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Genetic variants within obesity-related genes are associated with tumor recurrence in patients with stages II/III colon cancer

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Abstract

Objective—Obesity is an established risk factor for colorectal cancer (CRC) incidence and it is also linked to CRC recurrence and survival. Polymorphisms located in obesity-related genes are associated with increased risk of developing several cancer types including colorectal cancer. We evaluated whether SNPs in obesity-related genes may predict tumor recurrence in colon cancer patients.

Methods—Genotypes were obtained from germline DNA from 207 patients with stage II or III colon cancer at the Norris Comprehensive Cancer Center. Nine polymorphisms in eight obesity-related genes (PPAR, LEP, NFKB, CD36, DRG1, NGAL, REGIA and DSCR1) were evaluated. The primary endpoint of the study was 3-year recurrence rate. Positive associations were also tested in an independent Japanese cohort of 350 stage III CRC patients.

Results—In univariate analysis, for PPAR rs1801282, patients with a CC genotype had significantly lower recurrence probability (29± 4% standard error, SE) compared to patients with a CG genotype (48% ± 8% SE), HR: 1.77; 95%CI, 1.01-3.10; p=0.040. For DSCR1 rs6517239, patients with an AA genotype had higher recurrence probability than patients carrying at least one allele G (37% ± 4% SE vs 15% ± 6% SE), HR: 0.51, 95% CI, 0.27-0.94; p=0.027. This association was stronger in the patients bearing a left-sided tumor (HR: 0.34; 95%CI, 0.13-0.88; p=0.018). In the Japanese cohort no associations were found.

Conclusion—This hypothesis generating study suggests a potential influence of polymorphisms within obesity-related genes in the recurrence probability of colon cancer. These interesting results should be further evaluated.

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Keywords

colon cancer; polymorphisms; obesity; recurrence; adjuvant

Introduction

Recurrence of colon cancer after surgery occurs in approximately 30-45% of the patients with stages II and III [1]. Adjuvant 5-fluorouracil (5-FU) has demonstrated to reduce recurrence rates, and the addition of oxaliplatin to 5-FU can further diminish the risk of relapse in stage III colon cancer patients [2, 3]. Despite this reduction, the majority of patients are treated unnecessarily due to the lack of predictive factors to determine which patients will benefit from adjuvant treatment. Therefore, it is of high importance to identify biomarkers that will allow the selection of those patients benefiting from adjuvant treatment in order to avoid unnecessary toxicities and increased costs.

The mechanisms responsible for relapse after treatment are not completely understood. Many factors and processes such as dormancy, epithelial mesenchymal transition, immunosuppression and inflammation appear to impact on the probability of recurrence. Obesity has also been linked to colon cancer recurrence and survival as well as to colon cancer risk [4, 5]. Regarding recurrence and cancer specific survival, several studies have shown an increased risk of recurrence and a decrease in survival in obese patients. A study including 4,288 patients with Dukes B and C colon cancer reported an increased recurrence rate and shorter overall survival as well as colon cancer specific survival for patients with BMI ≥ 35 kg/m^2 [6]. More recently, it was suggested that overweight pre-diagnosis of colon cancer but not post-diagnosis was associated with an increased risk of cancer specific mortality [7]. It has also been pointed out by Meyerhardt *et al*. that this risk may be gender-specific, as this group reported a significant increase in mortality among obese women with stages II and III colon cancer treated with 5-FU based adjuvant chemotherapy [8]. However, negative results regarding the relationship between obesity and cancer risk and survival have also been published. In a study that included more than 1,000 patients with stage III colon cancer enrolled in a randomized adjuvant chemotherapy trial, no differences were found regarding recurrence or death based on a high BMI index [9].

