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Leptin and leptin receptor genes in relation to premenopausal breast cancer incidence and grade in Caucasian women

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Abstract

Body mass is inversely related to breast cancer risk among premenopausal women. Leptin, an essential cytokine regulating food intake, energy expenditure, glucose, and fat metabolism may be part of the mechanistic pathway. We investigated 50 tagging and candidate SNPs in the leptin (*LEP*) and leptin receptor (*LEPR*) genes for associations with premenopausal breast cancer incidence using 405 cases and 810 controls nested within the Nurses' Health Study II. We also examined associations between these SNPs and circulating leptin (among 910 women) and breast cancer grade (among 267 patients). Permutation tests were performed to adjust for multiple testing. We did not detect a significant association between SNPs in the *LEP* or *LEPR* gene and either breast cancer incidence or plasma leptin levels. Among cases, 14 SNPs of the *LEPR* gene were significantly associated with cancer grade, and rs1137101 (Q223R) survived multiple testing adjustment (adjusted $P = 0.04$). The G carriers of rs1137101 were more likely to have poorly differentiated than well-differentiated cancers. Our data suggest that common genetic variation in the *LEP* or *LEPR* gene has no strong association with premenopausal breast cancer risk. The *LEPR* gene might be associated with breast cancer grade.

Keywords

Premenopausal; Breast cancer; Leptin; Leptin receptor

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Conflict of interest The authors of this manuscript report no conflict of interest.

Introduction

The inverse association between body mass index (BMI) and the risk of breast cancer among premenopausal women has been observed in numerous studies, and body size during early phases of adult life seems to be particularly important [1]. This association is not explained by factors related to ovulation (menstrual cycle characteristics, infertility due to an ovulatory disorder, and probable PCOS) [1]. Leptin, an essential cytokine regulating food intake, energy expenditure, glucose and fat metabolism, and also a growth factor in normal and malignant breast cells, may be one of the factors that may explain the BMI-breast cancer association [2, 3].

Genetically obese mice lacking the leptin receptor gene (*Lepr*(db)/*Lepr*(db)) who overexpress TGF-α do not develop mammary tumors while lean mice having this gene have high mammary tumor incidence (69% for Lepr(+)-Lepr(db) mouse and 82% for Lepr(+)Lepr(+) mouse) [4]. In human studies, leptin and its receptor are expressed in both normal and malignant breast tissue [2], but their expression levels are higher in malignant breast tissue [5]. In an in vitro study, leptin increased the cell proliferation in both normal (HBL100) and malignant (T-47D) breast epithelium cells [2]. Leptin also plays a significant role in promoting breast cancer cell proliferation by amplifying estrogen signaling [6]. These observations suggest that leptin may be involved in breast carcinogenesis. However, the epidemiologic evidence between plasma leptin levels and premenopausal breast cancer is inconsistent and based on retrospective case–control studies [7-8]. Genetic epidemiology studies have mainly focused on a few candidate SNPs of *LEP* (rs7799039) and/or *LEPR* (rs1137101, rs1137100, rs1045895) genes, and mostly considered postmenopausal breast cancer [9-15]. In a recent study, rs1137100 and rs1137101 were associated with luminal A breast cancer (including both pre- and post-menopausal women), but this association was no longer significant after multiple testing adjustment [16].

We comprehensively investigated the association between 50 tagging and candidate SNPs in the leptin (*LEP*) and leptin receptor (*LEPR*) genes and premenopausal breast cancer risk in 405 Caucasian premenopausal breast cancer patients and 810 matched controls from the Nurses' Health Study II (NHSII). We present results after multiple testing adjustment. We also explored the association of these SNPs with cancer grade given the previous report that *LEPR*-109RR (rs1137100) was associated with breast tumor size [12]. The association between these SNPs and circulating leptin levels was also evaluated.

Materials and methods

Study population

We used a nested case–control design within the NHSII. The NHSII was established in 1989 when 116,609 female nurses aged 25–42 returned a baseline questionnaire. Participants have been followed biennially by questionnaire to update information on demographics, anthropometry, lifestyle information, medication, and newly diagnosed diseases. The race/ethnicity breakdown is 94% Caucasian, 2% Asian, 2% African American, and 2% Hispanic. Between 1996 and 1999, a subset of 29,611 NHSII cancer-free participants (ages 32–54 years) provided blood samples. These women were similar to the total cohort in age, body mass index, parity, age at menarche, smoking habits, and oral contraceptive use. Each person in the blood cohort provided either timed blood samples (initial 15-ml blood sample during the follicular phase of ovulation, and a second 30-ml blood sample during the luteal phase, $n = 18,521$) or a single 30-ml untimed sample ($n = 11,090$). Characteristics of the blood cohort and information about blood draw and storage in detail have been reported previously [17]. The follow-up rate of the blood cohort was 98% in 2005. Women who provided a timed sample were considered to be premenopausal. Among those providing an

