

RESEARCH ARTICLE

Association between leptin and leptin receptor gene polymorphisms and breast cancer risk in premenopausal and postmenopausal Mexican women

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Abstract

Purpose: This study aims to evaluate the association between leptin (-2548 G>A) and leptin receptor (p.K109R, p.Q223R, p.K656N) polymorphisms with breast cancer (BC) risk in premenopausal and postmenopausal Mexican women.

Material and methods: 274 women were included, 76 with BC in premenopause, 66 with BC in postmenopause, 108 without BC in premenopause, and 22 without BC in postmenopause. Four polymorphisms were genotyped by PCR, High Resolution Melting, and DNA direct sequencing. χ^2 , logistic regression analysis was performed with SPSS and XLSTAT software.

Results: There was statistically significant risk of BC in postmenopausal women with *LEPR* p.Q223R+p.R223R (OR: 8.4, 95% CI: 2.7-25.6, P <0.001), and in premenopausal women with *LEP*-2548G>A+ -2548A>A genotype (OR: 1.7, 95% CI: 1.0-3.0, P = 0.04).

Discussion and Conclusions: This is the first study from Mexico that includes four polymorphisms on *LEP* and *LEPR* genes. Our data indicate that both *LEPR* p.Q223R in postmenopausal women and *LEP*-2548G>A in premenopausal women have a strong association with BC, which suggests that these polymorphisms play a role in the development of BC in Mexican women. We detected coincidences and differences with respect to other populations. This highlights the importance of conducting genetic epidemiologic studies to identify the risk of gene polymorphisms and BC for each population.

Keywords: leptin, leptin receptor, breast cancer, premenopause, postmenopause

INTRODUCTION

Breast cancer (BC) is a heterogeneous and complex disease due to an interaction between environmental factors and genetic susceptibility. The major risk factors for developing BC include being of female sex, receiving hormone replacement therapy during menopause, having received ionizing

radiation, being of an early age at first menstruation, having children late or none at all, being of older age, having a family history of BC, and being obese (1). Obesity is a well-established risk factor for postmenopausal BC (2) The adipocyte dysfunction in obese individuals stimulates insulin resistance, ineffectual lipolysis, metabolic disease, and the

release of mitogenic factors/ proinflammatory cytokines (3).

Leptin, a cytokine of the adipocytes encoded by the leptin (*LEP*) gene, interacts with the leptin receptor (*LEPR*), which is present in breast and other tissues (4). *LEP* and *LEPR* are overexpressed in BC tissue (5, 6). *LEP* 2548G>A polymorphism seems to influence leptin mRNA expression in breast cancer cell lines affecting signaling capacity and receptor functionality, increasing the *LEPR*-*LEP* binding capacity (7,8). This induces a cascade of events in the tumor microenvironment, causing the proliferation, angiogenesis, and invasion of the cell (9, 10). QR and RR genotypes of the *LEPR* p.Q223R polymorphism have been associated with modest risk for BC in non-obese premenopausal women (11), whereas QR and QR+QQ genotypes of the *LEPR* p.Q223R polymorphism with BC in overweight postmenopausal women (12).

The RR genotype of the *LEPR* p.K109R polymorphism has been associated with large tumors in obese premenopausal women (13). The *LEP* -2548G>A polymorphism has been associated with modest risk of BC in obese postmenopausal women (14). In a previous study, no statistical differences were found between BC and *LEP* 2548G>A in obese or non-obese Mexican women (15).

We hypothesize that single nucleotide polymorphisms (SNP), -2548G>A, located in the promoter of the *LEP* gene, and p.K109R, p.Q223R, p.K656N, located in the coding zone of *LEPR* gene, could influence BC risk, according to menopausal status (16–20) in Mexican women.

MATERIAL AND METHODS

Patient population

The study included 274 women (mean age of 42.5±10.4 years), 142 with BC (mean age of 47.3±9.1 years), and 132 healthy controls (mean age of 37.3±9.2 years); 184 were premenopausal (mean age of 36.4±6.8 years) and 90 were postmenopausal (mean age of 53.2±5.9 years). The stage of the patients was III according to the breastcancer.org. All of the unrelated subjects were born in Mexico, with at least three generations of Mexican parents. All women resided in metropolitan areas. The protocol was approved by the Ethics Committee of the General Hospital of Mexico. Informed consent

was obtained from all subjects. Eating habits, reproductive and menstrual histories, exogenous hormone use, familial history of cancer, physical activity, alcohol and smoking histories, weight in kilograms (Kg), height in meters (m), and body mass index (BMI = Kg/m²) were determined in all subjects.