The rationale for the relationship between obesity and the increased risk of colorectal cancer, cancer recurrence and decreased survival is not fully elucidated. The biological mechanisms underlying this association include several processes such as inflammation, modulation of the energy balance, as well as growth factor and hormones signaling pathways, which influence tumorigenesis and cancer progression. Fat tissue is an endocrine organ that produces the polypeptide hormones adipokines. The adipokine leptin, which is directly associated with the amount of adipose tissue, has been reported to induce cancer progression by activation of PI3K, MAPK, I-CAM1 and STAT3 pathways [10, 11]. Specifically in colon cancer, leptin promotes motility and invasion by activating PI3K in colon cancer cell lines [12]. A higher percentage of adipose tissue is also associated with increased levels of interleukins and TNF-α, hence a chronic state of inflammation [13]. Inflammatory cytokines contribute to carcinogenesis through activation of the NF-κβ

signaling cascade responsible for inducing expression of genes associated to cell proliferation, apoptosis, metastases and angiogenesis [14].

Interestingly, in normal weight population, fat tissue potentiates an anti-inflammatory environment through the nuclear transcription factors peroxisome proliferator activated receptors (PPAR). PPAR-gamma antagonizes inflammation through the inhibition of NF-κβ activity as well as the attenuation of expression of various cytokines involved in cancer promotion and progression such as IL-1, IL-8 and TNF-α [15, 16]. Particularly in colorectal cancer, PPAR-gamma regulates the transcription of several genes implicated in carcinogenesis such as developmentally-regulated GTP-binding protein (DRG-1), regenerating gene IA (REGIA), neutrophil gelatinase-associated lipocalin (NGAL) or down syndrome critical region 1 gene (DSCR1) [17-20].

Based on the lack of factors to predict colon cancer recurrence and the implication of obesity in the risk and prognosis of this cancer, the aim of this study was to investigate polymorphisms located in obesity-related genes as biomarkers to predict recurrence in stages II and III colon cancer.

Material and Methods

Study population

A total of 207 patients with high-risk stage II and stage III colon cancer treated with 5-FU based adjuvant chemotherapy at the Norris Comprehensive Cancer Center or at the Los Angeles County Hospital, University of Southern California (USC), Los Angeles, USA were analyzed. As an exploratory cohort, a total of 350 Japanese patients with stage III cancer treated with adjuvant FOLFOX at the Cancer Institute Hospital (CIH), Tokyo, Japan were evaluated. Baseline characteristics are shown in table 1.

Follow up consisted of physical examination, blood test including carcinoembryonic antigen and CT scan every 3 months for the first two years and every 6 months thereafter. All patients participating in the study signed informed consent for the collection of samples as well as for the analysis of the molecular correlates. The present study was approved by the Institutional Review Boards of the University of Southern California and the Cancer Institute Hospital in Japan.

Selected polymorphisms and genotyping

Nine common polymorphisms located in eight obesity-related genes were selected. The polymorphisms selected had a reported functionality (NCBI Pubmed) or were considered potentially functional (F-SNP and SNP Info NIH databases). The minimum minor allele frequency (MAF) cut off for the selection was 0.1 for Caucasian population. Table 2 shows the characteristics of the selected polymorphisms.

DNA was extracted from peripheral blood in the majority of the patients. In cases where peripheral blood was not available, DNA was extracted from formalin-fixed, paraffin-embedded tissue. Most of the samples were tested using PCR- based restriction fragment length polymorphism (PCR-RFLP) analysis. In cases where no matching enzyme was found,

samples were analyzed by direct DNA Sanger sequencing. To ensure quality of the results, 5% of the samples for each SNP analyzed by PCR-RFLP were re-tested by direct DNA sequencing.

Statistical analysis

The endpoints of the study were time to recurrence (TTR) and 3-year recurrence rate. TTR was calculated from the diagnosis (USC) or surgical (Japanese) date to the day of first documented recurrence. TTR was censored at the time of last follow-up or death if patients were relapse-free. With 207 patients available for analysis, this study had an 80% power to detect a minimum hazard ratio (HR) of 1.92-2.16 for TTR in the dominant model considering a MAF of 0.1-0.5 and 2.03-3.24 in the recessive model considering a MAF of 0.25-0.5. The association of the SNP with the time to recurrence (TTR) was also analyzed using Kaplan-Meier curves and log-rank test. For multivariable analysis a Cox regression analysis was fitted adjusting by stage, type of adjuvant chemotherapy and stratified by race.

