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Breast Cancer Res Treat. Author manuscript; available in PMC 2013 February 13.

Published in final edited form as:

Breast Cancer Res Treat. 2010 April ; 120(3): 745–752. doi:10.1007/s10549-009-0503-1.

Common genetic variations in the *LEP* and *LEPR* genes, obesity and breast cancer incidence and survival

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Abstract

Objective—Obesity is a strong risk factor for breast cancer in postmenopausal women and adverse prognostic indicator regardless of menopausal status. Leptin is an important regulator of adipose tissue mass and has been associated with tumor cell growth. Leptin exerts its effects through interaction with the leptin receptor (LEPR). We investigated whether genetic variations in the leptin (*LEP*) and *LEPR* genes are associated with risk of breast cancer, or once diagnosed, with survival.

Methods—The polymorphisms *LEP* G-2548A and *LEPR* Q223R were characterized in population-based study consisting of mostly European-American women. The study examined 1,065 women diagnosed with first, primary invasive breast cancer between 1996 and 1997. Controls were 1,108 women frequency matched to the cases by 5-year age group.

Results—A modest increase in risk of developing breast cancer was associated with the *LEP* -2548AA genotype when compared to the *LEP* -2548GG genotype (age-adjusted OR=1.30; 95% CI=1.01–1.66). This association was stronger among postmenopausal women who were obese (OR=1.86; 95% CI=0.95–3.64) although the interaction was of borderline statistical significance (*P*=0.07). We found no evidence of an association with polymorphisms of either *LEP* or *LEPR* in relation to all-cause or breast cancer-specific mortality among women with breast cancer (mean follow-up time=66.7 months). The effects of these genotypes on breast cancer risk and mortality did not vary significantly when stratified by menopausal status.

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Conclusions—In summary, our results show that a common variant in *LEP* may be associated with the risk of developing breast cancer supporting the hypothesis that leptin is involved in breast carcinogenesis.

Keywords

Leptin; leptin receptor; polymorphism; obesity; breast cancer

Introduction

Obesity is a growing public health problem with increasing incidence and prevalence not only in the United States, but also worldwide [1]. Previous reports on the relationship between obesity and breast cancer have consistently shown an increased risk in postmenopausal women, but little evidence of an increase in premenopausal women [2]. Among breast cancer patients, obesity at diagnosis has been consistently shown to adversely affect survival among both pre-and postmenopausal women [3]. The mechanism by which obesity exerts its effects is thought to be through endogenous hormone pathways, particularly through increased production of estrogens that occur through conversion of androstenedione in peripheral adipose tissue [4].

Obesity is caused by a chronic energy imbalance and is thought to be regulated in large part by the leptin-signaling pathway which, together with insulin, regulates medium- to long-term food intake and energy expenditure [5]. White adipose tissue is the primary production site of leptin, which is found in circulation and is considered one of the main signals that affect food intake and body weight. It is encoded by the leptin (*LEP*) gene [6] and its expression and secretion are highly correlated with body fatness and adipocyte size [7]. Leptin exerts its biologic effects through selective binding with the leptin receptor (LEPR), which is expressed in many tissues, including the mammary gland [8].

In addition to its role in obesity, leptin has also been shown to stimulate the growth of human breast cancer cells *in vitro* and has shown angiogenic qualities that acts through regulation of vascular endothelial growth factor (VEGF) and VEGF receptor-2 (VEGF-R2) expression [9,10]. Furthermore, leptin may modify estrogenic activity by inducing aromatase activity, thereby increasing the amount of androstenedione converted to estrone in adipose tissue [11,12]. Results from epidemiologic studies of breast cancer, however, have provided little evidence for an association with circulating leptin levels [13–15].

Genetic mutations of *LEP* and *LEPR* in mice have been shown to produce a phenotype resulting in morbid obesity [6,16,17]. In humans, *LEP* and *LEPR* polymorphisms, including a G to A substitution at nucleotide -2548 upstream of the ATG start site of *LEP* (G-2548A) and an A to G substitution at codon 223 in exon 6 (Q223R) of *LEPR* [18–20], may be related to obesity, as well as enhanced gene expression and increased circulating levels of leptin [20,21].