Genotyping analysis of LEP and LEPR polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes (using the protocol of peripheral blood DNA extraction, Wizard-Promega). Oligonucleotides for the *LEP* gene, SNP (-2548G>A) were: forward 5'-TTCCTGTAATTTCCCATGAGAAC-3' and reverse 5'-TCTCAGCACTTAGGGAGACT-3'; while the oligonucleotides for the SNP in *LEPR* gene (p.K109R) were: forward 5'-GAAACTGCTCCTTATGTGCAGA-3' and reverse 5'-TGCTTACCTATTTGT TGAAAACTAAA-3'; oligonucleotides for the SNP in the *LEPR* gene (p.Q223R) were: forward 5'-CGACACTCTCCTTATGTG TTTGA-3' and reverse 5'-ATATTTATGGGGTGAAGTGAACATTAG-3'; and for the SNP in the *LEPR* gene (p.K656N) were: forward 5'-GAGGACCTGAATTTTGGAGAATAA-3' and reverse: 5'-GAATACCTTCCAAAGTAAAGTGACA-3'. The PCR products obtained from the cases/controls were sequenced on an ABI 3730 Automated Sequencer (PE Biosystems, Foster City, CA, USA). Genotyping by PCR and High Resolution Melting (HRM) analyses were done on the LS-32 (Idaho Technology) and analyzed with the software LightScanner Primer Desing, as previously described elsewhere (21, 22).

Statistical analyses

The agreement of genotype frequencies with Hardy-Weinberg equilibrium (HWE) expectations and allelic frequencies were analyzed with the chi-square test (χ^2). Binary logistic regression was used to determine OR (odds ratio), 95% confidence interval (CI), and significant differences (*P* value) of the genotypic frequencies among BC patients and healthy controls, between premenopausal versus postmenopausal women, and among BMI >30 versus BMI < 30. Postmenopausal status was considered by absence of menses for at least 1 year or according to the average of the Mexican population if the record was not obtained (23). For analysis, because patients with the homozygous minor allele or genotype were uncommon in our sample, these patients were included with their respective heterozygotes. This is

