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The *LEP* G-2548A Polymorphism is not Associated with Breast Cancer Susceptibility in Obese Western Mexican Women

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Abstract

Breast cancer incidence and the prevalence of obesity have considerably increased in Mexican women. The *LEP* G-2548A polymorphism has been associated with obesity and breast cancer risk in several ethnic populations. We hypothesize that G-2548A *LEP* polymorphism could be associated with breast cancer risk in women Mexican population. A total of 319 women were included in this study, 130 breast cancer patients and 189 control women. The data collected included anthropometric measurements and menopausal status, and a blood sample was obtained to evaluate SNPs using a PCR-RFLP analysis. No statistically significant associations were identified between breast cancer risk and the *LEP* G-2548A polymorphism in obese or normal-weight subgroups regardless of menopausal status. Our findings suggest that the *LEP* G-2548A polymorphism is not associated with breast cancer risk in obese western Mexican women. Our results indicate that, for western Mexican women, the *LEP* G-2548A polymorphism does not affect breast cancer susceptibility in obese or normal-weight subgroups regardless of menopausal status.

Keywords: Leptin; Polymorphism; Obesity; Breast cancer

Introduction

Leptin is a multifunctional adipokine produced primarily by white adipose tissue. The main role of leptin is the regulation of food intake and energy balance at the hypothalamic level [1], and plasma leptin is highly correlated with body mass index in rodents and obese humans [2]. Additionally, recent *in vitro* studies have shown that leptin stimulates breast cancer growth [3] and that leptin and the leptin receptor are overexpressed in breast cancer tissue and lymph node metastases [4,5].

Mammes et al. were the first to report an association between the *LEP* G-2548A polymorphism and overweight and variations in leptin levels [6]. Obesity is a well-established risk factor for postmenopausal breast cancer [7], and the prevalence of obesity in Mexican adults has increased markedly [8]. We hypothesised that the *LEP* G-2548A polymorphism is associated with breast cancer risk in Mexican women. In the present study, we evaluated obese and normal-weight breast cancer patients for the *LEP* G-2548A polymorphism.

Materials and Methods

Study Population

This study included 319 unrelated female residents from western Mexico (130 breast cancer patients and 189 controls). All participants provided written informed consent, and the study was performed in accordance with the Helsinki Declaration.

Data and sample collection

Data were collected on anthropometric measurements and menopausal status, and a blood sample was taken. The patients and controls were categorised as obese (BMI >30 kg/m^2) or normal weight (BMI <24.99 kg/m^2).

Genomic DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol [9]. The *LEP* G-2548A polymorphism was identified using polymerase chain reaction (PCR). The PCR products were digested with 3 U of the restriction enzyme CfoI (Promega®). Homozygous and heterozygous (*LEP*-2548AA and GA, respectively) genotype samples were included in each run.

Statistical analysis

The allele and genotype frequencies are presented as percentages. The χ^2 test was used to evaluate Hardy-Weinberg equilibrium and

Genotype	Patients n= 130	Controls n=189	OR IC (95%)	p
Co-dominant model	n (%)	n (%)		
GG*	37 (28.5)	48 (25.4)		
GA	71 (54.6)	95 (57.2)	0.96 (0.57-1.64)	0.90
AA	22 (16.9)	46 (24.3)	0.62 (0.31-0.12)	0.15
Dominant model				
GG*	37 (28.5)	48 (25.4)		
GA + AA	93 (71.5)	141 (74.6)	0.85 (0.51-1.41)	0.54
Recessive model				
GG* + GA	108 (83.1)	143 (75.7)		
AA	22 (16.9)	46 (24.3)	0.66 (0.35-1.11)	0.11
Allele				
G	145 (55.8)	195 (51.0)		
A	115 (44.2)	187 (49.0)		0.23

OR: Odds Ratio; CI: Confidence Interval.
Categorical variables were compared using the χ^2 test

Table 1: Genotype distribution and allele frequencies of the LEP G-2548A polymorphism in control subjects and patients with breast cancer.

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	BMI ≤ 30 kg/m²		OR IC (95%)	p	BMI ≤ 25 kg/m²		OR IC (95%)	p
Genotype	**Patients n= 73**	**Controls n=99**			**Patients n=57**	**Controls n=90**		
Co-dominant model	n (%)	n (%)			n (%)	n (%)		
GG	17 (23.3)	25 (25.3)			20 (35.1)	23 (25.6)		
GA	42 (57.5)	48 (48.5)	1.28 (0.61-2.70)	0.50	29 (50.9)	47 (52.2)	0.70 (0.33-1.51)	0.37
AA	14 (19.2)	26 (26.2)	0.79 (0.32-1.93)	0.60	8 (14.0)	20 (22.2)	0.40 (0.16-1.27)	0.13
Dominant model								
GG*	17	25			20	23		
GA+AA	56	74	1.11 (0.54-2.25)	0.76	37	67	0.63 (0.30-1.13)	0.21
Recessive model								
GG*+GA	59	73			49	70		
AA	14	26	0.66 (0.32-1.38)	0.2	8	20	0.57 (0.23-1.40)	0.21
Allele								
G	76 (52.1)	98 (49.5)						
A	70 (49.4)	100 (50.5)		0.63	69 (60.5)	93 (51.7)		
					45 (39.5)	87 (48.3)		0.13

OR: Odds Ratio; CI: Confidence Interval.
Categorical variables were compared using the χ^2 test

Table 2: Genotype distribution and allele frequencies of the G-2548A *LEP* polymorphism in breast cancer patients and control women, stratified into obese (BMI ≥ 30 kg/m²) and normal-weight (BMI ≤ 25 kg/m²) groups.