To assess the associations between *LEP* and *LEPR* polymorphisms and risk of developing breast cancer and subsequent survival, we used data from the Long Island Breast Cancer Study Project (LIBCSP). In addition, we evaluated whether the breast cancer associations with *LEP* and *LEPR* polymorphisms varied according to body mass index (BMI).

Materials and methods

This study draws upon data that was collected from participants as part of the LIBCSP, a population-based study of English-speaking residents of Nassau and Suffolk counties of

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Long Island, NY [22]. The study reported here utilizes resources from both the case-control and follow-up studies of the LIBCSP, as described below.

Study Population

Case-control study—Eligible case participants were women newly diagnosed with a first, primary *in situ* or invasive breast cancer between August 1, 1996, and July 31, 1997. Cases were identified using a rapid reporting system established specifically for the LIBCSP and were confirmed by physicians' and medical records. The attending physician was contacted to confirm study eligibility and to seek permission to contact the patient. Controls were women who were residents of the same two counties, frequency-matched by 5-year age group to the expected age distribution of cases. Potentially eligible control women were identified by Waksberg's method of random digit dialing (RDD) [23] for those under 65 years of age, and by Health Care Finance Administration (HCFA) rosters for those 65 years of age and older. Institutional Review Board (IRB) approval of the study protocol was obtained from each collaborating institution and participating hospital and written informed consent was obtained from each participant prior to the interview. A total of 1,508 women with breast cancer, of which 1,273 had invasive breast cancer, and 1,556 control women participated in the case-control study interview.

Follow-up study—Vital status through the end of 2002 among the case women (n = 1508) was determined through the National Death Index (NDI).

Data collection

Baseline, case-control data—The lifetime weight and most of the covariate data used in this analysis were collected as part of the LIBCSP baseline case-control interview. The main questionnaire was administered in-home by a trained interviewer and took approximately 2 h to complete. Information obtained from the main questionnaire includes reproductive and menstrual history, exogenous hormone use, family history of cancer, physical activity, smoking history, alcohol intake, and demographic characteristics (http://epi.grants.cancer.gov/LIBCSP/projects/Questionnaire.html). Descriptive characteristics for the entire LIBCSP study have been previously published [22].

After completing the interview and an additional informed consent form, participants were asked to donate blood samples. From each participant, about 40 cc of blood was obtained (5 EDTA-treated lavender-top tubes). The number of control women with a completed interview who donated a blood sample was 1,141 (73.3%). Among women with available DNA (1,065 cases and 1,108 controls), women were primarily Caucasian (94.4, 92.4%, respectively), postmenopausal (67.3, 64.6%), had at least a high school diploma (88, 91.5%), and had never used hormone replacement therapy (HRT) (70.3, 72.3%) [22].

Additionally, as part of the baseline case-control study, medical records of the cases were abstracted for tumor stage, estrogen receptor (ER) status, progesterone receptor (PR) status, and initial course of treatment.

Follow-up data among case women—Follow-up information on completed course of treatment for the initial breast cancer diagnosis was obtained by trained interviewer via telephone from 1,098 case participants or their proxy (8%) in 2002–2004. There were 410 cases without follow-up interview data due to nonresponse, refusal, were untraceable or were deceased without an identifiable proxy.

As part of the LIBCSP follow-up, updated medical records were retrieved and abstracted for 598 (39.8%) women. Trained abstractors reviewed medical records to determine each

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participant's tumor size and nodal status information for their initial breast cancer diagnosis. In addition, medical records were reviewed to determine the treatment regimen for each breast cancer case and this data was compared with the respondent's self-reported treatment regimen from the baseline interview and from the follow-up interview. A high concordance was found between information abstracted from medical records and self-reported radiation therapy (Kappa=0.97), chemotherapy, (Kappa=0.96) and hormone therapy (Kappa=0.92). Thus, self-reported treatment at the baseline and follow-up interviews was used for this analysis.