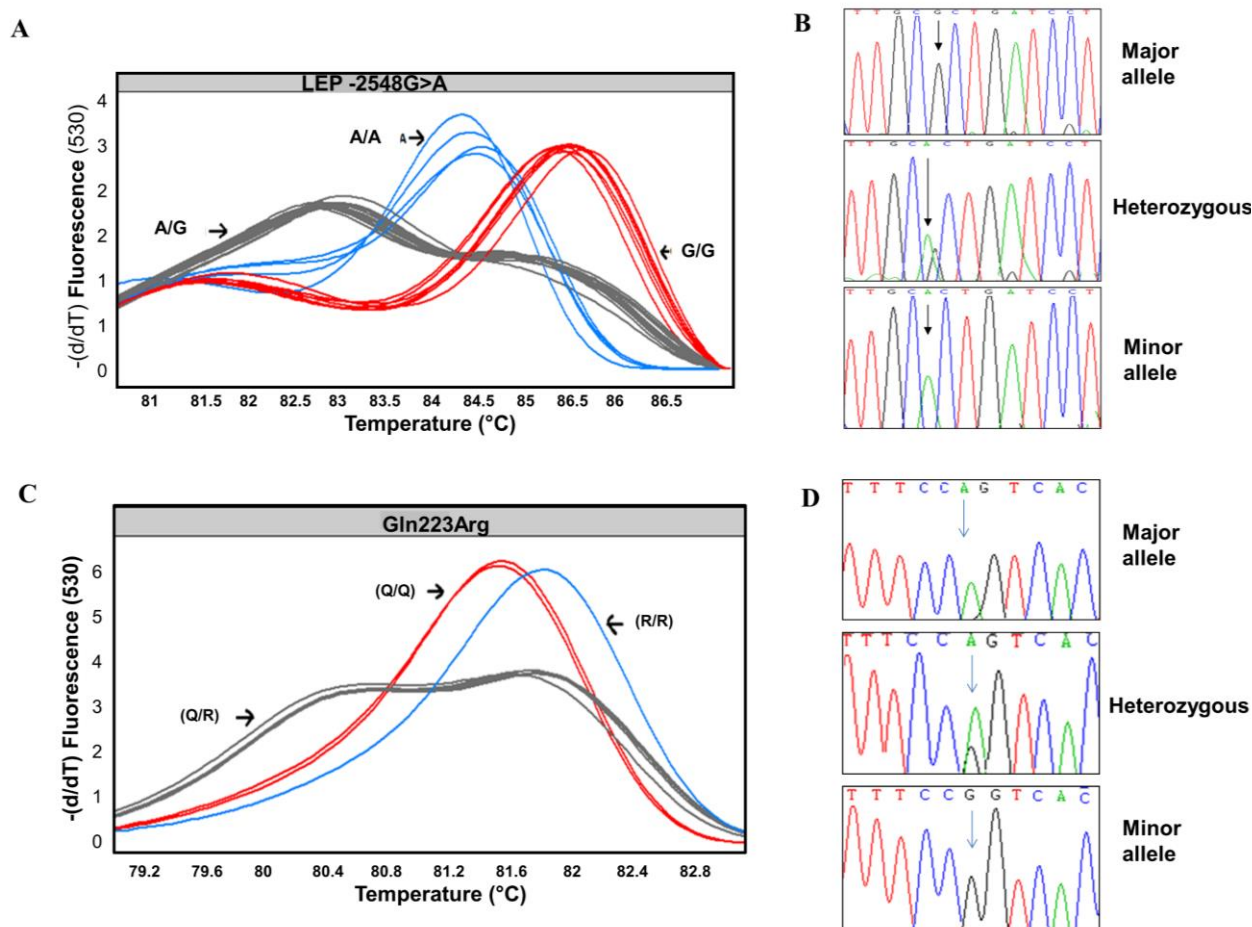


Figure 1. Melting curve analysis (A and C) and electropherograms of a partial sequence of the (B) *LEP* (-2548G>A) and (D) *LEPR* (p.Q223R) gene polymorphisms in the BC patients and healthy controls are showed. Wild-type homozygous, heterozygous and mutant homozygous genotypes are represented by colors different in the melting curves. The black arrows show the SNPs sites in the electropherograms. Abbreviations: R, Arginine; Q, glutamine.

consistent with analysis techniques reported elsewhere (24, 25). *P* value <0.05 was considered statistically significant. These analyses were carried out with SPSS (v. 22.0) and XLSTAT (v. 2015) software.

RESULTS

Distribution of genotype and allele frequencies of LEP and LEPR gene polymorphisms

HRM curve and sequencing analysis of the four SNPs in the 274 Mexican women showed heterozygous and homozygous curves, suggesting the presence of allelic variation (Figure 1). HRM variation detection sensitivity was 100% concordant with 274 variations,

as confirmed through DNA direct sequencing analysis.

The genotype distribution and allele frequencies of the *LEP* (-2548 G>A) and *LEPR* p.K109R, p.Q223R, p.K656N polymorphisms in all patients with BC, and in the healthy controls, are shown in Table 1. The genotype frequencies were in HWE in both patients and healthy controls for *LEP* (-2548 G>A) (*P* = 0.32, *P* = 0.31), *LEPR* p.Q223R, (*P* = 0.22, *P* = 0.40) and *LEPR* p.K656N (*P* = 0.75, *P* = 0.75); there was not Hardy-Weinberg equilibrium for *LEPR* p.K109R (*P* = 0.035, *P* = 0.042).

Association between LEP and LEPR polymorphisms with overall BC risk

Table 1 Genotypic and allelic frequency of *LEP* and *LEPR* polymorphisms and their associations with risks of breast cancer (BC) in patients with BC and healthy controls

Genotype	P (n=142) n (%)	HC (n=132) n (%)	OR	(95 % CI)	P value
<i>LEP</i> -2548G>A					
GG	40 (28.2)	40 (30.3)	Ref		
GA+AA	102 (71.8)	92 (69.6)	0.9	(0.5–1.6)	0.77
G allele	144 (50.7)	156 (59.1)	Ref		
A allele	140 (49.3)	108 (40.9)	1.4	(1.2–1.5)	0.18
<i>LEPRA</i> 326G (p.K109R)					
AA	94 (66.2)	60 (45.5)	Ref		
AG+GG	48 (33.8)	72 (54.5)	0.4	(0.2–0.7)	0.002*
A allele	230 (81.0)	172 (65.1)	Ref		
G allele	54 (19.0)	92 (34.9)	0.4	(0.3 –0.5)	<0.001*
<i>LEPRA</i> 668G (p.Q223R)					
AA	58 (40.8)	32 (24.2)	Ref		
AG+GG	84 (59.1)	100 (75.7)	0.5	(0.3–0.9)	0.024*
A allele	168 (59.1)	140 (53.0)	Ref		
G allele	106 (40.9)	124 (47.0)	0.6	(0.5–0.7)	0.31
<i>LEPRG</i> 1968C (p.K656N)					
GG	104 (73.2)	98 (74.2)	Ref		
GC+GG	38 (22.6)	34 (24.2)	0.9	(0.3–2.5)	0.44
G allele	240 (84.5)	228 (86.3)	Ref		
C allele	44 (15.5)	36 (13.6)	1.2	(1.0 –1.3)	0.62