χ^2 test was used to analyze deviations from Hardy-Weinberg equilibrium in each ethnic group. Chi-square test was used to analyze the polymorphisms distribution as well as the baseline patients' characteristics. The inheritance model of these polymorphisms is not known; therefore we assumed a co-dominant, additive, dominant or recessive model wherever appropriate.

SAS 9.4 (SAS Institute, Cary, NC, USA) was used to perform all the analyses. Case-wise deletion was applied when patients with missing SNPs were excluded in the analyses. All tests were 2-sided at a significance level of 0.05. *P* values were not adjusted for multiple hypothesis testing.

Results

The median follow-up of this series was 3.9 years (range from 0.4 to 16.8). During this follow-up period, ninety patients (38.4%) presented tumor recurrence and the probability of 3-year recurrence was 33% (± 4% SE). The median overall survival of this series has not been reached. In the Japanese cohort, the median follow up was 5.0 years (range 0.3-8.6) and the 3-year recurrence probability was 29% (± 2% SE).

Genotyping was successful in > 90% of the analyzed samples for each SNP. Quality control re-sampling with DNA Sanger sequencing yielded a concordance of >99%. All the studied polymorphisms but two (rs28362491 and rs12006030) were within the probability limits of the Hardy-Weinberg equilibrium.

Polymorphisms in obesity-related genes and their association with recurrence

Detailed information of the studied SNPs with the 3-year recurrence probability is shown in Table 3.

PARPγ rs1801282 correlated with the 3-year recurrence probability: patients with a CC genotype had a significantly lower recurrence probability of 29% (±4% standard error, SE) compared to 48% (±8% SE) for patients with a CG genotype with a hazard ratio (HR) of

1.77; 95%CI, 1.01-3.10; *p*=0.040. In multivariate analysis, this association did not retain significance after adjusting for the relevant clinical parameters (HR: 1.68; 95% CI, 0.94-2.99; *p*= 0.08). The MAF for this polymorphism in Asian population is only 4%, therefore it was not analyzed in the Japanese cohort. The MAF for this SNP across race groups in the USC population varied significantly (p=0.023), being the variant allele more frequent in among the Hispanic population (data not shown).

DSCR1 rs6517239 was also associated with the 3-year recurrence probability in the dominant model: patients with a homozygous wild-type genotype (AA) had higher 3-year recurrence rate than patients carrying at least one variant allele G (37% ±4% vs 15% ±6%, respectively; HR: 0.51, 95% CI, 0.27-0.94; *p*=0.027). In multivariate analysis, this polymorphism had a trend towards an association with the recurrence probability (HR: 0.54; 95% CI, 0.29-1.03; *p*= 0.058).

In the Japanese cohort, the evaluation of DSCR1 rs6517239 and the 3-year recurrence probability did not show any association (p=0.53).

Subgroup analysis by gender and tumor location

Detailed information of these analyses is shown in Table 4. Evaluation of the selected polymorphisms by tumor location demonstrated a stronger association of the DSCR1 rs6517239 polymorphism with the 3-year recurrence probability. In patients bearing left-sided tumors, those with an AA genotype had a 3-year recurrence probability of 44% (±6% SE) compared to patients with an AG or GG genotypes whose probability of recurrence at 3-years was 11% (±8% SE) (HR: 0.34; 95%CI, 0.13-0.88; *p*=0.018). This difference retained statistical significance in multivariate analysis (HR: 0.36; 95%CI, 0.14-0.96; *p*= 0.040). Subgroup analyses in the Japanese cohort for the DSCR1 rs6517239 did not yield any association.

Analysis by gender revealed that the association of PARPγ rs1801282 with the 3-year recurrence was due mainly to the female population. Females carrying a CC genotype had lower 3-year recurrence rate (27% ±5% SE) compared to females with a CG genotype (64% ±13% SE) with a HR: 2.29; 95%CI, 1.09-4.82; *p*= 0.022, whereas no difference was found in the male population subgroup (*p*=0.53). However, in multivariate analysis this difference did not remain significant (HR: 1.94; 95% CI, 0.87-4.34; *p*= 0.11).