Study outcome for the follow-up analyses—For the LIBCSP follow-up study, the National Death Index (NDI) was used to ascertain all-cause and breast cancer-specific mortality among case participants. Case participants were followed from diagnosis until December 31, 2002 for a mean of 66.7 months (range, 2.7 – 88.6). Among the 1,508 women diagnosed with breast cancer, 198 (13.1%) deaths occurred. Based on International Classification of Diseases (ICD) codes 174.9 and C-50.9 listed as a primary or secondary code on the death certificate, 128 (64.6%) deaths were due to breast cancer. There were nine additional cancer deaths arising in areas of common breast tumor metastases, including the brain and lung. Cardiovascular disease was the second most common cause of death, accounting for 21% of all deaths.

Genotyping

DNA was isolated from blood cells and genotyped with the use of template-directed primer extension with detection of incorporated nucleotides by fluorescence polarization in a 96 microwell-based format. Master DNA 96 well plates containing 10 ng/μl were used to make replica plates containing 25 ng DNA/well. For polymerase chain reaction (PCR) amplification, the primers for *LEP* G-2548A (rs7799039) (forward 5′-TCCCGTGAGAACTATTCTTCTTTTG-3′, reverse 5′-CCTGCAACATCTCAGCACTTAGG-3′) and for *LEPR* Q223R (rs1137101) (forward 5′-AACAGCCAAACTCAACGACA-3′, reverse 5′-GCCACTCTTAATACCCCCAGT-3′) gave 93 and 251 bp products, respectively. Conditions for amplification were 0.2μl (10 pmol/ μl) forward and reverse primers, 0.2 μl 25mM $MgCl_2$ (only for *LEPR*), 1 μl 10× PCR buffer, 0.1 μl (5u/ml)Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN), 0.2 μl (10mM) dNTPs (Roche) and water added to the total volume of 10 μl.

Thermocycling conditions for *LEP* G-2548A were initial denaturation at 94° for 5 min followed by 35 cycles of 94° for 30 s, 60.5 ° for 35 s72 ° for 30 s, followed by final extension at 72 ° for 5 min. Thermocycling conditions for *LEPR* Q223R were initial denaturation at 94 ° for 5 min followed by 35 cycles of 94° for 30 s 60 ° for 45 s, 72 ° for 30 s, followed by final extension at 72 ° for 5 min. Primers and dNTPs were digested with 1 unit of shrimp alkaline phosphatase (1u/μl, Roche) after the addition of 1 μl of 10× SAPbuffer and 1 unit E.Coli exonuclease I (10 u/ μl, United States Biochemical, Cleveland, OH) and 7.9 μl of water for 45 min at 37°, followed by heating at 95° for 15 min. Forward extension primers were SNPS 5′-TTGTTTTGCGACAGGGTTGC-3′ and 5′-ATCACATCTGGTGGAGTAATTTTCC-3′ for *LEP* and *LEPR*, respectively. Acycloprime FP SNP Detection kit C/T contained the ddNTPs labelled either with R110 or TAMRA (Perkin Elmer Life Sciences, Boston MA). To 7 μl of reaction mixture was added 0.05 μl Acycloprimer enzyme, 1 μl C/T Terminator mix, 2 μl 10× reaction buffer, 0.5 μl extension primer (10 pmol/ μl) and 9.45 μl water. Extension was carried out by heating at 95° for 2 min followed by 30 cycles for *LEP* G-2548A and 20 cycles for *LEPR* of 95° for 15 s and 55° for 30 s. Plates were read on a Perkin Elmer Victor instrument. In addition to assay specific quality control samples, 10% of samples were reassayed after relabeling to keep laboratory personnel blinded to identity.

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The genotype distributions of the *LEP* -G2548A and *LEPR* Q223R genotypes were in Hardy-Weinberg equilibrium among controls (*P*=0.12 and 0.33, respectively). The frequencies of the common alleles among control women for the *LEP* and *LEPR* genotypes were consistent with those found in other Caucasian populations [24–26].

