Association of Leptin Receptor (LEPR) Q223R Polymorphism with Breast Cancer

By C.Anuradha, P.Madanranjit, D.Surekha, D.Raghunadharao, N.Santhoshi Rani & S.Vishnupriya

Osmania University, Hyderabad

Abstract – The leptin receptor (LEPR) is a member of the cytokine receptor family with two cytokine domains and a single trans membrane domain, which plays an important role in body weight homeostasis through regulation of food intake and energy expenditure. It also influences other pathways like hematopoiesis, reproduction, angiogenesis, and immune processes upon interacting with ligand leptin. Leptin is an adipocytokine produced in adipose tissue. Since obesity is one of the known risk factor as well as the LEPR expression and leptin levels were shown to be associated with the development of mammary ductal carcinoma, an attempt has been made to evaluate the role of LEPR Q223R polymorphism with breast cancer susceptibility in south Indian population as well as with confounding epidemiological and clinical factors. The present study included 194 breast cancer cases and 186 age matched control samples for the analysis of LEPR Q223R polymorphism through PCR-RFLP method.

Keywords : Leptin, LEPR, PCR RFLP, Breast cancer.

GJMR-B Classification : FOR Code: 111201, 730108, NLMC Code: QZ 20.5, QZ 275

ASSOCIATIONOFLEPTINRECEPTORLEPRQ223RPOLYMORPHISMWITHBREASTCANCER

Strictly as per the compliance and regulations of:

Association of Leptin Receptor (LEPR) Q223R Polymorphism with Breast Cancer

C.Anuradha[α], P.Madanranjit[σ], D.Surekha[ρ], D.Raghunadharao[ω], N.Santhoshi Rani[¥] & S.Vishnupriya[§]

Abstract -The leptin receptor (LEPR) is a member of the cytokine receptor family with two cytokine domains and a single trans membrane domain, which plays an important role in body weight homeostasis through regulation of food intake and energy expenditure. It also influences other pathways like hematopoiesis, reproduction, angiogenesis, and immune processes upon interacting with ligand leptin. Leptin is an adipocytokine produced in adipose tissue. Since obesity is one of the known risk factor as well as the LEPR expression and leptin levels were shown to be associated with the development of mammary ductal carcinoma, an attempt has been made to evaluate the role of LEPR Q223R polymorphism with breast cancer susceptibility in south Indian population as well as with confounding epidemiological and clinical factors. The present study included 194 breast cancer cases and 186 age matched control samples for the analysis of LEPR Q223R polymorphism through PCR-RFLP method. The frequency of RR genotype was significantly elevated in breast cancer compared to control subjects ($\chi2$= 6.567; df=2, p= 0.037)). Similar trend was also observed with respect to high BMI and post menopausal status. No significant association was found with stage of the disease.

Keywords : *Leptin, LEPR, PCR RFLP, Breast cancer.*

I. INTRODUCTION

The leptin receptor (LEPR) is a single transmembrane protein, belongs to class I cytokine receptor family. Due to alternative splicing during transcription of LEPR gene different isoforms of LEPR are formed with varied length of intracellular domain, but all the isoforms have identical extracellular and transmembrane domains [Lee et al., 1996; Tartagia, 1997]. Six isoforms derived from LEPR transcription have been identified so far, and a long isoform, LEPR-b, is reported to be responsible for signal transduction [Zabeau et al., 2003].

Leptin, a 16 kDa cytokine acts as a regulator of body weight and energy balance, a product of ob gene synthesized from white adipose tissue which influences food intake and energy expenditure [Campfield et al., 1995; Halaas et al., 1995]. Leptin exerts its physiological