Discussion

In the present work, polymorphisms within obesity-related genes were associated with the recurrence probability in high-risk stage II and stage III resected colon cancer patients treated with 5-FU-based adjuvant chemotherapy. Presence of at least one variant allele of PPARγ rs1801282, correlated with a higher 3-year recurrence rate, whereas the presence of a least one variant allele of DSCR1 rs6517239 correlated with a lower 3-year recurrence rate. These associations were more pronounced in the female population for PPARγ rs1801282 and in the patients with left-sided tumors for DSCR1 rs6517239.

PPARγ is a critical regulator of metabolism acting through transcriptional factors in adipose tissue. Additionally, PPARγ also acts as a tumor suppressor by binding with retinoid X receptor α and signaling antiangiogenic and antiproliferative pathways [21]. Particularly in colorectal cancer, *in vitro* data suggest that a perturbation of the correct function of PPARγ promotes colorectal cancer carcinogenesis. Colorectal cancer cell lines treated with PPARγ agonists induce differentiation and growth arrest through PTEN activation and cyclin D suppression [22-24]. Moreover, β-catenin, a highly important protein for colorectal carcinogenesis part of the Wnt signaling, is downregulated by PPARγ [25]. In the PPARγ gene, the variant allele of the PPARγ rs1801282 C>G polymorphism correlates with reduced PPARγ transactivation activity [26]. These in vitro data are consistent with our results as patients bearing a G allele had significantly higher recurrence probability, which suggests a reduction in PPARγ's tumor suppressor activity for patients harboring the variant allele. This association was stronger in the female population. This fact underlines the gender differences in colorectal cancer and the incompletely clarified relationship between estrogen, obesity and colorectal cancer [27, 28]. This association did not retain significance in the multivariate analysis. The differences in the MAF among the races in the USC population may have influenced these results. Therefore, further analyses in more homogeneous populations are needed to clarify the value of this SNP. To date, no previous works had evaluated this polymorphism as a recurrence biomarker in cancer patients. Nonetheless, epidemiological studies evaluating cancer risk have yielded inconsistent results for PPARγ rs1801282 as it has been associated with a reduced risk of gastric cancer but an increased risk of colorectal cancer [29].

DSCR1 is one of the genes regulated by PPARγ [20]. DSCR1 codifies for calcipressin, a protein able to modulate the calcineurin-NFAT pathway [30]. The calcineurin-NFAT signaling has important roles in physiological processes such as regulating glucagon synthesis in pancreatic cells [31]. Additionally, DSCR1 has also been shown to be involved in many pathological conditions such as Alzheimer's disease and Down Syndrome [32]. DSCR1 might also play a critical role in cancer development. Epidemiological studies have underlined that Down syndrome's individuals develop significantly less solid tumors than healthy controls [33]. Presumably, genes located in chromosome 21 are responsible for this fact. DSCR1 is one of the genes located in chromosome 21 and its increased expression due to an extra copy is able to suppress angiogenesis and tumor growth through inhibition of the calcineurin-NFAT signaling [34]. Moreover, DSCR1 expression is induced by VEGF representing a potential angiogenesis regulation mechanism [35]. In this study, the rs6517239 polymorphism within DSCR1 was associated with the recurrence probability and this association was stronger in patients with left side tumors. Growing evidence indicates that left and right side colorectal cancer tumors are different molecular entities [36, 37]. Left-side tumors rely more in growth factor pathways to develop and progress, which may explain the stronger association of DSCR1 rs6517239 in patients with left-sided tumors [38]. Although no functionality has been described for this polymorphism, *in silico* analysis revealed this SNP affects a DNA binding site [39, 40]. This association was not validated in the Japanese series, despite of the similar minor allele frequencies for this SNP between the cohorts, which might be explained by several reasons. First, the differences in the initial stage that included stages II and III in the American cohort vs only stage III in the Japanese

cohort. This fact influenced the adjuvant treatment that was mainly 5-FU monotherapy for the American series compared to FOLFOX in the Japanese. Second, ethnical background might also have played a role due to different obesity rates in these populations, which is explained not only by life style but also by clear genetic differences.