untimed sample, a woman was considered as premenopausal if she (a) reported that her periods had not ceased or (b) had a hysterectomy but had at least one ovary remaining and was ≤47 (for non-smokers) or 45 (for smokers) years old. A woman was considered postmenopausal if she (a) reported that her natural menstrual periods had ceased permanently or (b) had a bilateral oophorectomy. All others were considered to be of unknown menopausal status. We selected cases and controls from the blood cohort. We included both incident and prevalent cases. Incident cases were cancer free before blood draw and were diagnosed with breast cancer after blood collection but before 2005. Prevalent cases (34% of the total cases) were those diagnosed with breast cancer before they donated their blood. Among prevalent cases, the average duration between breast cancer diagnosis and blood draw was 3.36 (SD = 2.2) years. Cases were reported and confirmed by medical review or by verbal confirmation of the diagnosis by the nurse participant. Due to the high confirmation rate (99%) by pathology reports all self-reported cases were included in the analyses. For patients, breast cancer grade was categorized as well differentiated, moderately differentiated and poorly differentiated (http://www.ccrcal.org/Vol_1/BloomRichardsonGradeForBreastCancer_CA.htm). Two controls were matched to each case on age (±2 years), menopausal status at blood collection and diagnosis (premenopausal, postmenopausal, and unknown), and ethnicity (African American, Asian, Hispanic, Caucasian, Other). For blood collection, we matched on month and year (±2 months), time of day (±2 h) and fasting status (<2, 2–4, 5–7, 8–11,>12 h). In addition, cases providing timed samples were matched on the luteal day of the blood collection (date of next period minus date of blood draw at luteal phase, ±1 days). For each matching variable, >90% of case–control pairs had exact matches. Only premenopausal women were included in the present analyses. Because the majority of our samples are Caucasians (94%), we restricted our analyses to Caucasian women. Overall, 405 Caucasian cases (128 prevalent and 251 incident) who were premenopausal at cancer diagnosis and 810 matched Caucasian controls were included in this study. For the analysis of leptin level, we used a subset of 910 women who were premenopausal and cancer free at the time of blood draw; 305 of them became breast cancer cases later.

This study was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard School of Public Health. Returning a completed questionnaire and returning a blood samples by mail was considered implied consent by the IRB.

SNP selection and genotyping

For each gene, we included SNPs from 20 kb upstream of the transcription start point to 10 kb downstream of the transcription end point with minor allele frequency ≥0.05 based on the HapMap CEU panel (release 21, Mar08, on NCBIB36 assembly, dbSNP b126) (www.hapmap.org). To save genotyping costs and analyses burden, we selected pairwise tagging SNPs for further study using the algorithm implemented in tagger (http://www.broad.mit.edu/mpg/tagger/) [18]. We gave priority to the previously reported SNPs, rs7799039 (−2548 G > A, replaced by rs10487506 due to genotyping feasibility) and rs2167270 (19 A > G) [19] for the *LEP* gene, and rs7602 (IVS2 + 6890 G > A, replaced by rs9436302 due to genotyping feasibility), rs1045895 (IVS2 + 6920 G > A), rs1137101 (Gln223Arg A > G) [10], rs1137100 (Lys109Arg A > G) [12], rs3790419 (Ser343Ser T > C), rs8179183 (Lys656Asn G > C), and rs1805096 (Pro1019Pro C > T) [20] for the *LEPR* gene. In total, 10 tagging SNPs of the *LEP* gene and 40 of the *LEPR* gene were chosen. Together with SNPs from other genes, high throughput genotyping was performed at the Massachusetts Institute of Technology Broad Institute Center for genotyping and analysis using the Illumina Golden Gate with Bead Express (Vera Code) technology. Call rates for each SNP were greater than 97%. All SNPs had minor allele frequency greater than 0.05 among controls and none of them departed from HWE ($P > 0.05$). QC samples from 40

individuals, each with two or more replicates, were used to evaluate the concordance rate (CR). 34 of these SNPs (68%) had CR of 100%; 10 had CR of 99.5% and the other 6 had CR >97.6%.

Leptin measurement

Circulating leptin was measured using an ultrasensitive ELISA assay (R&D Systems, Minneapolis, MN) by Dr. Nader Rifai's laboratory at Children's Hospital in Boston. Using sample concentrations of 65.7, 146, and 581 pg/ml, the intra-assay coefficients of variations (CVs) were measured as 5.4, 4.2, and 3.5%, respectively. The inter-assay CVs for the same sample concentrations were 3.3, 3.0, and 3.2%. The limit of detection for this assay is reported as 7.8 pg/ml [21]. One-year intraclass correlation coefficient for leptin has been reported to be 0.82 [22] and the 4-year intraclass correlation has been reported to be 0.74 [23]. Samples from cases and matched controls were assayed together with the laboratory blinded to case–control status. The order of each case–control set was randomly determined. Each batch included blinded replicate samples to assess laboratory precision.