action through binding to leptin receptor (LEPR) and exerts negative feedback mechanism to control food intake and body weight [Malik et al., 1996]. However, its expression was identified in other tissues and cells, including immune cells, placenta, endometrium, stomach and lung [Caldefie-Chezet et al., 2001 Ebenbichler et al., 2002; Kitawaki et al., 2000; Breidert et al., 1999; Tsuchiya et al., 1999]. In addition, leptin receptor expression has been detected in pathological conditions such as acute myeloid leukemia, hepatocelluar carcinoma, gastric cancer cells and breast cancer [Konopleva et al., 1999; Wang et al., 2004; Mix et al., 2004; Ishikawa et al., 2004; Garofalo et al., 2006]. The binding of leptin to LEPR activates the JAK/STAT (Janus kinase signal transducer and activates of transcription), the Ras/ERK1/2 signaling cascade (a member of the MAPK family), and the PI-3K/Akt/GSK3 growth/anti-apoptotic pathways [Zabeau et al., 2003]. In addition, leptin binding to receptor has shown to transactivate HER2/neu, and also increase the expression of vascular endothelial growth factor (VEGF) [Eisenberg et al., 2004; Garofalo et al., 2006].

In humans, several single nucleotide polymorphisms (SNPs) have been described in the LEPR gene [Chung et al., 1997]. An A to G transition at nt 668 from the start codon that converts a glutamine to an arginine at codon 223 (Q223R) in the LEPR gene [Gotoda et al., 1997] alters amino acid charge from neutral to positive that could affect the functionality of the receptor and modifies its signaling capacity, which is associated with higher mean circulating levels of leptin [Quinton et al 2001., Yiannakouris et al., 2001]. This polymorphism is located within the region encoding the extracellular domain of the leptin receptor; the amino acid change affects all forms of the receptor. It has been demonstrated that individuals homozygous for the G (R223R) allele is associated with variation in ligand binding activity than the carriers of the A (Q223Q) [Quinton et al 2001]. Several studies had shown the relationship between LEPR Q223R gene polymorphism with BMI, insulin resistance, Prostate cancer, Postmenopausal breast cancer

[Snoussi et al., 2006]. It can activate ERα and ERα-dependent transcription in a ligand-independent manner [Catalano et al., 2004]. Since BMI is known to be associated with breast cancer as well as with LEPR polymorphism, the present study has been planned to evaluate the role of LEPR polymorphism in the pathogenesis of breast cancer development.

Author [ρ, ¥]: Department of Genetics, Osmania University, Hyderabad 500007, India.
Author [α, σ] : AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam530003, India.
Author [ω] : Department of Medical Oncology, Nizams Institute of Medical sciences, Hyderabad 500082, India.
Author [§] : Ph.D. Head & Professor Department of Genetics Osmania University Hyderabad 500007, Andhra Pradesh, INDIA Phone (Off) 91-40-2768-2335,91-40-2709-5178 Fax: 91-40-2709-5178
Corresponding Author : E-mail : sattivishnupriya@gmail.com

II.   MATERIAL AND METHODS

a) Study population

The study comprises of 194 breast cancer patients from Nizam's institute of medical sciences (NIMS) and 186 age matched women with out any familial history of malignancies to serve as controls. Clinical information such as stage of the breast cancer, menopausal status at the time of onset, hormonal receptor status (ER, PR), Her2 status and BMI was noted down from the hospital records with the help of medical oncologist Ethical committee approval from the institute is obtained before initiation of the study. All the participants were informed about the study and consent was taken. 5ml of blood sample was collected into 10 ml   EDTA vacutainer tube from both patients and controls. All the samples were stored at -20∘ C. Genomic DNA was extracted from leukocyte nuclei by the salting-out method [Lahiri et al., 1991] and used for amplification of Q223R polymorphism in exon 6 of LEPR gene.

b) LEPR Q223R Polymorphism

PCR-RFLP was carried out for the identification of LEPR Q223R polymorphism using gene specific oligonucleotide primers previously described [Gotoda et al., 1997]. The amplified product was digested with 5 units of Msp1enzyme (New England Biolabs, USA) at 37°C for overnight and electrophoresed on 3% agorose gel. A–G substitution at nucleotide 668 in exon six of LEPR gene introduces a recognition site for MspI. Digestion by MspI produces one band of 80 bp in the normal LEPR sequence (QQ). Three separate bands of 80, 58 and 22 bp in the heterozygous individual (QR), and two separate bands of 58 and 22 bp in mutant homozygotes (RR) were observed.

c) Statistical Analysis

All the statistical analyses were performed using SPSS software15.0 (Statistical Package for the Social Science). Chi square test was done to test the significance of genotype association with the occurrence of disease. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. All the P values were two sided and the level of significance was taken as $P < 0.05$.