Overall, study provides the first insight on the potential implication of germline variants within obesity-related genes on colon cancer recurrence after curative treatment. Moreover, these data underlines the potential influence of tumor location in the value of these polymorphisms as biomarkers. However, the lack of a more similar validation cohort and adjustment for multiple testing makes this a hypothesis generating study. Although these results are not statistically robust, we believe that these polymorphisms deserve further evaluation in other populations with bigger sample size and in prospective clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics and treatment of USC and Japanese cohorts

	USC* (n=207)		Japanese (n=350)	
	n	**%**	**n**	**%**
Age, years				
<55	74	35.8	70	20.0
55-64	75	36.2	101	28.9
≥65	58	28.0	179	51.1
Sex				
Female	96	46.4	175	50.0
Male	111	53.6	175	50.0
T				
T1	2	1.0	0	
T2	12	5.8	5	1.4
T3	164	79.2	232	66.3
T4	25	12.1	113	32.3
Tx	4	1.9		
N				
Negative	97	46.9	0	
N1	61	29.5	229	65.4
N2	49	23.7	121	34.6
Grade				
Well	11	5.3	75	21.4
Moderate	132	63.8	236	67.4
Poor/undifferentiated	47	22.7	24	6.9
Missing	17	8.2	15	4.3
Stage				
II	97	46.9	0	
III	110	53.1	229	65.4
IIIC			121	34.6
N of resected lymph nodes				
≤12	60	29.0	47	13.5
>12	129	62.3	302	86.5
Missing	18	8.7	1	
Tumor side				
Right	98	47.3	110	31.4
Left	101	48.8	238	68.0
Left and right	3	1.5	2	0.6
Missing	5	2.4		
Adjuvant treatment				

	USC* (n=207)		Japanese (n=350)	
	n	**%**	**n**	**%**
Fluoropyrimidines	130	62.8	206	58.9
5-FU/LV/Oxaliplatin	58	28.0	68	19.4
5-FU/LV/Irinotecan	19	9.2	0	
None			76	21.7
Ethnicity				
Asian	28	13.5	350	100.0
African American	14	6.8		
Caucasian	110	53.1		
Hispanic	55	26.6		

Table 2

Characteristics of the selected polymorphisms

GENE	rs number	Change/Position	Function/Association	MAF
NFκβ	rs28362491	-/ATTG ins upstream	Altered mRNA expression[41]	0.46
Leptin	rs7799039	A>G upstream	Influence on leptin expression[42]	0.50
CD36	rs3211882	-/TGT intron	NA	0.41
DRG1	rs5749272	T>C downstream	NA	0.47
NGAL	rs12006030	G>C intron	NA	0.2
PPARγ	rs1801282	C>G Pro12Ala	Reduced transactivation activity[26]	0.08
	rs3856806	C>T His447His	Higher leptin levels[43]	0.11
REG1A	rs10165462	T>C 5'UTR	NA	0.36
DSCR1	rs6517239	A>G intron	NA	0.14

Abbreviations: MAF: minor allele frequency; NFκβ: nuclear factor κβ; DRG1: developmentally-regulated GTP-binding protein; NGAL: neutrophil gelatinase-associated lipocalin; PPARγ: peroxisome proliferator activated receptor gamma; REG1A: regenerating gene IA DSCR1: Down syndrome critical region 1.

