

Associations among Lipids, Leptin, and Leptin Receptor Gene Gln223Arg Polymorphisms and Breast Cancer in China

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Abstract We evaluated the relationship among the leptin receptor (LEPR) gene Gln223Arg polymorphism, body mass index (BMI), waist and hip circumference ratio (WHR), dietary structure, lifestyle, and other biomarkers with breast cancer and determined whether they could be effective for the prevention and treatment of breast cancer. The Gln223Arg polymorphisms in the LEPR gene were investigated in blood deoxyribonucleic acid (DNA) available for 240 breast cancer cases and 500 controls. Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism. Leptin, insulin were determined by enzyme-linked immunosorbent assays. We found that the serum levels of leptin, insulin, triglyceride (TG), free cholesterol (FCH), apolipoprotein (APO) A1, and BMI were significantly higher in breast cancer cases than the controls, while physical activity was clearly less in breast cancer cases ($P < 0.02 \sim P < 0.001$, respectively). Moreover, there were significant association between the Gln223Arg genotype and breast cancer risk; homozygotes for AA and heterozygotes for AG, AG+GG genotypes had been proved to increase the risk of breast cancer, and their corresponding odds ratio were 7.14 (95% confidence interval [CI]=1.92–25.64), 1.33 (95% CI=1.03–2.70), and 2.04 (95% CI=1.09–3.82). Interestingly, logistic regression analysis showed that LEPR gene Gln223Arg polymorphism and elevated leptin, insulin, TG, FCH, APOA1, WHR, and reduced APOB increased the risk of developing breast cancer, respectively. And, it also suggested that LEPR gene Gln223Arg polymorphisms, elevated leptin, insulin, TG, FCH, APOA1, WHR, and reduced APOB should play a major role in the development of breast cancer.

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Introduction

In recent years, the incidence of breast cancer have increased dramatically in China; the mortality of breast cancer rose 38.7% for women living in urban areas and 39.1% for rural women between 1991 and 2000 [1]. Reasons for this increase and risk factors of breast cancer have been reported and its association with the improvement of people's quality of life and changes in the dietary structure and lifestyle [2].

Epidemiological studies have found that overweight and obesity might be associated with increased risk of breast cancer in postmenopausal women [3–5]. Among the middle-aged and old-aged women of the USA and other Western countries, weight increment has been considered as a risk factor for increased breast cancer [6]. Overweight and obesity because of less physical activity and the greater availability of high-fat, energy-dense foods are the main results from environmental and societal changes in industrialized countries. In China, the traditional dietetic habits are based on plant protein foods. But, Chinese diets in the recent 10 years has been replaced by more animal protein in terms of the growth of economy and improvement of living standard. Hence, overweight and obesity are increasingly affecting people's health. The pathogenesis of overweight and obesity are a positive chronic imbalance between energy intake and expenditure mediated through the leptin-signaling pathway [7].

Leptin is an adipocyte-derived cytokine that regulates food intake and modulates immune and inflammatory responses through its receptor. Thereby, it can regulate body weight homeostasis. The weight-regulating effects of leptin are mediated through the binding and activation of the long isoform of its receptor (LEPR-b) in the hypothalamus [8]. Thus, the high circulating levels of leptin found in obese individuals suggest that obesity may be related to a leptin-resistant state. Leptin and its receptor are the key players in regulating energy balance and control body weight [9].

Many epidemiological studies have found that leptin correlates to body fat extent and several types of cancer. Our previous studies have demonstrated that the elevated serum levels of insulin, triglycerides (TG), apolipoprotein A1 (APOA1), and reduced level of high-density lipoprotein cholesterol (HDL-C) may be correlated with increased risk of breast cancer [10]. Tessitore et al. have reported that serum leptin was significantly elevated in breast cancer patients compared with the controls [11], Stattin et al. pointed out that leptin is a risk factor for colon cancer and that leptin may provide a link between obesity and colon cancer [12]. Somasundar et al. demonstrated that leptin acts as a growth factor for prostate cancer in vitro and enhanced prostate cancer cell migration by leptin [13].

Leptin exerts its physiological action through the leptin receptor (LEPR), a member of the cytokine family of receptors. LEPR was initially identified in the brain, which explained the negative feedback mechanism of controlling food intake and body weight [14]. Further studies, however, have demonstrated that LEPR is also identified in malignant cells of diverse origins, including lung and gastric carcinomas and leukemic cells [15–17]. In addition, leptin can regulate the proliferation and invasiveness of colonic and renal epithelial cells [18–19], and the expression of leptin in pituitary adenomas showed a positive correlation with the invasiveness of tumors [20].