Genotype	Patients n= 46	Controls n=144	OR IC (95%)	p
Pre-menopausal women	**n (%)**	**n (%)**		
GG*	12 (25.5)	35 (24.3)		
GA	26 (26.3)	73 (50.7)	1.0 (0.47- 2.33)	0.92
AA	8 (17.4)	36 (25.0)	0.64 (0.23-1.77)	0.39
Allele frequencies				
G	50 (54.3)	143 (49.7)		
A	42 (45.7)	145 (50.3)		0.43
Post-menopausal women	n=84	n=45		
GG*	25 (29.8)	13 (28.9)		
GA	45 (53.5)	22 (48.9)	1.0 (0.45-2.46)	0.88
AA	14 (16.6)	10 (22.2)	0.72 (0.25-2.06)	0.55
Allele frequencies				
G	95 (56.5)	48 (53.3)		
A	73 (43.5)	42 (46.6)		0.62

OR: Odds Ratio; CI: Confidence Interval.
Categorical variables were compared using the χ^2 test

Table 3: Genotype distribution and allele frequencies of the LEP G-2548A polymorphism in control subjects and patients with breast cancer according to menopause status.

significant associations between breast cancer and genotype. Odds ratios (ORs) were used to express the risk of breast cancer associated with a particular genotype. These statistical analysis were performed using SPSS for Windows software, version 15.0. Significance was assumed for $p< 0.05$.

Results

Genotype and allele frequencies of the *LEP* G-2548A polymorphism

The observed genotype frequencies were in Hardy-Weinberg equilibrium in both the patient and control groups (p=0.22 and p=0.94, respectively). The genotype and allele frequencies did not differ between breast cancer patients and controls (Table 1). An association test showed no statistically significant correlation between the *LEP* G-2548A polymorphism and breast cancer risk. To determine the influence of obesity on the relationship between the *LEP* polymorphism and breast cancer risk, the patient and control groups were further stratified into obese and normal-weight subgroups. A lower frequency of the homozygous genotype AA was observed in normal-weight breast cancer patients (14%). However, there were no differences in polymorphism frequency between obese and normal-weight patients and controls (Table 2). There was no significant relationship between the *LEP* G-2548A polymorphism and breast cancer risk regardless of menopausal status (Table 3).

Discussion

In this study, we found no association between the *LEP* G-2548A polymorphism and breast cancer risk (Tables 1 and 2). By contrast, studies conducted in other countries have associated the *LEP* G-2548A polymorphism with obesity [6,10-12] and breast cancer risk, although some have shown inconsistent results [10,13-16].

An association between the *LEP* G-2548A polymorphism and breast cancer risk has been shown in previous studies. For example, Tunisian women carrying the *LEP*-2548AA genotype have a threefold increase in breast cancer susceptibility (OR=3.17; 95% CI=1.47-6.96, p=0.001) [17]. A modest increase in the risk of developing breast cancer has been associated with the *LEP* -2548AA genotype (OR=1.30; 95% CI=1.01–1.66) in European-American women [18].

Obesity is a risk factor for postmenopausal breast cancer [7]. We classified breast cancer patients and controls according to menopause status and observed no significant changes in *LEP* G-2548A genotype frequencies (Table 3), which is in agreement with other studies [15,17]. The discrepancies observed between this and other studies may be attributable to several potential effect modifiers, such as differences in ethnicity, sample size, BMI, age and sex.

A few reports have described the functional effect of the G-2548A *LEP* polymorphism. Hoffstedt et al. reported that this polymorphism

influences leptin expression, possibly at the transcriptional level, and therefore also leptin secretion by adipocytes [19]. More recently, Terrasi et al. conducted a functional analysis of the *LEP* G-2548A polymorphism in breast cancer cell lines and demonstrated differences in leptin mRNA expression [20]. We were unable to evaluate the leptin levels in our samples, but other authors have reported variations in leptin values according to *LEP* G-2548A genotypes and alleles [6,10,11,16].

Conclusions

The *LEP* G-2548A polymorphism has been associated with obesity and breast cancer risk in several ethnic populations. However, this study does not support an association between the *LEP* G-2548A polymorphism and breast cancer risk in western Mexican women.

Competing Interests

The author(s) declare that they have no competing interests.

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