Table 3

Association of the analyzed SNPs and the 3-year recurrence probability

Polymorphism	*N*	Median TTR (95%CI)	3-year recurrence probability ± SE*	HR (95%CI)† Univariate	*P*† value	HR (95%CI)† Multivariate	*P*† value
NF-κβ rs28362491					0.45		0.55
ATTG/ATTG	64	7.1 (2.7, 11.3+)	0.36±0.06	1 (Reference)		1 (Reference)	
-/ATTG	82	12.4+ (4.9, 12.4+)	0.31±0.06	0.71 (0.41, 1.23)		0.77 (0.44, 1.36)	
-/-	49	5.2 (3.5, 16.8+)	0.29±0.07	0.91 (0.51, 1.64)		1.05 (0.57, 1.93)	
Leptin rs7799039					0.38		0.12
A/A	48	4.9 (3.4, 10.6+)	0.28±0.07	1 (Reference)		1 (Reference)	
A/G	93	10.7 (2.7, 16.8+)	0.38±0.06	0.91 (0.52, 1.60)		0.78 (0.43, 1.44)	
G/G	47	9.4 (5.4, 12.2+)	0.22±0.07	0.63 (0.32, 1.25)		0.45 (0.20, 0.98)	
CD36 rs3211882					0.69		0.87
TGT/TGT	67	5.7 (2.7, 10.7+)	0.38±0.07	1 (Reference)		1 (Reference)	
-/TGT	94	10.7 (4.9, 16.8+)	0.30±0.05	0.81 (0.48, 1.36)		0.91 (0.53, 1.57)	
-/-	31	9.4 (3.2, 11.3+)	0.25±0.08	0.81 (0.39, 1.70)		0.81 (0.38, 1.76)	
DRG1 rs5749272					0.33		0.28
T/T	58	5.4 (2.7, 9.0+)	0.38±0.07	1 (Reference)		1 (Reference)	
T/C	79	10.7 (4.8, 11.3+)	0.28±0.05	0.66 (0.37, 1.16)		0.62 (0.35, 1.12)	
C/C	48	6.6 (2.7, 16.8+)	0.36±0.08	0.85 (0.46, 1.58)		0.83 (0.44, 1.59)	
NGAL rs12006030					0.37		0.59
C/C	121	9.4 (5.7, 16.8+)	0.29±0.05	1 (Reference)		1 (Reference)	
C/G	79	5.4 (3.4, 10.9+)	0.35±0.06	1.24 (0.77, 1.98)		1.15 (0.70, 1.90)	
PPARrs1801282					**0.040**		0.080
C/C	168	9.4 (5.7, 12.4+)	0.29±0.04	1 (Reference)		1 (Reference)	
C/G	34	3.4 (1.5, 16.8+)	0.48±0.10	1.77 (1.01, 3.10)		1.68 (0.94, 2.99)	
PPAR rs3856806							
C/C	159				0.83		0.76
C/T‡	39	6.6 (4.9, 12.4+)	0.32±0.04	1 (Reference)		1 (Reference)	
T/T‡	2	7.1 (2.7, 16.8+)	0.34±0.08	0.94 (0.52, 1.68)		1.10 (0.59, 2.06)	
REGIA rs10165462					0.27		0.21
T/T	77	6.6 (3.5, 12.2+)	0.35±0.06	1 (Reference)		1 (Reference)	
T/C	100	10.7 (4.9, 16.8+)	0.27±0.05	0.73 (0.44, 1.19)		0.71 (0.43, 1.18)	
C/C	21	2.8 (1.4, 6.3+)	0.55±0.15	1.26 (0.55, 2.88)		1.34 (0.56, 3.18)	
DSCR1 rs6517239					**0.027**		0.058

Polymorphism	*N*	Median TTR (95%CI)	3-year recurrence probability ± SE*	HR (95%CI)† Univariate	*P*† value	HR (95%CI)† Multivariate	*P*† value
A/A	149	6.6 (4.0, 16.8+)	0.37±0.04	1 (Reference)		1 (Reference)	
A/G‡	47	9.4 (5.7, 10.7+)	0.15±0.06	0.51 (0.27, 0.94)		0.54 (0.29, 1.02)	
G/G‡	4						

Abbreviations: NF-κβ: nuclear factor kappa-light-chain enhancer of activated B cells; DRG1: developmentally regulated GTP-binding protein; NGAL: neutrophile gelatinase-associated lipocalin; PPAR: peroxisome proliferator activated receptor; REGIA: regenerating gene IA; DSCR1: Down syndrome critical region 1.

*Greenwood SE.

+Estimates were not reached.

†Based on log-rank test in the univariate analysis and based on Wald test within multivariate Cox proportional hazards model adjusting for stage and type of adjuvant therapy and stratified by race.

‡In the dominant model.

Table 4

Subgroup analyses. Association of the analyzed SNPs and the 3-year recurrence probability according to tumor location and gender.