Statistical analysis

Logistic regression was used to examine the association between each SNP and premenopausal breast cancer incidence. SNP genotypes were coded additively as the copies of the minor allele (0, 1, 2). For comparison with previously studied candidate SNPs, we also used codominant models to estimate distinct effects for heterozygote and homozygote carriers of the minor allele. We originally adjusted for matching factors (age at diagnosis), and further adjusted for height, BMI at age 18, and BMI at blood draw. In addition, we adjusted for as many potential confounding factors as possible (age at menarche, parity, age at first birth, benign breast disease, and family history). In a separate step, we also adjusted for plasma leptin to explore its influence on the results. Since an association between the SNPs and premenopausal breast cancer incidence may be restricted to obese women [9], we performed subgroup analyses among overweight and obese women (BMI at blood draw ≥25 and ≥30 kg/m^2, respectively). We also performed the analyses among subjects with BMI at age 18 ≥ 21 kg/m^2, since BMI at adolescence is a stronger predictor of breast cancer incidence than current BMI [1]. Because we included prevalent cases which may induce survival bias, sensitivity analyses were performed restricting to incident cancer cases. We also performed sensitivity analyses excluding carcinoma in situ (30% of the total cases). We examined the association between these SNPs and leptin levels by linear regression, adjusting for age of blood draw, and further adjusted for BMI at blood draw. To ensure normality, we log transformed leptin levels.

Among 267 women with invasive breast cancer, we examined the association between the 50 SNPs and cancer grade. With patients of predominantly well-differentiated tumor as reference group, we calculated the odds ratio of moderately differentiated and poorly differentiated breast cancer groups separately/increase of one copy of minor allele for each SNP. To take multiple tests into consideration, we compared the minimum observed *P* value over all single SNPs tested to the distribution of minimum *P* values generated by permuting case–control status 2,000 times. These analyses were carried out using Plink 1.06-dos version (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml). Descriptive analyses for the study population were performed using SAS software.

Results

We included a total of 405 premenopausal breast cancer cases and 810 controls, all of whom were self-reported Caucasian. Table 1 presents their characteristics at time of blood draw. Compared to controls, cases had a higher percentage of family history of breast cancer (18.0

vs. 9.8%) and history of benign breast disease (24.9 vs. 18.3%). Cases and controls had a similar distribution of age at blood draw, leptin levels, height, BMI at 18, BMI at blood draw, age at menarche, parity and age of first birth. Among women with invasive breast cancer for whom information on cancer grade was available, 38.2% had poorly differentiated, 46.1% moderately differentiated, and the 15.7% well differentiated cancer.

Association between SNPs and premenopausal breast cancer incidence

We did not find significant association between any of the 50 SNPs and premenopausal breast cancer incidence (permutation *P* value = 0.94) (Table 2). The odds ratios (95% confidence intervals) for minor alleles of previously reported SNPs were 0.97 (0.79–1.20) for rs9436302, 0.99 (0.84–1.18) for rs1045895, 0.86 (0.71–1.05) for rs1137100, 0.94 (0.79–1.11) for rs1137101, 1.01 (0.80–1.26) for rs8179183, 0.94 (0.79–1.13) for rs1805096, and 1.04 (0.88–1.23) for rs10487506. Because some cancer cases were prevalent, we performed sensitivity analyses restricted to 251 incident cases and the results did not appreciably change. Restricting analyses to invasive breast cancer cases did not change the results appreciably either. We did not appreciably detect any significant association while conducting subgroup analyses restricting to either overweight women, obese women, or those with a BMI at 18 greater than 21 kg/m^2.

Association between SNPs and circulating leptin levels

We did not detect significant association between any of the examined SNPs and plasma leptin levels when taking multiple comparison into account (permutation *P* value = 0.67). We observed three SNPs (rs7540807, rs1887285, and rs11208659) in the *LEPR* gene with *P* values 0.04 before adjusting for multiple testing. Each copy of the minor alleles for these SNPs was associated with average changes in leptin levels of −10.8, −11.5, and +13.3%, respectively (Table 2). Further adjustment for BMI at blood draw did not appreciably change the results.

Association between SNPs and grade among cancer patients

Our data suggested that at least one SNP in the *LEPR* gene was associated with poorly (vs. well) differentiated breast cancer (permutation *P* value = 0.04) (Table 3). The top SNP rs1137101 survived multiple testing correction (*P* = 0.002; permuted *P* = 0.04). Patients with the G allele were more likely to develop poorly differentiated breast cancer than those with the A allele: the odds ratio (95% confidence interval) for each copy of the G allele was 2.45 (1.40–4.31). For the previously reported SNP rs1137100 [12], the odds ratio (95% confidence interval) for each copy of the G allele was 1.97(0.99–3.89) for moderately differentiated and 2.67(1.31–5.47) for poorly differentiated cancer compare to well-differentiated cancer patients. For rs1627238, in LD with the top SNP (rs2767485) of a GWAS study on sOB-R [24], the odds ratio (95% confidence interval) for each copy of the A allele were 0.60 (0.31–1.14) for moderately differentiated and 0.49 (0.25–0.96) for poorly differentiated breast cancer.