Statistical methods

Case-control analyses—Odds ratios (ORs) and 95% confidence intervals (CI) were calculated using unconditional logistic regression models [27] with the statistical software package SAS version 8.1 (SAS Institute Inc., Cary, NC). All models were adjusted for age at diagnosis. Additional factors considered as potential confounders included: variables related to demographic factors (race, income, education, marital status, religion), reproduction (parity, age at first live birth, breast feeding), and menstruation (age at menarche, menopausal status), exogenous hormone use was also considered (hormonal birth control, hormone replacement) as was medical history (benign breast disease, family history of breast cancer), and lifestyle factors (alcohol consumption, dietary fat and total caloric intake, cigarette smoking, BMI and physical activity). Using manual backward elimination, potential confounders were removed from models beginning with those with the highest *P*-value. Variables remained in the final models if their inclusion changed the estimate of effect by >10% [27].

Effect measure modification on the multiplicative scale between categorical body size variables and other covariates was evaluated using the log likelihood ratio test to compare logistic regression models with and without the cross-product terms [28]. Body size measures included BMI in the year prior to diagnosis which was categorized based on World Health Organization guidelines [29]. Because there were very few cases considered to be underweight (BMI <18.5) and including them in the reference group did not alter the results, three levels of BMI were used to categorize women: ideal weight (BMI <24.9); overweight (BMI 25–29.9); obese (BMI   30). Menopausal status was determined using information provided by the subject about the date of her last menstrual period, prior surgical information on hysterectomy and oophorectomies, cigarette smoking status, and use of hormone replacement [22]. Postmenopausal status was defined as having a last menstrual period more than 6 months before the reference date or if both ovaries were removed before the reference date. For women with unknown menopausal status, women were categorized as postmenopausal based on the 90th percentile for age at menopause in the control population, calculated according to smoking status; these women were categorized as postmenopausal if the age at reference was   54.8 years of age and a smoker, and   55.4 years if a nonsmoker. We were unable to assign menopausal status to 3.2% of women (30 cases, 63 controls), who were excluded from analyses specific to menopausal status.

Survival analysis—Cox proportional hazards regression [28] was used to estimate Hazard Ratios (HR) and 95% confidence intervals (CI) for the risk of all-cause and breast cancer-specific mortality. The results for overall and 5-year survival were similar; therefore only overall survival is reported.

Effect measure modification on the multiplicative scale between genotype and selected covariates was evaluated using the log likelihood ratio test to compare proportional hazards regression models with and without the cross-product terms [28]. We evaluated models stratified by obesity at diagnosis (<30, 30+) and menopausal status (premenopausal, postmenopausal). Associations were also evaluated by stratification on the tumor characteristics, ER status (negative, positive), PR status (negative, positive), tumor stage (*in situ*, invasive), nodal status (node-negative, node-positive) and tumor size (<2 cm,   2 cm).

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All models were adjusted for age at diagnosis. In addition to consideration of the covariates listed above for the case-control analyses, for the survival analyses we also considered as potential confounders other factors including history of comorbidities reported at the baseline interview (high cholesterol, history of blood clots, diabetes, hypertension, previous myocardial infarction and stroke), tumor characteristics (tumor stage, tumor size and nodal status) and treatment undergone for the original breast cancer diagnosis. Adjustment for covariates did not alter the estimates of effect by more than 10% and therefore associations reported are adjusted for age only.

To further explore possible confounding by tumor characteristics, we conducted separate analyses restricted to women for whom we have complete tumor characteristic and tumor treatment data. There were no differences in the estimated effects or evidence of confounding by these variables for the relationship between genotype and mortality (data not shown).

Results

Case-control analysis

A 30% increased association was observed in subjects with the *LEP* -2548AA genotype compared to those with the *LEP* -2548GG genotype (OR=1.30; 95% CI=1.01–1.66) (Table 1). No significant association emerged for *LEPR* Q223R genotypes. Adjustment for covariates did not materially alter these associations. Results were similar for both pre- and postmenopausal women (data not shown).

We explored the potential interaction between *LEP* G-2548A and *LEPR* Q223R genotypes (Table 2). There was little evidence of effect modification of the *LEP* -G2548A genotype by *LEPR* Q223R. Although the OR for having the *LEP* -2548AA genotype was slightly stronger among those who carried the *LEPR* 223GG genotype (OR=1.54; 95% CI=1.00–2.37), we did not observe statistically significant interaction between the two polymorphisms in the case-control analyses (P=0.57).