In humans, several polymorphisms have been identified in the *LEP* and *LEPR* genes: a G to A substitution at nucleotide –2548 upstream of the ATG start site in the *LEP* gene 5' promoter region and an A to G substitution at nucleotide 668 from the start codon 223 in exon 6 (Q223R) of the *LEPR* gene coding for the extracellular region common to all isoforms of *LEPR*. Enhanced gene expression and increased circulating leptin levels have

been reported in subjects carrying the *LEPR* 223R or *LEP* (-2548) A alleles [43]. However, the expression of *LEPR* in various cancers has not been fully investigated, and the precise role of leptin in the development and promotion of cancer remains unknown.

In this study, we evaluate the associations of genotype polymorphisms in the *LEPR* gene and breast cancer. To examine the potential relationship between obesity and breast cancer, we studied the relationship of the effects of increased body mass index (BMI), waist and hip circumference ratio (WHR), lipids, insulin, and leptin to breast cancer independently and in conjunction with each other. A case-control study was conducted in the Shanxi Cancer Hospital, in Taiyuan, China, from 2001 to 2005. In addition, to assess whether heritable traits are associated with the breast cancer, we studied Gln223Arg polymorphisms in the *LEPR* gene in a subset of the case and controls that had deoxyribonucleic acid (DNA) available for analysis.

Materials and Methods

Study Subjects

In a study conducted in Shan Xi Area between 2001 and 2005, 240 breast cancer patients (mean age 45 years, range 22–78 years) who were initially identified by pathology in Shanxi Cancer Hospital (Taiyuan, China) and 500 controls matched by age, regional occupation, and race were collected at the same time. All the patients were not performed by any treatment including operation, chemotherapy, and radiotherapy.

All the participants completed in-person interviews. The investigated information included education (less than junior school, high-school graduate, more than high-school graduate), family history of breast cancer (have, naught), smoking status (never smoked, three and more cigarettes smoked per day), alcohol consumption (none, two drinks, or more per week), physical active (none, three, or more per week), vegetable consumption (250 g or more per day), fruits consumption (500 g or more per week), energy intake (250 kcal/day) and total fat intake density (g/1,000 kcal/day) were obtained from all the subjects. Detailed dietary and other data were not collected in this study.

Biological Samples

Blood samples were drawn from the elbow vein, and before the subjects were sampled, they were prescribed an overnight fast. Sera were obtained from blood samples by processing of clotting and centrifugation. Serum samples can be stored at 2–8°C for 24 h and could be stored at -20°C for a longer time until analysis. Genomic DNA was extracted from 5 ml peripheral blood using Qiagen DNA extraction Kits.

Genotyping Assay

The Gln223Arg polymorphism in the *LEPR* gene was detected using polymerase chain reaction (PCR) amplification with primers forward: 5'-ACCCTTTAAGCTGGGTGTCC CAAATGA-3', 2 μ l (12.5 mM), and reverse: 5'-CTAGCAAATATTTTGTAAGCAATT-3', 2 μ l (12.5 mM). For the PCR-restriction fragment length polymorphism (PCR-RFLP) assay, the PCR reaction was performed in total 50 μ l using 10 μ l genomic DNA, 11 μ l sterilized double-distilled H₂O (ddH₂O), and 25 μ l a-PCR Master. PCR cycling consisted of an initial denaturation at 94°C for 5 min, 94°C for 60 s at 55°C for 60 s, and at 72°C for 60 s. The final extension was for at 72°C. PCR product (10 μ l) was added to 2 μ l 6 \times PCR

buffer and mixed. The reaction mixture was loaded onto the 2.0% gelose gel and electrophoresed for 30–40 min, at 100 V. The PCR amplification product was scored in 440 bp.

PCR product (10 μ l) was added to 15 μ l 10 \times PCR buffer, 2 μ l *Msp*I, and 23 μ l sterile ddH₂O. The reaction mixture was kept overnight at 37°C and was loaded onto the 2.0% gelose gel and electrophoresed for 30–40 min, at 100 V. Digested products were separated on a 2% NuSieve gel stained with ethidium bromide and visualized with UV light. Alleles were scored as either A or G (presence or absence of the restriction site, respectively). The resulting fragments (AA 440 bp, GG 300 and 140 bp, GA 300, 140, and 440 bp).