Abbreviations: P, patients with breast cancer; HC, healthy controls; OR, Odds ratio; CI, confidence interval, adjusted for age; n, number of patients; *P, statistical significance;

Analyses to determine the association of *LEP* and *LEPR* polymorphisms with overall BC risk indicated statistical differences ($p < 0.05$) with low OR in patients with BC, and in carriers of *LEPR* 109KR+RR and *LEPR* 223QR+RR genotypes. No statistical differences were observed for *LEP* (-2548G>A) and for *LEPR* p.K656N polymorphisms (Table 1).

Association between *LEP* and *LEPR* polymorphism with BC risk according menopausal status

Analyses of stratification of the premenopausal and postmenopausal patients to detect BC risk showed statistical differences for the *LEPR* 223QR+RR genotype, with a higher BC risk in postmenopausal women (OR: 8.4, 95% CI: 2.7-25.6, $P < 0.001$, whereas the *LEP*-2548 GA+AA genotype indicated statistical differences with a higher risk of BC in premenopausal women (OR: 1.7, 95% CI: 1.0-3.0, $P = 0.04$). No risk was observed with the remaining *LEP* and *LEPR* gene polymorphisms and BC (Table 2).

When we added the condition of BMI > 30 or BMI < 30 into the analysis, in addition to the menopausal status, we observed a higher risk of BC in postmenopausal women, predominantly in those with BMI > 30 (OR 4.5 95% CI: 2.1-9.5, $P < 0.001$) versus BMI < 30 (OR 2.6 95% CI: 1.2-5.7, $P = 0.015$) (Table 3).

Cumulative analyses of the studied polymorphisms showed that *LEP* -2548, *LEPR* 326 y *LEPR* 668 have higher risk for BC (OR: 9.0, 95% CI 2.48-32.5, $P = 0.001$) (Table 4).

DISCUSSION

In this study, *LEPR* p.Q223R (QR+RR) established a statistical difference with a high OR for BC in postmenopausal women, and *LEP* -2548G>A (GA+AA) had a statistical difference with a higher OR in premenopausal women with BC. These data show the involvement of *LEP* and *LEPR* gene

Table 2. Genotypic frequency distribution of *LEP* and *LEPR* Polymorphisms and BC risk according at menopausal status

Genotype	PREMENOPAUSAL				POSTMENOPAUSAL			
	P	HC	OR (95 % CI)	P Value	P	HC	OR (95 % CI)	P Value
<i>LEPG2548G>A</i>								
GG	22	32	Ref		18	8	Ref	
GA+AA	54	76	1.7 (1.0–3.0)	0.04*	48	16	2.2 (0.8–6.1)	0.10
<i>LEPRA326G</i>								
AA	68	52	Ref		26	8	Ref	
AG+GG	8	56	0.1 (0.08–0.4)	<0.001*	40	16	0.2 (0.07–0.8)	0.02*
<i>LEPRA668G</i>								
AA	38	16	Ref		20	16	Ref	
AG+GG	38	92	0.4 (0.23–0.8)	0.01*	46	8	8.4 (2.7–25.6)	<0.001*
<i>LEPRG196C</i>								
GG	62	78	Ref		42	20	Ref	
GC+CC	14	30	1.0 (0.48–2.3)	0.85	24	4	2.9 (0.83–10.3)	0.09

Abbreviations: P, patients with breast cancer; HC, healthy controls; OR, Odds ratio; CI, confidence interval, adjusted for age; n, number of patients; f, frequency; BMI, body mass index; *P, statistical significance; Ref, reference.

polymorphisms in BC risk in the Mexican population. Contrary to a previous study in Mexican patients (15), our study detected a higher risk of BC in postmenopausal women with a BMI>30, suggesting obesity contributes significantly to the risk of BC in postmenopausal women, but not in premenopausal women (Table 3). This could be due to a bias in the sample selection in the previous Mexican study.