We also conducted analyses to determine the influence of obesity on the relationship between the *LEP* and *LEPR* polymorphisms and breast cancer in both pre- and postmenopausal women (Table 3). There was no evidence that the estimated risks for carrying the *LEP* -2548AA genotype differed according to obesity status among premenopausal women. However, among postmenopausal women who were considered obese at diagnosis (BMI 30), there was a moderate increase in the risk estimate for women with the *LEP* -2548AA genotype (OR=1.86; 95% CI=0.95–3.64) when compared to women who have the *LEP* -2548GG genotype, although the interaction was not statistically significant on the multiplicative scale (P=0.07). There was no evidence for modification by BMI of the association between *LEPR* genotypes and breast cancer risk.

Survival analysis

We evaluated the relation between the *LEP* -G2548A and *LEPR* Q223R genotypes and mortality (Table 4). There was little or no association between the *LEP* -G2548A or *LEPR* Q223R genotypes on the risk of either breast cancer-specific or overall death.

Additional survival analyses to determine associations with breast cancer mortality of the *LEP* -G2548A or *LEPR* Q223R genotypes were conducted according to tumor prognostic factors such as stage, node status, tumor size or ER and PR status (data not shown). No meaningful associations were observed for most indicators for either the *LEP* or *LEPR* genotypes. Only PR status was found to be related to the *LEP* polymorphism, where breast

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cancer cases with the *LEP* -2548AA genotype were observed to have a higher frequency of PR positive tumors (*P*=0.03).

Discussion

In this large population-based study, women with the *LEP* -2548AA genotype were observed to have a 30% increased risk of developing breast cancer, an association that was not modified by menopausal status. Although there was some suggestion that the effect was stronger among obese postmenopausal women, the interaction was not statistically significant. The *LEP* -2548AA genotype was not, however, associated with survival among those with a breast cancer diagnosis. We were not able to demonstrate an effect of the *LEPR* Q223R polymorphism on breast cancer development or prognosis.

Leptin is thought to be a growth factor and has been associated with stimulation of normal and tumor cell growth, tumor migration and invasion, enhanced aromatase activity and involvement with angiogenesis (reviewed in [9]). Higher circulating levels of leptin have been directly correlated with BMI and insulin, and have been associated with prognostic indicators such as advanced tumor stage, a higher tumor grade at diagnosis, as well lack of expression for both estrogen and progesterone receptors [30]. There is recent evidence that leptin is expressed in both normal and cancerous mammary tissue, however LEPR is generally only detected in mammary carcinoma cells [31]. When found in tumors, both leptin and LEPR are strongly associated with poorer prognosis having higher occurrence of distant metastasis and lower survival [31]. There is further evidence that tumors expressing LEPR have a worse prognosis among those who also have a high serum or mRNA levels of leptin [32]. These observations have lent support to the suggestion that leptin may play a role in breast cancer etiology and prognosis.

One hospital-based study conducted in Tunisia has reported on the *LEP* G-2548A and *LEPR* Q223R polymorphism genotypes [33]. This Tunisian sample of 308 cases and 222 controls reported a threefold increase in risk of developing breast carcinoma for those who carried the *LEP* -2548AA genotype (OR=3.17; 95% CI=1.47–6.96), with GA heterozygous carriers having an intermediate risk (OR=1.45, 95% CI=0.99–2.11), compared with women who were homozygous for the *LEP* -2548 G allele. In our population-based sample of mostly European-American women we observed that the *LEP* -2548AA genotype was associated with a somewhat more modest 30% increase in the risk of developing breast cancer when compared to those with the *LEP* -2548GG genotype. We did not, however, observe an increased association with breast cancer risk for carrying a single A allele. Whether the differences in the magnitude of the association with the *LEP* genotype between the two studies is due to differences in ethnicity, or the differences in sample size, is not clear. While the functional status of the *LEP* G-2548A polymorphism is unknown, previous reports have indicated that the A allele is associated with increased mRNA expression and higher circulating levels of leptin [21]. Additionally, the A allele of the *LEP* -2548 polymorphism has also been linked to other cancers, such as those of the lung and prostate [24,34].