Statistical Analysis

SAS Statistical Software (version 8.5) was used to conduct the statistical analyses. Analyses included the evaluation of the distribution of the genotypes in the cases and controls, the independent associations of genetic polymorphisms with breast cancer risk, and the joint effect of genotypes on breast cancer risk. The observed genotype frequencies in the breast cancer cases and controls were tested for the Hardy–Weinberg equilibrium (HWE), and the difference between the observed and expected frequencies was also tested for significance using the χ^2 test. A significant difference among the serum levels of leptin, insulin, lipids substance, BMI, and WHR in breast cancer cases and controls were assessed by *t* tests. Difference were taken to be significant at $P < 0.05$. χ^2 statistics were used to evaluate case–control differences in the distribution of LEPR gene Gln223Arg genotype, and odds ratios (OR) and 95% confidence intervals (CI) were used to measure the strength of the association between Gln223Arg polymorphisms and breast cancer risk in logistic regression models. Because age, race, and region were matched in controls and cases, matched logistic regression models were not used. Correlations were done to determine whether leptin, insulin, and lipids levels were associated with BMI and WHR. Other potential factors such as lifestyle, diet, total physical activity score, family history of breast cancer, number of cigarettes smoking per day, and volume of alcohol intake per week were evaluated by unconditional logistic regression models. The association between risk of breast cancer and serum levels of insulin, lipids, leptin, genotypes frequencies, BMI, and WHR were determined as ORs and 95% CIs based on the unconditional logistic regression analysis.

Results

Serum Levels of Leptin, Insulin, Lipids, and Other Factors in Patients with Breast Cancer

Table 1 compares the breast cancer cases and controls with the several plasma markers including leptin, insulin, TG, FCH, and APOA1. Leptin, insulin, TG, FCH, and APOA1 levels were significant higher, and the apolipoprotein B (APOB) levels were significant lower in breast cancer cases compared with the controls ($P < 0.02 \sim P < 0.001$, respectively). The levels of HDL-C and low-density lipoprotein cholesterol (LDL-C) were not significantly difference between the two groups ($P > 0.05$, respectively). There were significant differences of WHR, BMI, and physical activity in breast cancer cases compared with the controls ($P < 0.01$ and $P < 0.005$, respectively). Moreover, the results have also showed that the serum leptin level was significantly positive in relation to BMI and WHR ($r = 0.468$, $P < 0.001$; $r = 0.423$, $P < 0.001$; respectively), whereas serum insulin

Table 1 Serum Levels of Leptin, Insulin, Lipids, and Other factors in Patients with Breast Cancer

Variable	Breast cancer cases (<i>n</i> =240)	Healthy controls (<i>n</i> =500)	<i>P</i> value
Age (year)	45 (22–78)	44 (20–75)	
BMI (mean±SD, kg/m ²)	25.05±3.55	23.36±3.06	0.005
WHR (mean±SD, cm)	84.37±7.20	80.82±5.57	0.01
Leptin (mean±SD, ng/ml)	18.97±9.97	13.31±7.81	0.001
Insulin (mean±SD, mU/l)	13.30±10.56	6.34±4.48	0.01
T-Chol (mean±SD, mmol/l)	4.71±0.98	4.84±1.03	0.35
F-Chol (mean±SD, mmol/l)	1.49±0.47	1.31±0.38	0.002
TG (mean±SD, mmol/l)	1.48±0.59	1.17±0.36	0.01
HDL-C (Mean±SD, mmol/l)	1.34±0.38	1.31±0.26	0.51
LDL-C (mean±SD, mmol/l)	3.03±0.80	3.23±0.85	0.07
APOA1 (mean±SD, mmol/l)	1.53±0.32	1.31±0.25	0.001
APOB (mean±SD, mmol/l)	0.72±0.19	0.81±0.23	0.02

BMI Body mass index, *WHR* waist and hip circumference ratio, *T-Chol* total cholesterol, *FCH* free cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *APOA1* apolipoprotein A1, *APOB* apolipoprotein B

level was unrelated to BMI and WHR ($r=0.164$, $P>0.05$; $r=0.054$, $P>0.05$, respectively). There were no finds of a family history of breast cancer in patients and controls because of the small sample size in this study.

Comparison of Case and Controls on Demographics and Dietary Factors

Table 2 compares the demographics, diet, and lifestyle with the risk of breast cancer. The value of the patients were from predisease. The cases and controls were assessed for potential factors including education (less than junior school, high-school graduate, more than high-school graduate), family history of breast cancer (have, naught), smoking status

Table 2 Comparison of Case and Controls on Demographics and Dietary Factors

Variable	Breast cancer cases (<i>n</i> =240)	Healthy controls (<i>n</i> =500)	<i>P</i> value
Physical activity, % (h/w)	63 (26.60)	76 (35.16)	0.018
Region (%)			
City	125 (52.10)	270 (54.0)	
Country	115 (47.91)	230 (46.0)	
Education (%)			
Less than junior school	138 (57.5)	284 (56.8)	
High school	84 (35.0)	175 (35.0)	
More than high school	18 (7.50)	41 (8.2)	
Alcohol intake (%)	8 (3.33)	20 (4.98)	0.07
Cigarettes smoking (%)	5 (2.13)	11 (2.24)	0.56
Fruits consumption (%)	166 (69.15)	351 (70.20)	0.43
Vegetable consumption (%)	122 (50.83)	246 (49.22)	0.35
Energy intake (%)	138 (57.64)	262 (52.34)	0.18
Total fat intake (%)	149 (58.33)	246 (49.20)	0.02