III.   RESULTS AND DISCUSSION

LEPR a cytokine receptor involved in the homeostatic control of appetite, weight, metabolism and reproductive functions in women [Friedman et al., 1998], via activating signal transduction pathways through ligand binding with leptin [Banks et al., 2000]. LEPR spans over 70 kb and includes 20 exons which encode for 1,165 amino acid protein that belongs to the superfamily of cytokine receptors and requires all its extracellular subdomains functional for transmitting the signal. LEPR presents two basic isoforms, a short

intracellular domain variant, unable to transmit a signal, which is present in a variety of tissues; and a second one with a long intracellular functional domain, capable of activating JAK2 and STAT3, and perhaps other signaling pathways [Sweeney et al., 2002].

The LEPR Q223R polymorphism corresponding to A to G transition in exon 6 of the gene is known to result in several functional consequences due to altered charge distribution from neutral to positive charge. The Q223R polymorphism lies within the first of two putative leptin-binding regions in the extracellular domain of the receptor and therefore, the amino acid change affects all isoforms of the receptor and may be associated with impaired LEPR signaling capacity [Yiannakouris et al., 2001].

The genotype and allele frequencies of the LEPR Q223R SNP in 194 breast cancer patients and 186 healthy controls are shown in Table1. The frequency of LEPR 223 RR genotype was significantly elevated in breast cancer as compared to control group ($\chi 2 = 6.567$; df=2, p= 0.037) (Table 1) LEPR 223RR genotype with more efficient binding capacity to leptin might trigger cellular JAK2 /STAT3, the Ras/ERK1/2 signaling pathways there by initiating the tumorogenesis. Leptin when binds to receptor also acts synergistically with VEGF (vascular endothelial growth factor) and fibroblast growth factor 2 (FGF- 2) to promote angiogenesis [Sierra-Honigmann et al., 1998]. It has effect on the expression of several genes involved in the angiogenesis (MMP-2 and MMP-9) [Park et al., 2009; Zhang et al., 2007].

The homozygous RR genotype frequency was elevated considerably in post menopausal patients (11.34%) compared to pre-menopausal patients (6.19%) (Table2.1). There was no significant association of LEPR Q223R polymorphism with Estrogen and Progesterone receptor status among breast cancer patients (Table2.2, 2.3), however the RR genotype frequencies were elevated in both ER-ve (8.2%) and PR-ve cases (8.97%) compared to ER+ve(6.85%) and PR+ve cases(5.88%) (Table2.2, 2.3).

With reference to BMI of patients (Table.3), a considerable elevation in the RR genotype and R allele frequency in obese patients (10.66%, 36.07%) compared to patients with lower BMI (3.45%, 27.59%) was observed. Further when non-carriers of R allele (i.e Q/Q) and carriers of R allele (i.e. Q/R+R/R) were compared, R allele carrier frequency was significantly higher (61.48%) in obese subjects when compared to less weight group (33.34%). Many reports had suggested that Q223R LEPR polymorphism was associated with BMI and overweight. Thompson et al, 1997 first reported this polymorphism was associated with obesity [Bruce Thompson et al., 1997].

When the data was stratified based on the stage of the disease and genotype frequency (Table. 5). A higher percentage of patients with stage II breast

cancer carried RR genotype (12.5%) than patients with advanced stage cancer (6.98%) (OR, 0.39; 955 CI, 0.14-1.08) although the OR was not statistically significant due to a small sample size.

The impact of Q223R polymorphic variants on human body composition had also been reported in two recent studies [Chagnon et al., 1999; Chagnon et al., 2000]. In the Que´bec Family Study, Chagnon et al., 1999 observed a significant sibling pair linkage between the Q223R polymorphism and fat mass (FM). Although no association between body composition variables and the Q223R polymorphism was observed, there was a weak, but significant, association between the Q223 allele and free fat mass (FFM) in lean males when the analysis was performed by BMI and gender groups (Chagon et al., 1999). In the Heritage Family Study cohort, stronger evidence of an association between the Q223R polymorphism and human adiposity was reported among Caucasians, although no reciprocal linkages were detected [Chagnon et al., 2000]. In particular, middle-aged Caucasian males who were carriers of the R223 allele had significantly higher BMI, %FM, and plasma leptin levels than noncarriers.