Circulating leptin levels have been associated with cancers of the lung and prostate and with non-Hodgkin lymphoma [24,34,35]. However, despite evidence that leptin stimulates breast cancer cell proliferation *in vitro* [36], epidemiologic support for the association of circulating leptin levels in breast cancer development and progression has been limited. One investigation found an inverse relationship with breast cancer development in premenopausal women only [14], whereas no association was observed in two other studies [15,37]. With regard to breast cancer prognosis, one study reported no association between leptin levels and either disease-free or overall survival [30]. Why these studies failed to show evidence that leptin levels are associated with breast cancer is unclear. One possibility

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may be that the leptin levels in these studies were measured in circulation which could vary substantially from tissue concentrations found in the breast.

Although systemic levels of leptin do not appear to be associated with breast cancer risk, there has been shown to be a positive correlation between circulating levels and tumor levels [32] and there is evidence that leptin and leptin receptor play a role in breast cancer locally [31]. The Tunisian study by Snoussi *et al.* found an increased risk of breast cancer and worse prognosis among women carrying the R variant of the *LEPR* Q223R polymorphism [33] while another small Korean study found no association for the leptin receptor variant (OR=0.59; 95% CI=0.19–1.81) [38]. In our study we were not able to show a relationship between the *LEPR* Q223R polymorphism and either breast cancer risk or prognosis, which is consistent with two [39,40] of three [20] previous studies which show a lack of an association with serum leptin levels. Our lack of association between the *LEPR* Q223R polymorphism and breast cancer risk and prognosis may be due to a lack of functionality of the polymorphism. A recent study found no association between serum leptin concentrations and *LEPR* Q223R genotypes [38] and a recent meta-analysis of 18 studies indicated that there is no association between the *LEPR* Q223R polymorphism and obesity [41]. We were not, however, able to confirm whether circulating levels of leptin are associated with variants of LEP and LEPR, as serum leptin levels were not measured in this study.

We observed a slightly increased association with breast cancer risk for the *LEP* -2548AA genotype among obese postmenopausal subjects (BMI 30), an interaction that approached statistical significance (P=0.07). BMI is a well-known risk factor for breast cancer in postmenopausal women and is also negatively associated with survival among both pre- and postmenopausal breast cancer cases [42]; these observations have been confirmed among the LIBCSP participants in our study [43,44]. Leptin is a key hormone in obesity and regulation of energy balance, and whose levels are highly correlated with fat mass and BMI [45]. Due to the close correlation of leptin and BMI, we hypothesized that the association between the *LEP* and *LEPR* genotypes association with higher levels of circulating leptin and breast cancer might behave similarly. However, while the effects of BMI on breast cancer risk differ according to menopausal status (with obesity often associated with a decreased risk of breast cancer in premenopausal women [2]), we did not observe any difference in the association between *LEP* and *LEPR* and breast cancer risk according to menopausal status, although there was some suggestion that the effect of the *LEP* AA genotype was modified by obesity among postmenopausal women only.

To the best of our knowledge this is the first report among European-American women that a variant in the promoter region of the *LEP* gene at locus -2548 is associated with a 30% increase in risk of breast cancer development, and the association may be more profound among obese women. These results should be confirmed in additional studies to further evaluate the potential interaction between this polymorphism, obesity and breast cancer development.

Acknowledgments

This work supported by the University of North Carolina Nutrition and Cancer Training Grant NCI T32CA72319; National Cancer Institute and the National Institutes of Environmental Health and Sciences Grant nos. UO1CA/ES66572, UO1CA66572, P50CA52283, P30ES09089, P30ES10126, and 5T32CA009330-25; and the Breast Cancer Research Fund.

For their valuable contributions, the authors thank: members of the Long Island Breast Cancer Network; the participating institutions; our NIH and NIEHS collaborators; members of the External Advisory Committee to the population-based case-control study. We also wish to acknowledge and thank the following LIBCSP collaborators for their contributions with various aspects of our study efforts including: Amanda Golembesky, Ph.D. and Julie Britton, Ph.D.