(never smoked, three, and more cigarettes smoked per day), alcohol consumption (none, two drinks, or more per week), physical active (none, three, or more per week), vegetable consumption (250 g or more per day), fruits consumption (500 g or more per week), energy intake (250 kcal/day), and total fat intake density (g/1,000 kcal/day), which were obtained from all the subjects. Detailed diet and other data were not collected in this study. All the duration was more than 6 months. The results demonstrated that the region, cigarette smoking, alcohol intake, energy intake, and fruit and vegetable consumption were not statistically different between the breast cancer cases and controls ($P>0.05$, respectively) and total fat intake was significant higher in breast cancer cases than the controls ($P<0.05$).

Gln223Arg Genotype Distribution and Allele Frequencies in Breast Cancer Cases and the Controls

The genotype distribution and allele frequencies for the LEPR Gln223Arg polymorphisms in all the studied population follow the HWE, and our allele frequency for G and A was concordant with the studies on Korea, Japan, and the Shanghai population. Analyses showed that there was no deviation from HWE ($P>0.05$) [21]. The distributions of the genotypes of GG, GA, and AA in breast cancer cases were 69.14%, 17.02%, and 13.84% compared with that of the controls, 82.03%, 15.63%, and 2.34%. The significance difference was observed between the two groups ($\chi^2=11.16$ $P=0.004$). Similar significance differences were also found in allele gene frequencies for G and A of the patients, which were 77.66% and 22.34% compared with 89.84% and 10.16% of the controls. ($\chi^2=12.41$ $P=0.001$, see Table 3).

In the breast cancer cases, we also observed an increased risk in the distribution of homozygotes for Arg/Arg, heterozygotes Gln/Arg, and Arg/Arg+Gln/Arg, their corresponding ORs being 7.14 (95%CI=1.92–25.64, $P=0.001$), 1.33 (95%CI=1.03–2.70, $P=0.03$), and 2.04 (95%CI=1.09–3.82, $P=0.004$), respectively, (see Table 3). There were significant associations of the main effects of the Cln223Arg polymorphisms and breast cancer risk. Thus, our data suggested that carriers of the Arg/Arg, Arg/Gln, and Arg/Arg+Arg/Gln genotypes in humans have a high risk of breast cancer.

Relationship among Leptin, LEPR Genes Gln223Arg Genotype, and Other Biomarkers and Breast Cancer

Unconditional logistical regression models were used ($\alpha=0.05$, $\beta=0.10$) to evaluate the relationship between the risk of breast cancer and BMI, WHR, and serum levels of

Table 3 Associations Between LEPR Gene Gln223Arg Genotype and Breast Cancer

Genotype	Breast cancer cases	Controls	χ^2	P value
GG	166 (69.17)	410 (82.03)		
GA	41 (17.08)	78 (15.60)		
AA	33 (13.75)	12 (2.37)	11.16	0.004
G	373 (77.70)	898 (89.84)		
A	107 (22.30)	102 (10.16)	12.41	0.001
GG OR (95% CI)	1.0			
AG	1.30 (1.03–2.70)			0.03
AA	7.14 (1.92–25.60)			0.001
AG+AA	2.04 (1.09–3.82)			0.04

biomarkers. It showed that the Gln223Arg polymorphism and high levels of leptin and insulin would increase the risk of breast cancer, their corresponding ORs being 4.87(95% CI=1.30–18.22, $P=0.019$), 1.53 (95% CI=1.13–2.07, $P=0.006$), and 1.11 (95% CI=1.02–1.29, $P=0.02$), respectively. The high levels of FCH, TG, and APOA1 significantly increased the risk of breast cancer (OR=15.19, 95%CI=4.11–56.19, $P=0.0001$; OR=5.54, 95%CI=2.33–9.89, $P=0.016$; OR=12.73, 95% CI=2.44–66.31, $P=0.003$, respectively). An inverse relation between serum level of APOB and the risk of breast cancer was observed (OR=0.05, 95%CI=0.003–0.63, $P=0.021$). In our study, high WHR can increase the risk of breast cancer (OR=3.68, 95%CI=1.34–10.11, $P=0.011$); however, BMI was not related to the risk of breast cancer (OR=1.13, 95%CI=0.67–4.54, $P=0.17$; see Table 4). There were no significant association between any of the variants including education, region, cigarette smoking, alcohol intake, energy intake, total fat intake, and physical activity and breast cancer risk.