Discussion

We did not find a significant association between 50 tagging and candidate SNPs in the *LEP*/*LEPR* genes and cancer incidence (permutation *P* = 0.94) or leptin levels (permutation *P* = 0.16) in 405 premenopausal Caucasian breast cancer cases and 810 Caucasian controls (Table 2). Among 267 cancer patients, we observed several SNPs in the *LEPR* gene that were associated with breast cancer grade, and SNP rs1137101 survived multiple test adjustments.

A recent study did not find associations between tagging SNPs in the *LEP* and *LEPR* genes and incidence of breast cancer; positive association between two candidate SNPs Lys109Arg (rs1137100) and Gln223Arg (rs1137101) were observed with Luminal A breast cancer, but not basal like breast cancer, and the observed significance did not passmultiple comparison testing [16]. Other previous studies mainly focused on seven candidate SNPs (*LEP* G-2548A [9, 14], *LEPR* Gln223Arg [9-11, 13-15], *LEPR* Lys109Arg [12, 15], *LEPR* IVS2 +6890 [10], *LEPR* IVS2 +6920 [10] *LEPR* Lys656Asn [15], *LEPR* Pro1019Pro [15]). The *LEPR* Gln223Arg (rs1137101) is the best studied SNP, but the results are inconsistent. Studies in Nigerian women [13] and Tunisian women [14], and recent report in Caucasians [16] observed positive associations between the G allele of rs1137101 and breast cancer incidence, while a study in Chinese women [11] found an inverse association. A study among Korean women [15] and 3 studies in Caucasian women [9, 10] (including ours) did not find any significant association. The difference may be due to either false positive findings in some studies or effect modification by different characteristics of these study populations such as ethnicity, menopausal status, subtypes of breast cancer, or others. For *LEP* G-2548A (rs7799039), both previous studies [9, 14] found positive association between the AA genotype and breast cancer risk, one in Tunisian and the other in Caucasian women. A positive association with Lys109Arg (rs1137100) was observed in a study in Caucasions [16], but not in studies of other ethnicities [12, 15]. For the *LEPR* IVS2 +6920 (rs1045895 G > A), a cohort study in Caucasian postmenopausal women detected a negative association between carriers of the A allele and breast cancer risk [10]. No other significant association was detected for the other 3 SNPs (rs7602, rs8179183, rs1805096), but the studies had small sample sizes (less than 90 participants in a case-control studie, and 61 cases in a cohort study) [10, 15].

We did not find an association between any of these SNPs and risk of premenopausal breast cancer even before adjusting for multiple tests. At the 0.05 α level, we had more than 59% power to detect a SNP with an effect size (R_G) greater than 1.15 and MAF ≥ 0.2, and more than 80% power to detect a SNP with an effect size of 1.2 and MAF ≥0.2. Our study did not reveal any significance finding at the 0.05 α level, suggesting no strong association between common SNPs in these two genes and the incidence of premenopausal breast cancer.

A recent GWAS study identified over 100 genotyped and imputed SNPs in the *LEPR* gene associated with plasma soluble leptin receptor ($P < 10^{-8}$), including 9 SNPs examined in our study (rs9436302, rs11808888, rs1627238, rs1171279, rs6697315, rs10158279, rs1137100, rs1137101, rs4655537) [24]. None of these SNPs was significantly associationed with breast cancer incidence in our study.

We hypothesized that genetic variants in the *LEP* gene may be associated with circulating and tissue-specific leptin levels, so that observed associations between *LEP* variants and breast cancer risk may be used to draw inferences about the causal relation between leptin and breast cancer risk. However, our data did not detect any association between these tagging SNPs of the *LEP* gene and circulating leptin levels (Table 2). This suggests that the association between variants in the *LEP* gene and the concentration of circulating leptin is modest at best, which makes genetic variation of the *LEP* gene a weak instrumental variable for testing a causal relation between leptin levels and breast cancer risk. Therefore we should be careful interpreting the null results between SNPs in the *LEP* gene and breast cancer.

Breast cancer grade is based on three morphologic features: degree of tumor tubule formation, tumor mitotic activity, and nuclear pleomorphism of tumor cells. Poorly differentiated tumors tend to grow rapidly and spread faster than tumors with a lower grade (moderately and well differentiated). Our data suggested multiple SNPs in the *LEPR* gene

might be associated with poorly differentiated breast cancer (Table 3). The top SNP rs1137101 (Gln223Arg, A > G) survived multiple testing correction ($P = 0.002$; permuted $P = 0.04$). Patients with the G allele were more likely to develop poorly differentiated (vs. well differentiated) breast cancer than those with the A allele, the odds ratio (95% confidence interval) for each copy of the G allele was 2.45 (1.40–4.31). The rs1137101 is a non-synonymous SNP in exon 6 (Gln223Arg) of the *LEPR* gene coding for the extracellular region common to all isoforms of *LEPR*. In a previous study of 89 postmenopausal Caucasian women, the GG genotype was associated with higher leptin-binding activity compared with other geno-types [25]; and according to a recent GWAS in the Nurses' Health Study [24], the G allele was associated ($P < 5 \times 10^{-8}$) with a lower risk of plasma soluble leptin receptor, which is highly correlated with the expression levels of the leptin receptor ubiquitously expressed in most tissues [24]. These evidences suggest the rs1137101 or some SNP in LD with it might be associated with breast cancer grade through their impact on *LEPR* binding capacity or expression levels. Thus leptin and/or the leptin receptor might be involved in the underlying mechanism of breast cancer grade.