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Table 1

LEP (G-2548A) and *LEPR* (Q223R) genotype ORs and 95% CI in breast cancer cases and controls in the Long Island Breast Cancer Study Project

Genotype	Cases (%)	Controls (%)	OR* (95% CI)
LEP G-2548A			
GG	341 (32)	360 (33)	Ref.
GA	492 (47)	561 (51)	0.91 (0.75–1.10)
AA	226 (21)	180 (16)	1.30 (1.01–1.66)
LEPR Q223R			
AA	173 (16)	187 (17)	Ref.
AG	521 (50)	551 (50)	1.00 (0.78–1.27)
GG	355 (34)	360 (33)	1.04 (0.81–1.34)

*Odds ratio (OR) adjusted for age

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Table 2

ORs and 95% CI for the breast cancer association between the *LEP* (G-2548A) genotype stratified by the *LEPR* (Q223R) genotype for all women combined

Genotype	Cases/ Controls	OR* (95% CI)	Cases/ Controls	OR* (95% CI)	Cases/ Controls	OR* (95% CI)	*P*§
	LEPR Q223R AA		*LEPR* Q223R AG		*LEPR* Q223R GG		
LEP G–2548A							
GG	50/62	Ref.	177/180	Ref.	111/116	Ref.	
GA	82/88	1.18 (0.73–1.92)	242/280	0.86 (0.65–1.12)	159/187	0.87 (0.62–1.22)	
AA	41/36	1.43 (0.80–2.58)	99/87	1.11 (0.78–1.59)	82/55	1.54 (1.00–2.37)	0.57

* Odds ratio (OR) adjusted for age

§ *P*-value for interaction

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Table 3

ORs and 95% CI for the breast cancer association between *LEP* (G-2548A) and *LEPR* (Q223R) genotypes stratified by obesity according to menopausal status

Genotype	Cases/ Controls	OR* (95% CI)	Cases/ Controls	OR* (95% CI)	$P^{§}$
		BMI < 30		BMI 30	
Premenopausal women					
LEP G-2548A					
GG	99/109	Ref.	12/17	Ref.	
GA	140/148	0.99 (0.69–1.43)	20/35	0.81 (0.32–2.04)	
AA	57/48	1.31 (0.81–2.11)	10/11	1.34 (0.43–4.17)	0.86
LEPR Q223R					
AA	54/63	Ref.	9/8	Ref.	
AG	137/150	1.09 (0.70–1.68)	20/26	0.68 (0.22–2.07)	
GG	100/91	1.25 (0.78–1.99)	14/28	0.44 (0.14–1.38)	0.18
Postmenopausal women					
LEP G-2548A					
GG	163/153	Ref.	56/59	Ref.	
GA	223/276	0.75 (0.56–0.99)	97/70	1.40 (0.86–2.27)	
AA	111/96	1.07 (0.75–1.53)	37/19	1.86 (0.95–3.64)	0.07
LEPR Q223R					
AA	76/81	Ref.	27/26	Ref.	
AG	255/262	1.02 (0.71–1.46)	95/81	1.12 (0.60–2.09)	
GG	161/180	0.94 (0.64–1.37)	68/42	1.54 (0.79–3.01)	0.28

*Odds ratio (OR) adjusted for age

§ *P*-value for interaction

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Table 4

LEP (G-2548A) and *LEPR* (Q223R) genotype HRs and 95% CI in breast cancer cases in the Long Island Breast Cancer Study Project

		Breast Cancer Mortality		Overall Mortality	
Genotype	**Cohort**	**Deaths**	**HR* (95% CI)**	**Deaths**	**HR* (95% CI)**
LEP G-2548A					
GG	341	27	Ref.	43	Ref.
GA	492	32	0.82 (0.49–1.37)	58	0.92 (0.62–1.36)
AA	226	13	0.72 (0.37–1.40)	29	0.98 (0.61–1.58)
LEPR Q223R					
AA	173	10	Ref.	23	Ref.
AG	521	37	1.23 (0.61–2.48)	62	0.85 (0.53–1.37)
GG	355	23	1.11 (0.53–2.33)	43	0.87 (0.53–1.45)

* Hazard ratios (HR) adjusted for age