Discussion

Recently, epidemiological studies have shown that overweight and obesity might be associated with increased death rates for cancers at multiple specific sites [22]. Previous studies have consistently shown a positive association between adiposity and increased risk of cancer of the endometrium, kidney, colon, and gallbladder in women and breast cancer in postmenopausal women [23]. These results encouraged us to evaluate the level of the serum leptin and the expression of its receptor gene in breast cancers because leptin plays a major role in the regulation of weight and adiposity. In this study, we found a significant elevation of leptin levels in breast cancer cases compared with the controls and observed a positive association with the risk of breast cancer.

Our studies have shown that BMI and WHR of the patients with breast cancer are significantly higher than the controls, and leptin has a positive association with both BMI and WHR. It has been also demonstrated that the total fat intake was higher in breast cancer cases than the controls. The high levels of serum leptin and insulin derived from the increased adipose tissue are considered to contribute to the formation of breast cancer in those obese patients. Moreover, circulating leptin is an essential factor which can regulate fat metabolism. Therefore, it can be hypothesized that leptin itself might be involved in the development of breast cancer. In fact, serum leptin levels have been shown to be significantly higher in breast cancer patients than the controls. Moreover, the most recent reports indicated that higher serum leptin levels are associated with advanced stage breast cancer [24–25], although not in premenopausal patients [26]. Our result is consistent with

Table 4 Association Between Risk of Breast Cancer and Serum Levels of Leptin, Insulin, Lipids, WHR, and LEPR Gene Polymorphism Based on a Case and Control Analysis

Variable	β	OR	95% CI	Wald	P value
Leptin	0.43	1.53	1.13–2.07	7.63	0.006
Polymorphism	1.58	4.87	1.30–18.22	5.52	0.019
Insulin	0.103	1.11	1.02–1.29	5.13	0.02
WHR	1.30	3.68	1.34–10.11	6.40	0.011
FCH	2.72	15.19	4.11–56.19	16.62	0.001
TG	0.61	5.54	2.33–9.89	5.75	0.016
APOA1	2.54	12.73	2.44–66.31	9.13	0.003
APOB	−3.09	0.05	0.003–0.63	5.29	0.021

those reports. Makoto et al. have clearly recognized that leptin was positive in both normal epithelial and carcinoma cells. However, the expression of leptin was significantly stronger in carcinoma than in normal epithelium as judged by its staining intensity [27]. The expression of leptin in normal and malignant mammary glands has been reported at the messenger ribonucleic acid (mRNA) level [28]. O'Brien et al. [9] reported that leptin mRNA expression was found to be higher in three different breast cancer cell lines (MCF-7, T470, and MDAMB 231) than in adipose tissue. These findings agree with our results and suggest that leptin production by the epithelium of mammary glands is enhanced during tumor genesis. The relationship between obesity and breast cancer susceptibility has been verified in mouse experiments [29–30]. Recently, Clear et al. [31] have shown a direct contribution of leptin to the development of mammary tumors in a transforming growth factor-transgenic and leptin-deficient mouse model. However, the results about the relationship between serum leptin level and breast cancer is still controversial. Mantzoros et al. observed that leptin did not appear to increase the risk of premenopausal breast cancer. In addition, Petridou et al. have found no relationship between serum leptin levels and breast cancer in postmenopausal women [32–33]. Obesity is known as a risk factor for the women's breast cancer. But, the molecular mechanisms involved are not clear.

Genetic mutations in the leptin gene of ob/ob mice and the LEPR gene in db/db mice produce a phenotype characterized by morbid obesity and immune dysfunction [34–36]. Makoto et al. also clearly recognized cytoplasmic as well as membranous expression of functional LEPR in most of the breast carcinoma cells but not in normal epithelium [27]. In humans, several polymorphisms linked to an obese phenotype have been identified in the leptin and LEPR gene [37–38]: an A to G nucleotide change at position 19 in the 5' untranslated region and a G to A substitution at nucleotide –2548 upstream of the ATG start site, both in the leptin gene, and an A to G transition at nucleotide 668 from the start codon that converts a glutamine to an arginine at codon 223 (Q223R) in the LEPR gene [39–41]. This glutamine to arginine substitution occurs within the first of two putative leptin-binding regions and may be associated with impaired LEPR signaling capacity [42]. Enhanced gene expression and increased circulating leptin levels have been reported in those with the LEPR223RR and LEP-2548AA genotypes [43], whereas the leptin 19GG variant has been reported to confer lower plasma leptin levels in morbidly obese women. In the present study, our results have suggested that leptin may contribute to the risk of breast cancer. And, some genes may regulate the serum level of leptin. Therefore, we assessed LEPR gene Gln223Arg polymorphisms. The frequencies for the A allele was 0.22 in breast cancer, while in controls the frequencies of this allele was only 0.10. It has shown a significant difference in the two groups. The evaluation of genetic polymorphisms in the LEPR gene along this pathway suggests that at least one allele of the LEPR gene Gln223Arg (*GA/AA* genotypes) may increase risk of breast cancer. Recently, Snoussi et al. has reported that leptin and LEPR polymorphisms are associated with increased risk and poor prognosis of breast carcinoma [44]. However, the relationship between LEPR gene polymorphisms and breast cancer is still controversial. Woo et al. have found no relationship between Gln223Arg polymorphisms in the LEPR gene and breast cancer [45]. The *GA/AA* genotypes of the LEPR gene Gln223Arg in combination with lipids, insulin, and leptin also appear to increase risk of breast cancer.