Conclusions

Our study suggests no strong association between common genetic variants in the *LEP*/*LEPR* genes and the incidence of premenopausal breast cancer in Caucasian women. The potential association of the *LEPR* gene with breast cancer grade deserves further consideration.

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Table 1

Characteristics at blood draw of 1215 participants of the Nurses' Health Study who were included in this nested case-control study population and information on cancer grade among women with invasive breast cancer

	Cases (*n* = 405)	**Controls (*n* = 810)**
Age diagnosis (y), mean (sd)	46.0 (4.6)	-
Age blood draw (y), mean (sd)	44.8 (4.1)	44.6 (4.0)
Log10 (Leptin) (pg/ml), mean (sd)	4.17 (0.3)	4.17 (0.3)
Height (cm), mean (sd)	165.6 (6.5)	165.1 (6.5)
BMI at age 18 (kg/m^2), mean (sd)	20.9 (2.9)	21.1 (2.9)
BMI at blood draw (kg/m^2), mean (sd)	25.4 (5.2)	25.8 (5.9)
Age at menarche (y), mean (sd)	12.4 (1.4)	12.4 (1.4)
Parity, median (interquartile range)	2 (1–2)	2 (1–3)
Age at first birth (y), mean (sd)	26.8 (4.6)	26.2 (4.4)
Family history *n* (%)		
Yes	73. (18.0)	79 (9.8)
No	332 (82.0)	731 (90.3)
Benign breast disease *n* (%)		
Yes	101 (24.9)	148 (18.3)
No	304 (75.1)	662 (81.7)
Grade, *n* (%[a])		
Total number = 267		
Well differentiated	42 (15.7)	
Moderately differentiated	123 (46.1)	
Poorly differentiated	102 (38.2)	

[a]Percentage based on 267 cases with this information

Table 2

Association between SNPs and incidence of pre-menopausal breast cancer and circulating leptin levels among 405 women with breast cancer and 810 women free of breast cancer participation in the Nurses' Health Study II

Gene	SNP	Minor allele	Major allele	MAF	With breast cancer			With leptin		
					N	Odds ratio (95% CI)	*P*	*N*	Percentage change per minor allele (%, 95% CI)[a]	*p*
LEPR	rs4592284	G	C	0.43	1208	1.04 (0.88–1.23)	0.65	904	1.2 (−5.5, 8.3)	0.74
LEPR	rs4655762	A	G	0.32	1212	1.02 (0.85–1.22)	0.84	907	4.6 (−2.8, 12.6)	0.23
LEPR	rs2148683	G	A	0.47	1209	1.02 (0.86–1.21)	0.80	904	−1.8 (−8.4, 5.3)	0.62
LEPR	rs7540807	C	A	0.1	1215	0.89 (0.67–1.18)	0.43	910	−10.8 (−20.2, −0.3)	0.04[b]
LEPR	rs913199	A	C	0.45	1213	1.01 (0.86–1.20)	0.89	909	0.5 (−6.1, 7.6)	0.88
LEPR	rs9436729	C	A	0.47	1201	1.01 (0.85–1.19)	0.94	898	0.8 (−6.0, 8.0)	0.83
LEPR	rs17127601	G	A	0.14	1213	0.98 (0.77–1.26)	0.89	908	−6.8 (−15.6, 3.0)	0.17
LEPR	rs3790436	C	G	0.44	1211	1.00 (0.84–1.18)	0.96	906	2.9 (−4.1, 10.3)	0.43
LEPR	rs3806318	G	A	0.25	1206	0.96 (0.79–1.17)	0.72	900	0.6 (−7.1, 8.9)	0.89
LEPR	rs12145690	C	A	0.44	1203	0.97 (0.82–1.15)	0.7	902	2.7 (−4.1, 10.1)	0.44
LEPR	rs4655802	G	A	0.42	1205	1.03 (0.87–1.23)	0.72	901	2.7 (−4.5, 10.4)	0.47
LEPR	rs9436738	A	G	0.13	1214	1.04 (0.82–1.33)	0.74	910	−6.9 (−15.7, 2.9)	0.16
LEPR	rs9436740	A	T	0.28	1206	1.15 (0.95–1.39)	0.17	902	5.7 (−2.3, 14.3)	0.17
LEPR	rs3790433	A	G	0.26	1192	1.06 (0.87–1.28)	0.57	900	−1.9 (−9.2, 6.0)	0.63
LEPR	rs17127618	G	C	0.15	1214	1.02 (0.81–1.28)	0.89	909	3.8 (−5.5, 14.2)	0.44
LEPR	rs9436301	G	A	0.24	1212	1.01 (0.83–1.23)	0.91	907	−4.0 (−11.3, 3.9)	0.31
LEPR	rs9436302[c]	A	G	0.21	1215	0.97 (0.79–1.20)	0.79	910	−0.2 (−8.2, 8.6)	0.96
LEPR	rs1887285	G	A	0.09	1214	0.95 (0.71–1.26)	0.71	908	−11.5 (−21.2, −0.8)	0.04[b]
LEPR	rs1045895c	A	G	0.4	1204	0.99 (0.84–1.18)	0.94	905	3.1 (−3.8, 10.5)	0.39
LEPR	rs6657868	A	G	0.38	1212	0.99 (0.83–1.18)	0.90	908	1.6 (−5.3, 9.0)	0.66
LEPR	rs6704167	T	A	0.43	1213	1.01 (0.85–1.19)	0.95	908	−0.1 (−6.6, 6.8)	0.97
LEPR	rs7513047	A	G	0.48	1209	0.97 (0.82–1.16)	0.76	907	1.1 (−5.6, 8.3)	0.75
LEPR	rs11808888	A	G	0.14	1214	1.04 (0.82–1.33)	0.74	910	−6.7 (−15.4, 3.0)	0.17
LEPR	rs6672331	C	G	0.03	1214	0.69 (0.39–1.19)	0.18	910	−3.7 (−21.5, 18.2)	0.72

