83	0.46–1.79
P^b	13/66		0.43		0.54

^a Adjusted for race/ethnicity/study-site; BMI (categorical <18.5 kg/m²; 18.5 and <25 kg/m²; 25 and <40 kg/m²; 40 kg/m²); 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