Both leptin and LEPR gene polymorphisms have been found to correlate with breast cancer, and we also found that insulin was the risk factor of breast cancer. Our results showed that the serum level of insulin was clearly higher in breast cancer cases than the controls. The mechanism underlying insulin-induced breast cancer involves the insulin-like growth factor-I (IGF-I) system, and insulin decreases IGF-binding protein (IGFBP)

production and secretion, thereby increasing the bioavailability of IGF [46, 47]. Epidemiological evidence indicated that an increase in the ratio of IGF-I to IGFBP-3 in circulation was associated with an increase risk for the development of breast cancer, and IGFBP-3 polymorphisms may be association with the level of IGFBP-3 protein and an increased risk of breast cancer [48].

In addition, similar positive associations were observed in our study for the lipids. In the analysis of logistic regression, levels of lipids (FCH, TG, and APOA1) were positively associated with the risk of breast cancer. The risk of breast cancer, however, displayed an inverse relation with APOB. Little is known about the role of lipids in connection with breast cancer, although the lipid substance has been found to correlate with cardiovascular disease risk. Takahashi-Yauno et al. has reported that LEPR gene Arg223Gln polymorphism in Japanese men is associated with significant elevation of serum TG and LDL-C levels. Their data showed that the Arg/Arg homozygotes tend to show lower levels of serum HDL-C. This polymorphism tended to show an attenuated response to a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor in term of the cholesterol-lowering effect. Their results suggest that LEPR may serve as a novel modifier gene for hypercholesterolemia in Japanese men [49]. We reported that serum levels of FCH, TG, and APOA1 were positively associated with the risk of breast cancer. Moreover, when the insulin, leptin, LEPR gene polymorphism, and lipids variants are evaluated together, a more substantial and significant effect on breast cancer risk is observed.

Coskun et al. [50] and our previous study [51] had shown that serum leptin levels are similar in patients with metastatic and nonmetastatic breast cancer. This may suggest that leptin functions in an autocrine manner at the tumor site to support the development of metastasis of breast cancer.

In our study, there was no correlation between smoking and drinking and the risk of the breast cancer. For the result, we think it may be the reason that fewer Chinese women have the habit to drink and smoke. The low rate of smoking and drinking led to low numbers of smokers and drinkers in both the two research groups, which could affect the result of the statistics.

In conclusion, Gln223Arg polymorphisms in the LEPR gene have modest independent effects on breast cancer risk, and levels of leptin, insulin, and lipids were association with the risk of breast cancer. These data suggest that obesity may contribute to the increasing incidence of breast cancer. We assessed these genotypes in conjunction with diet and lifestyle factors in order to better understand the disease pathway and its importance in breast cancer. Further studies of the association between obesity, diet, and breast cancer are warranted to confirm our results and to clarify the biological mechanism that underlies the observed associations. Our findings are novel and may have significant public health implications for identifying high-risk women for the prevention of breast cancer.

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References

1. Xu GW (2005) Chinese breast cancer deaths jump 40% since one child abortion policy. Life Site News, October 13 (Beijing)
2. Shu X, Jin F, Dai Q, Rong J, Shi JR, Potter JD, Brinton LA, Hebert JR, Ruan ZX, Gao YT, Zheng W (2001) Association of body size and fat distribution with risk breast cancer among Chinese women. *Int J Cancer* 94:499–454