Gene	SNP	Minor allele	Major allele	MAF	With breast cancer			With leptin		
					N	Odds ratio (95% CI)	*P*	*N*	Percentage change per minor allele (%, 95% CI)[a]	*p*
LEPR	rs11208659	G	A	0.08	1214	1.30 (0.96–1.76)	0.09	909	13.3 (0.3, 28.1)	0.04[b]
LEPR	rs1627238	A	G	0.17	1210	1.04 (0.83–1.30)	0.75	905	−4.2 (−12.5, 4.9)	0.36
LEPR	rs1171279	A	G	0.25	1213	1.15 (0.94–1.39)	0.17	908	1.5 (−6.3, 10.0)	0.71
LEPR	rs6697315	G	A	0.35	1215	0.98 (0.82–1.18)	0.85	910	2.9 (−4.2, 10.5)	0.44
LEPR	rs10158279	A	C	0.49	1209	1.00 (0.84–1.19)	1	906	0.5 (−6.2, 7.7)	0.88
LEPR	rs1137100c	G	A	0.27	1213	0.86 (0.71–1.05)	0.14	908	0.4 (−7.0, 8.4)	0.92
LEPR	rs3790429	A	T	0.17	1214	1.04 (0.84–1.30)	0.72	908	8.3 (−0.8, 18.1)	0.08
LEPR	rs6588152	A	T	0.21	1215	1.10 (0.90–1.35)	0.36	910	−1.9 (−9.6, 6.5)	0.65
LEPR	rs1137101[c]	G	A	0.46	1210	0.94 (0.79–1.11)	0.45	905	−1.6 (−8.2, 5.4)	0.64
LEPR	rs4655537	A	G	0.36	1213	1.06 (0.88–1.26)	0.54	909	−1.1 (−7.8, 6.1)	0.75
LEPR	rs3762274	G	A	0.39	1191	0.95 (0.80–1.13)	0.59	891	−2.5 (−9.1, 4.5)	0.47
LEPR	rs11585329	A	C	0.16	1212	0.90 (0.71–1.13)	0.37	907	7.0 (−2.3, 17.1)	0.15
LEPR	rs8179183[c]	C	G	0.18	1202	1.01 (0.80–1.26)	0.96	896	−0.1 (−8.9, 9.5)	0.98
LEPR	rs6690625	C	A	0.2	1214	0.88 (0.71–1.09)	0.24	909	−1.9 (−9.9, 6.8)	0.66
LEPR	rs10889569	A	T	0.38	1209	0.95 (0.79–1.13)	0.55	904	−1.2(−8.0, 6.0)	0.73
LEPR	rs1805096[c]	A	G	0.38	1211	0.94 (0.79–1.13)	0.52	906	−0.9 (−7.6, 6.4)	0.81
LEP	rs4731420	C	G	0.18	1212	0.83 (0.67–1.03)	0.1	908	−0.6 (−8.8, 8.3)	0.89
LEP	rs10954172	A	G	0.3	1213	1.02(0.85–1.22)	0.83	909	0.6 (−6.4, 8.1)	0.87
LEP	rs791600	A	G	0.41	1211	1.09 (0.92–1.30)	0.31	906	1.7 (−5.2, 9.0)	0.64
LEP	rs10487506[c]	A	G	0.44	1214	1.04 (0.88–1.23)	0.62	908	−1.4 (−7.9, 5.4)	0.67
LEP	rs10244329	A	T	0.5	1212	1.02 (0.86–1.21)	0.84	907	1.5 (−5.2, 8.7)	0.67
LEP	rs7795794	A	G	0.07	1215	0.99 (0.70–1.41)	0.96	910	−5.3 (−17.8, 9.2)	0.45
LEP	rs11760956	A	G	0.37	1184	0.99 (0.83–1.19)	0.93	891	3.2 (−3.9, 10.8)	0.39
LEP	rs2071045	G	A	0.24	1213	1.10 (0.90–1.34)	0.34	907	0.0 (−7.8, 8.5)	1.00
LEP	rs4731429	A	G	0.46	1211	0.97 (0.82–1.15)	0.72	906	0.4 (−6.3, 7.5)	0.92
LEP	rs10954176	G	A	0.47	1207	1.00 (0.84–1.18)	0.96	904	−2.3 (−8.8, 4.6)	0.50
					Permutation P = 0.94			Permutation P = 0.67		