Polymorphism	*N*	Median TTR (95%CI)	3-year recurrence probability ± SE*	HR (95%CI)† Univariate	*P*† value	HR (95%CI)† Multivariate	*P*† value
LEFT-SIDED TUMORS							
NFKBrs28362491					0.34		0.40
ATTG/ATTG	30	1.8 (1.4, 10.7+)	0.52±0.10	1 (Reference)		1 (Reference)	
-/ATTG	35	6.6 (2.4, 9.0+)	0.34±0.09	0.59 (0.28, 1.26)		0.60 (0.26, 1.35)	
-/-	30	5.2 (3.2, 16.8+)	0.25±0.08	0.65 (0.30, 1.45)		0.64 (0.28, 1.46)	
Leptinrs7799039					0.57		0.20
A/A	28	3.5 (1.5, 7.1)	0.38±0.10	1 (Reference)		1 (Reference)	
A/G	42	16.8+ (2.7, 16.8+)	0.35±0.09	0.68 (0.32, 1.45)		0.53 (0.23, 1.25)	
G/G	21	6.6 (1.6, 8.0+)	0.37±0.11	0.73 (0.30, 1.73)		0.41 (0.14, 1.21)	
CD36rs3211882					0.47		0.42
TGT/TGT	34	7.1 (2.3, 10.7+)	0.33±0.09	1 (Reference)		1 (Reference)	
-/TGT	39	4.9 (1.5, 16.8+)	0.46±0.09	1.46 (0.71, 3.00)		1.53 (0.73, 3.19)	
-/-	21	6.7+ (1.5, 6.7+)	0.26±0.10	0.96 (0.36, 2.57)		0.94 (0.34, 2.58)	
DRG1rs5749272					0.53		0.75
T/T	33	7.1 (1.8, 9.0+)	0.40±0.10	1 (Reference)		1 (Reference)	
T/C	35	7.2+ (3.5, 7.2+)	0.29±0.08	0.80 (0.36, 1.75)		0.75 (0.33, 1.69)	
C/C	26	5.2 (1.4, 16.8+)	0.47±0.11	1.25 (0.57, 2.75)		0.97 (0.42, 2.26)	
NGALrs12006030					0.21		0.51
C/C	64	7.1 (3.5, 16.8+)	0.32±0.07	1 (Reference)		1 (Reference)	
C/G	33	4.9 (1.6, 7.9+)	0.44±0.09	1.51 (0.79, 2.89)		1.26 (0.63, 2.53)	
PPARrs1801282					0.26		0.085
C/C	78	6.6 (3.5, 10.7+)	0.33±0.06	1 (Reference)		1 (Reference)	
C/G	19	2.7 (1.5, 16.8+)	0.52±0.12	1.51 (0.73, 3.10)		2.05 (0.91, 4.65)	
PPARrs3856806					0.89		0.20
C/C	76	5.7 (3.2, 10.7+)	0.36±0.06	1 (Reference)		1 (Reference)	
C/T, T/T‡	21	4.0 (1.5, 16.8+)	0.40±0.12	1.05 (0.50, 2.22)		1.84 (0.73, 4.64)	
REGIArs10165462					0.18		0.20
T/T	34	3.5 (1.8, 7.1)	0.42±0.10	1 (Reference)		1 (Reference)	
T/C	52	16.8+ (4.0, 16.8+)	0.31±0.07	0.60 (0.30, 1.18)		0.64 (0.31, 1.32)	
C/C	10	1.6 (0.5, 6.3+)	0.58±0.21	1.28 (0.42, 3.93)		1.76 (0.50, 6.20)	
DSCR1rs6517239					**0.018**		**0.040**