3. Lorincz AM, Sukumar S (2006) Molecular links between obesity and breast cancer. *Endocr Relat Cancer* 13:279–292
4. Berclaz G, Li S, Price KN et al (2004) Body mass index as a prognostic feature in operable breast cancer: the International Breast Cancer Study Group experience. *Ann Oncol* 15:875–884
5. Asseryanis A, Ruecklinger E, Hellan M, Kubista E, Singer CF (2004) Breast cancer size in postmenopausal women is correlated with body mass index and androgen serum levels. *Gynecol Endocrinol* 18:29–36
6. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625–1638
7. Hu X, Juneja SC, Maihle NJ, Cleary MP (2002) Leptin—a growth factor in normal and malignant breast cells and for normal mammary gland development. *J Natl Cancer Inst* 94:1704–1711
8. Laud K, Gourdou I, Pessemesse L, Peyrat JP, Djiane J (2002) Identification of leptin receptor in human breast cancer: functional activity in the T47-D breast cancer cell line. *Mol Cell Endocrinol* 188:219–226
9. O'Brien SN, Welter BH, Price TM, Clemson U, Glemson SG (1999) Presence of leptin in breast cell lines and breast tumors. *Biochem Biophys Res Commun* 259:695–698
10. Han CZ, Zhang XT, Du LL, Lui XY, Jing JX, Zhao XW, Yang X, Tian BG (2005) Serum levels of leptin, insulin, and lipids in relation to breast cancer in China. *Endocrine* 26:19–24
11. Tessitore L, Vizio B, Jenkins O, De Stefano I et al (2000) Leptin expression in colorectal and breast cancer patients. *Int J Mol Med* 5:421–426
12. Stattin P, Lukanova A, Biessy C, Soderberg S, Palmqvist R, Kaaks R, Olsson T, Jellum E (2004) Obesity and colon cancer: does leptin provide a link? *Int J Cancer* 109:149–152
13. Ribeiro R, Lopes C, Medeiros R (2006) The link between obesity and prostate cancer: the leptin pathway and therapeutic perspectives. *Prostate Cancer Prostatic Dis* 9:19–24
14. Malik KF, Young WS 3rd (1996) Localization of binding sites in the central nervous system for leptin (OB protein) in normal, obese (ob/ob), and diabetic (db/db) C57BL/6J mice. *Endocrinology* 137:1497–500
15. Tsuchiya T, Shimizu H, Hori T, Mori M (1999) Expression of leptin receptor in lung: leptin as a growth factor. *Eur J Pharmacol* 365:273–279
16. Mix H, Widjaja A, Jandl O, Cornberg M, Kaul A, Goke M, Beil W, Kuske M, Brabant G, Manns MP, Wagner S (2000) Expression of leptin receptor isoforms in the human stomach. *Gut* 47:481–486
17. Hino M, Nakano T, Yamane T, Ohta K, Takubo T, Tatsumi N (2000) Leptin receptor and leukemia. *Leuk Lymphoma* 36:457–461
18. Hardwick JC, Van Den Brink GR, Offerhaus GJ, Van Deventer SJ, Peppelenbosch MP (2001) Leptin is a growth factor for colonic epithelial cells. *Gastroenterology* 121:79–90
19. Attoub S, Noe V, Pirola L, Bruyneel E, Chastre E, Mareel M, Wymann MP, Gespach C (2000) Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3-kinase-, rho- and rac-dependent signaling pathways. *FASEB J* 14:2329–2338
20. Isono M, Inoue R, Kamida T, Kobayashi H, Matsuyama J (2003) Significance of leptin expression in invasive potential of pituitary adenomas. *Clin Neurol Neurosurg* 105:111–116
21. Zheng YM, Xiang KS, Zhang R, Jia WP, Lu JS, Tong JL (1999) Association of Gln223Arg variant in leptin receptor gene with metabolic abnormalities and hypertension in type 2 diabetes in Shanghai “Han” population. *Chinese Journal of Endocrinology and Metabolism* 38:174–178
22. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625–1638
23. Bianchini F, Kaaks R, Vainio H (2002) Overweight, obesity, and cancer risk. *Lancet Oncol* 3:565–574
24. Tessitore L, Visio B, Pesola D, Cecchini F, Mussa A, Argiles JM, Benedetto C (2004) Adipocytes expression and circulating levels of leptin increase in both gynaecological and breast cancer patients. *Int J Cancer* 24:1529–1535
25. Ozet A, Arpacı F, Yılmaz İlker M, Ayta H, Oztürk B, Komurcu S, Yavuz AA, Tezcan Y, Acikel C (2001) Effect of tamoxifen on the serum leptin level in patients with breast cancer. *Jpn J Clin Oncol* 31:424–427
26. Christo S, Bolhke K, Moschos S, Cramer W (1999) Leptin in relation to carcinoma in situ of the breast: a study of pre-menopausal women and controls. *Int J Cancer* 80:523–526
27. Makoto I, Joji K, Hirokazu N (2004) Enhanced expression of leptin and leptin receptor (LEPR) in human breast cancer. *Clin Cancer Res* 10:4325–4331
28. Smith-Kirwin SM, O'Connor DM, Johnston J, de Lancy E, Hassink SG, Funanage VL (1998) Leptin expression in human mammary epithelial cells and breast milk. *J Clin Endocrinol Metab* 83:1810–1813
29. Waxler SH, Taber P, Melcher LP (1953) Obesity and the time of appearance of spontaneous mammary carcinoma in C3H mice. *Cancer Res* 13:276–278
30. Heston WE, Vlahakis G (1962) Genetic obesity and neoplasia. *J Natl Cancer Inst (Bethesda)* 29:197–209