SNPs were coded as 0, 1, 2; results were adjusted for matching factor (age of diagnosis); further adjusting for other covariates did not change results substantially

[a] Calculated by $(10^{\beta}-1) \times 100\%$, where β is beta coefficients of linear regressions or the boundaries of their 95% confidence interval

[b] *P* value < 0.05, but none of them are significant after multiple testing adjustment by 2000 permutation

[c] These SNPs were reported previously or in LD with them (rs104875067 in LD with 799039, rs9436302 in LD with rs7602)

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Table 3

Association between SNPs and tumor grade among 267 women with invasive pre menopausal breast cancer in Nurses' Health Study II

Gene	SNP	Minor allele	Major allele	MAF	Grade 2 versus 1 (*N* = 123 + 42)		Grade 3 versus 1 (*N* = 102 + 42)	
					OR	*P*	*OR*	*P*
LEPR	rs4592284	G	C	0.43	1.50 (0.88–2.55)	0.13	1.32 (0.78–2.25)	0.30
LEPR	rs4655762	A	G	0.32	1.12 (0.64–1.96)	0.70	0.86 (0.49–1.53)	0.62
LEPR	rs2148683	G	A	0.47	0.93 (0.57–1.52)	0.77	0.88 (0.51–1.50)	0.63
LEPR	rs7540807	C	A	0.1	0.41 (0.20–0.87)	0.02[c]	0.49 (0.23–1.07)	0.08
LEPR	rs913199	A	C	0.45	0.62 (0.36–1.04)	0.07	0.62 (0.35–1.08)	0.09
LEPR	rs9436729	C	A	0.47	0.59 (0.35–1.01)	0.05	0.58 (0.33–1.03)	0.06
LEPR	rs17127601	G	A	0.14	0.36 (0.18–0.71)	0.003[c]	0.58 (0.30–1.13)	0.11
LEPR	rs3790436	C	G	0.44	0.75 (0.45–1.26)	0.28	0.65 (0.37–1.15)	0.14
LEPR	rs3806318	G	A	0.25	0.60 (0.34–1.05)	0.08	0.55 (0.30–1.00)	0.049[c]
LEPR	rs12145690	C	A	0.44	0.97 (0.58–1.62)	0.91	0.74 (0.41–1.36)	0.34
LEPR	rs4655802	G	A	0.42	1.02 (0.61–1.69)	0.94	1.09 (0.62–1.91)	0.78
LEPR	rs9436738	A	G	0.13	1.19 (0.55–2.54)	0.66	1.52 (0.67–3.44)	0.32
LEPR	rs9436740	A	T	0.28	1.05 (0.57–1.92)	0.88	0.84 (0.45–1.56)	0.58
LEPR	rs3790433	A	G	0.26	0.54 (0.30–0.97)	0.04[c]	0.51 (0.28–0.94)	0.03[c]
LEPR	rs17127618	G	C	0.15	0.48 (0.26–0.88)	0.02[c]	0.56 (0.29–1.07)	0.08
LEPR	rs9436301	G	A	0.24	0.62 (0.35–1.10)	0.10	0.59 (0.33–1.08)	0.09
LEPR	rs9436302[a]	A	G	0.21	0.58 (0.32–1.05)	0.07	0.66 (0.36–1.22)	0.19
LEPR	rs1887285	G	A	0.09	1.48 (0.59–3.75)	0.41	0.97 (0.38–2.50)	0.96
LEPR	rs1045895	A	G	0.4	0.90 (0.55–1.50)	0.69	0.92 (0.52–1.62)	0.77
LEPR	rs6657868	A	G	0.38	1.74 (0.99–3.06)	0.05	2.09 (1.13–3.87)	0.02[c]
LEPR	rs6704167	T	A	0.43	0.84 (0.50–1.41)	0.51	0.76 (0.44–1.30)	0.32
LEPR	rs7513047	A	G	0.48	0.81 (0.47–1.41)	0.46	0.77 (0.43–1.38)	0.38
LEPR	rs11808888[a]	A	G	0.14	0.44 (0.22–0.87)	0.02[c]	0.42 (0.20–0.85)	0.02[c]
LEPR	rs6672331	C	G	0.03	1.36 (0.25–7.52)	0.73	1.15 (0.16–8.35)	0.89
LEPR	rs11208659	G	A	0.08	1.02 (0.39–2.65)	0.98	1.09 (0.40–2.91)	0.87