Polymorphism	*N*	Median TTR (95%CI)	3-year recurrence probability ± SE*	HR (95%CI)† Univariate	*P*† value	HR (95%CI)† Multivariate	*P*† value
A/A	73	4.0 (1.8, 16.8+)	0.44±0.06	1 (Reference)		1 (Reference)	
A/G,G/G‡	24	10.7+ (5.2, 10.7+)	0.11±0.08	0.34 (0.13, 0.88)		0.36 (0.14, 0.96)	
FEMALE POPULATION							
NFKBrs28362491					0.36		0.48
ATTG/ATTG	27	9.4 (1.5, 9.4)	0.39±0.10	1 (Reference)		1 (Reference)	
-/ATTG	40	12.2+ (4.9, 12.2+)	0.24±0.07	0.61 (0.27, 1.39)		0.67 (0.29, 1.57)	
-/-	22	5.4 (1.4, 16.8+)	0.38±0.11	1.04 (0.44, 2.45)		1.13 (0.46, 2.78)	
Leptinrs7799039					0.24		0.13
A/A	23	4.9 (1.5, 7.7+)	0.37±0.10	1 (Reference)		1 (Reference)	
A/G	45	5.9 (2.4, 16.8+)	0.40±0.08	1.01 (0.46, 2.21)		0.88 (0.38, 2.04)	
G/G	22	12.2+ (4.8, 12.2+)	0.15±0.08	0.49 (0.17, 1.37)		0.35 (0.11, 1.06)	
CD36rs3211882					0.40		0.71
TGT/TGT	26	5.7 (1.8, 7.7+)	0.37±0.11	1 (Reference)		1 (Reference)	
-/TGT	51	16.8+ (4.8, 16.8+)	0.30±0.07	0.74 (0.33, 1.68)		1.01 (0.43, 2.40)	
-/-	10	5.9 (0.1, 9.4)	0.31±0.15	1.36 (0.48, 3.89)		1.51 (0.49, 4.66)	
DRG1rs5749272					0.59		0.42
T/T	30	5.9 (2.1, 9.0+)	0.34±0.09	1 (Reference)		1 (Reference)	
T/C	35	10.9+ (4.8, 10.9+)	0.26±0.08	0.75 (0.33, 1.74)		0.62 (0.25, 1.50)	
C/C	21	16.8+ (1.4, 16.8+)	0.48±0.12	1.19 (0.50, 2.84)		1.09 (0.43, 2.75)	
NGALrs12006030					0.96		0.54
C/C	59	9.4 (2.8, 16.8+)	0.36±0.07	1 (Reference)		1 (Reference)	
C/G	35	5.9 (4.8, 10.9+)	0.26±0.08	0.98 (0.49, 1.97)		1.27 (0.60, 2.69)	
PPARrs1801282					**0.022**		0.11
C/C	77	9.4 (5.4, 10.9+)	0.27±0.05	1 (Reference)		1 (Reference)	
C/G	17	2.4 (0.6, 16.8+)	0.64±0.13	2.29 (1.09, 4.82)		1.94 (0.87, 4.34)	
PPARrs3856806					0.85		0.99
C/C	75	5.9 (4.9, 10.9+)	0.30±0.06	1 (Reference)		1 (Reference)	
C/T, T/T‡	20	16.8+ (1.1, 16.8+)	0.43±0.12	1.08 (0.49, 2.39)		1.00 (0.44, 2.28)	
REGIArs10165462					0.64		0.82
T/T	34	5.9 (1.8, 12.2+)	0.35±0.09	1 (Reference)		1 (Reference)	
T/C	50	16.8+ (4.8, 16.8+)	0.30±0.07	0.76 (0.38, 1.55)		0.88 (0.39, 2.00)	
C/C	8	2.8 (0.5, 4.9+)	0.67±0.25	1.23 (0.35, 4.35)		1.34 (0.36, 5.01)	
DSCR1rs6517239					0.079		0.053
A/A	71	5.4 (2.8, 16.8+)	0.39±0.06	1 (Reference)		1 (Reference)	

Polymorphism	*N*	Median TTR (95%CI)	3-year recurrence probability ± SE*	HR (95%CI)† Univariate	*P*† value	HR (95%CI)† Multivariate	*P*† value
A/G,G/G‡	23	9.4 (5.7, 10.3+)	0.16±0.09	0.44 (0.17, 1.13)		0.37 (0.14, 1.01)	

*Greenwood SE.

+Estimates were not reached.

†Based on log-rank test in the univariate analysis and based on Wald test within multivariate Cox proportional hazards model adjusting for stage and type of adjuvant therapy and stratified by race.

‡In the dominant model.