31. Cleary MP, Phillip FC, Getzin SC, Jacobson TL, Jacobson MK, Christensen TA, Juneja SC, Grande JP, Maihle NJ (2003) Genetically obese MMTV-TGF- α /Lep(ob) Lep(ob) female mice do not develop mammary tumors. *Breast Cancer Res Treat* 77:205–215
32. Mantzoros CS, Bolhke K, Moschos S, Cramer CS (1999) Leptin in relation to carcinoma in situ of the breast: a study of pre-menopausal cases and controls. *Int J Cancer* 80:523–526
33. Petridou E, Papadiamantis Y, Markopoulos C, Spanos E, Dessypris N, Trichopoulos D (2000) Leptin and insulin growth factor I in relation to breast cancer. *Cancer Causes Control* 11:383–388
34. Friedman JM, Leibel RL, Siegel DS, Walsh J, Bahary N (1991) Molecular mapping of the mouse ob mutation. *Genomics* 11:1054–1062
35. Coleman DL (1978) Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* 14:141–148
36. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432
37. Mammes O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F (2000) Association of the G-2548A polymorphism in the 5' region of the LEP gene with overweight. *Ann Hum Genet* 64:391–394
38. Mattevi VS, Zembrzusi VM, Hutz MH (2002) Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. *Int J Obes Relat Metab Disord* 26:1179–1185
39. Hager J, Clement K, Francke S, Raison J, Lahlou N, Rich N, Pelloux V, Basdevant A, GuyGrand B, North M, Froguel P (1998) A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels. *Int J Obes Relat Metab Disord* 22:200–205
40. Lamas O, Marti A, Martinez JA (2002) Obesity and immunocompetence. *Eur J Clin Nutr* 56(3):S42–S45
41. Gotoda T, Manning BS, Goldstone AP, Imrie H, Evans AL, Strosberg AD, McKeigue PM, Scott J, Aitman TJ (1997) Leptin receptor gene variation and obesity: lack of association in a white British male population. *Hum Mol Genet* 6:869–876
42. Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS (2001) The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab* 86:4434–4439
43. Hoffstedt J, Eriksson P, Mottagui-Tabar S, Arner P (2002) A polymorphism in the leptin promoter region (–2548 G/A) influences gene expression and adipose tissue secretion of leptin. *Horm Metab Res* 34:355–359
44. Snoussi K, Strosberg AD, Bouauouina N, Ahmed SB, Helal AN, Chouchane L (2006) Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma. *BMC Cancer* 6:38–51
45. Woo HY, Park H, Ki CS, Park YL, Bae WG (2005) Relationships among serum leptin, leptin receptor gene polymorphisms, and breast cancer in Korea. *Cancer Lett* 9:1–6
46. Jerome L, Shiry L, Leyland JB (2004) Anti-insulin-like growth factor strategies in breast cancer. *Semin Oncol* 31:54–63
47. Allen NE, Appleby PN, Kaaks R, Rinaldi S, Davey GK, Key TJ (2003) Lifestyle determinants of serum insulin-like growth-factor-I (IGF-I), C-peptide and hormone binding protein levels in British women. *Cancer Causes Control* 14:65–74
48. Zefang R, Qiuyin C, Xiao-Ou S, Hui C, Chun LI, Herbert YU, Yu-Tang G, Wei Z (2004) Genetic polymorphisms in the IGFBP3 gene: association with breast cancer risk and blood IGFBP3 protein levels among Chinese women. *Cancer Epidemiol Biomark Prev* 13:1290–1295
49. Takahashi Yasuno A, Masuzaki H, Miyawaki T, Ogawa Y, Matsuoka N, Hosoda K, Inoue G, Yoshimasa Y, Nakao K (2003) Leptin receptor polymorphism is association with serum lipids levels and impairment of cholesterol lowering effect in Japanese men. *Diabetes Res Clin Pract* 62:169–175
50. Coskun U, Gunel N, Torunner FB et al (2003) Serum leptin, prolactin and vascular endothelial growth factor (VEGF) levels in patients with breast cancer. *Neoplasma* 50:41–46
51. Du L-L, Han C-Z, Liu X-Y et al (2006) Relationship between serum leptin level BMI and breast cancer incidence. *Cancer Res Clin* 18:307–310