Gene	SNP	Minor allele	Major allele	MAF	Grade 2 versus 1 (*N* = 123 + 42)		Grade 3 versus 1 (*N* = 102 + 42)	
					OR	***P***	***OR***	***P***
LEPR	rs1627238[a]	A	G	0.17	0.60 (0.31–1.14)	0.12	0.49 (0.25–0.96)	0.04[c]
LEPR	rs1171279[a]	A	G	0.25	0.71 (0.38–1.30)	0.26	0.59 (0.32–1.08)	0.08
LEPR	rs6697315[a]	G	A	0.35	1.44 (0.82–2.55)	0.21	1.92 (1.03–3.58)	0.04[c]
LEPR	rs10158279[a]	A	C	0.49	0.84 (0.50–1.42)	0.52	0.45 (0.25–0.79)	0.006[c]
LEPR	rs1137100[a,b]	G	A	0.27	1.97 (0.99–3.89)	0.05	2.67 (1.31–5.47)	0.007[c]
LEPR	rs3790429	A	T	0.17	1.24 (0.65–2.37)	0.51	0.65 (0.33–1.30)	0.23
LEPR	rs6588152	A	T	0.21	0.72 (0.38–1.38)	0.33	1.18 (0.65–2.15)	0.59
LEPR	rs1137101[a]	G	A	0.46	1.43 (0.84–2.43)	0.19	2.45 (1.40–4.31)	0.002[d]
LEPR	rs4655537[a]	A	G	0.36	0.51 (0.30–0.87)	0.01[c]	0.42 (0.23–0.75)	0.004[c]
LEPR	rs3762274	G	A	0.39	1.30 (0.76–2.20)	0.34	1.86 (1.05–3.31)	0.035[c]
LEPR	rs11585329	A	C	0.16	1.11 (0.53–2.34)	0.78	1.32 (0.62–2.85)	0.47
LEPR	rs8179183	C	G	0.18	1.40 (0.71–2.74)	0.34	0.77 (0.38–1.59)	0.48
LEPR	rs6690625	C	A	0.2	1.65 (0.81–3.34)	0.17	2.03 (0.95–4.35)	0.07
LEPR	rs10889569	A	T	0.38	1.77 (1.02–3.06)	0.04[c]	1.33 (0.77–2.30)	0.31
LEPR	rs1805096	A	G	0.38	1.69 (0.99–2.89)	0.06	1.27 (0.74–2.18)	0.38
LEP	rs4731420	C	G	0.18	1.53 (0.72–3.24)	0.27	1.61 (0.78–3.32)	0.20
LEP	rs10954172	A	G	0.3	1.20 (0.66–2.19)	0.54	1.25 (0.71–2.21)	0.44
LEP	rs791600	A	G	0.41	0.77 (0.46–1.30)	0.33	0.55 (0.32–0.95)	0.03[c]
LEP	rs10487506	A	G	0.44	0.91 (0.55–1.49)	0.70	0.69 (0.42–1.13)	0.14
LEP	rs10244329	A	T	0.5	1.35 (0.80–2.27)	0.26	1.38 (0.84–2.28)	0.21
LEP	rs7795794	A	G	0.07	0.53 (0.20–1.39)	0.20	0.82 (0.33–2.03)	0.66
LEP	rs11760956	A	G	0.37	1.32 (0.73–2.38)	0.37	1.32 (0.75–2.32)	0.34
LEP	rs2071045	G	A	0.24	1.19 (0.66–2.16)	0.57	0.68 (0.35–1.33)	0.26
LEP	rs4731429	A	G	0.46	0.92 (0.54–1.57)	0.76	1.21 (0.71–2.08)	0.49
LEP	rs10954176	G	A	0.47	0.84 (0.51–1.38)	0.49	0.87 (0.51–1.48)	0.60
					Permutation *P* = 0.09		Permutation *P* = 0.04	

Grade 1 well differentiated, *Grade 2* moderately differentiated; *Grade 3* poorly differentiated

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[a] 9 SNPs associated with plasma soluble leptin receptor in a GWAS study with Pvalue $< 5 \times 10^{-8}$

[b] The rs1137100 was previously reported to be associated with tumor size

[c] P<.05, but not significant after multiple testing adjustment by 2,000 permutation

[d] Significant after multiple testing adjustment by 2,000 permutation