In other populations, the *LEPR* p.K109R polymorphism has been associated with BC risk in obese premenopausal women (13); however, a meta-analysis suggested that the *LEP* p.K109R polymorphism did not affect risk of BC (26). *LEPR* p.Q223R and *LEP* -2548G>A polymorphisms have

been associated with BC in obese postmenopausal women (18,19), whereas *LEPR* p.Q223R and p.R223R polymorphisms are associated with BC in non-obese premenopausal women (14). Conversely, in our Mexican sample, *LEPR* p.Q223R was associated with BC in postmenopausal women; this demonstrates the importance of conducting these studies in several populations.

Five out of ten studies reported an association between the *LEPR* p.Q223R polymorphism with BC risk. In one of them, p.Q223R and p.R223R were associated with an increased BC risk and with shorter overall survival (16). Other studies reported an association between p.R223R with luminal A

Table 3. Breast cancer risk according at menopausal status and BMI status

Menopausal status with BMI status	OR	(95 % CI)	P value
Premenopausal-BMI<30	Ref		
Premenopausal-BMI>30	0.4	(0.2-0.7)	0.006*
Postmenopausal-BMI<30	2.6	(1.2-5.7)	0.015*
Postmenopausal-BMI>30	4.5	(2.1-9.5)	<0.001*

Abbreviations: OR, Odds ratio; CI, confidence interval, adjusted for age; BMI, body mass index; *P, statistical significance; Ref, reference.

Table 4. Cumulative effect of *LEP* and *LEPR* polymorphisms and breast cancer risk.

Interaction polymorphism	OR	(95 % CI)	P value
2548 by 326	0.14	(0.04-0.44)	0.001*
2548 by 326 by 668	9.00	(2.4-32.5)	0.001*
1968 by 2548 by 326 by 668	2.66	(0.71-9.9)	0.144

Abbreviations: OR, Odds ratio; CI, confidence interval, adjusted for age; *P, statistical significance.

subtype of BC (19), and tumor grade of BC (26), with poorly differentiated BC (20). In the present study, *LEPR* p.Q223R + p.R223R were associated with BC in obese postmenopausal women. Conversely, four studies showed no risk of BC with *LEPR* p.Q223R/p.R223R (14, 17, 27, 28), emphasizing the importance of the relationship between genetic backgrounds with environmental/epigenetic factors.

On the other hand, the *LEP* (-2548G>A) polymorphism has been analyzed in ten studies; in two of them, the A allele was associated with BC (16, 18, 29). Seven studies showed no association between BC and the *LEP* -2548G>A polymorphism (9, 17-19, 27, 28). In our population, we found statistical differences in the *LEP* (-2548G>A) (GA+AA) polymorphism and BC in premenopausal women.

Breast cancer is a complex and multifactorial disease, and gene-gene and gene-environmental interactions may contribute significantly to its occurrence (30). Many non-infectious diseases are polygenic and involve polymorphisms that predispose people to obesity, glucose intolerance, insulin resistance, and a higher risk of developing BC (14). Major risk factors for BC and linkage disequilibrium of *LEP* and *LEPR* polymorphisms in each population play a role in BC risk (12, 17, 18, 27, 28). In our study, the combination of *LEP* -2548, *LEPR* p.K109R, and *LEPR* p.Q223R polymorphisms have a higher risk for BC with OR of 9.0, 95% CI 2.48-32.5, P = 0.001 (Table 4). This demonstrates the cumulative effect of the gene-gene interaction and the complexity of determining the BC risk (31, 32).

The postmenopausal state involves hormonal changes such as decreases in estrogen. The importance of the *LEP* and *LEPR* gene polymorphisms in BC could be explained by the changes in the expression levels of leptin, with the effect on leptin receptors possibly at the

transcriptional level (20, 33). These changes stimulate the production of estrogen through the increase of aromatase expression in the cells of BC (34). Further studies of BC risk in several populations are necessary due to the different genetic background of each population.

In conclusion, we detected an association between *LEP* (-2548G>A) and BC in premenopausal women and *LEPR* p.Q223R with BC in obese postmenopausal women, which indicates that these polymorphisms play a role in the development of BC in Mexican women. One limitation of the present study is perhaps the sample size, even though there were statistical differences probably a larger sample could be a better indicator. We detected coincidences and differences with respect to other populations.

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ABBREVIATIONS

BC, breast cancer;
 BMI, body mass index;
 CI, confidence interval;
 HC, healthy controls;
 HRM, high resolution melting;
 LEP, leptin;
 LEPR, leptin receptor;
 OR, odds ratio;
 P, patients with breast cancer;
 PCR, polymerase chain reaction;

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