Nikos et al 2001 reported that, a higher percentage of homozygotes for R223 allele were found among the heavier subjects. R/R homozygotes had higher BMI (2±U; P=0.01) and higher fat mass values than the carriers of Q223 allele, the Q223R polymorphism was a significant predictor of 5% of the body composition variability [Yiannakouris et al., 2001]. Our study is in accordance with earlier studies, which infers that the presence of R 223 allele has a higher BMI than non carriers leading to obesity. Further obesity is a known epidemiological risk factor in the breast cancer development.

On the other hand, negative results also have been reported for the Q223R polymorphism with BMI in different Caucasian population including American [Silver et al., 1997], British [Gotoda et al., 1997] and Danish groups [Echwald et al., 1997].

However, the proximity and similarity of the Q223R polymorphism to the Q269P mutation, which causes obesity in the Zucker mouse model, raise the distinct possibility that the Q223R polymorphism may lead to subtle changes in signaling pathways that also predispose to a leptin-resistant state. Although the higher leptin levels in RR homozygotes provide supportive evidence for this hypothesis, future in vitro experiments involving expression of wild-type and mutant leptin receptors in cell lines are needed to evaluate the effect of the Q223R substitution on the functionality of the long isoform of the human LEPR [White et al., 1997; Chua et al., 1996].

Our study provides the support for the hypothesis that the Q223R polymorphism of LEPR gene is associated with obesity and BMI in accordance with earlier reports.

IV. Conclusion

In conclusion, our results suggest that the RR genotype of the LEPR Q223R polymorphism of the leptin receptor gene might be considered as a risk genotype for development of breast cancer. Thus, this study has demonstrated a modestly increased risk of breast cancer in obese women harboring the LEPR 223R allele of the LEPR Q223R polymorphism of the leptin receptor gene. To the best of our knowledge, ours is the first study to provide information on the role of LEPR Q223R polymorphism in breast cancer risk in Indian women, a population characterized by paucity of epidemiological literature on the determinants of breast cancer and other malignancies.

V. Declaration of Interest

No conflicts of Interest

VI. Acknowledgement

References Références Referencias

1. Banks AS, Davis SM, Bates SH, Myers MG Jr (2000). Activation of Downstream Signals by the Long Form of the Leptin Receptor. J Biol Chem 275:14563-72.
2. Breidert M, Miehlke S, Glasow A, et al (1999). Leptin and its receptor in normal human gastric mucosa and in Helicobacter pylori associated gastritis. Scand J Gastroenterol 34: 954-61.
3. Bruce Thompson D, Eric Ravussin, Peter H. Bennett and Clifton Bogardus(1997). Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians, Human Molecular Genetics 6(5): 675–79.
4. Caldefie-Chezet F, Poulin A, Tridon A, Sion B and Vasson MP (2001). Leptin: a potential regulator of polymorphonuclear neutrophil bactericidal action? J Leukoc Biol 69: 414-18.
5. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995). Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269: 546- 49.
6. Catalano S, Mauro L, Marsico S, Giordano C, Rizza P, Rago V,Montanaro D, Maggiolini M, Panno ML, Ando S(2004). Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells. J Biol Chem 279:19908-15 Chagnon. Y. C, WK Chung, L Pearusse1, M Chagnon1, RL Leibel and C Bouchard(1999). Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study. International Journal of Obesity 23: 278-86 Chagnon. Y. C, jack H. Wilmore,

lingrid B. Borecki, Jacques Gagnon, Louis pe´ Russe, M Chagnon,et al(2000). Associations between the Leptin Receptor Gene and Adiposity in Middle-Aged Caucasian Males from the Heritage Family Study. The Journal of Clinical Endocrinology & Metabolism 85(1).

7. Chua SC, White DW, Wu-Peng XS, et al (1996). Phenotype of fatty due to Gln269Pro mutation in the leptin receptor (Lepr). Diabetes 45:1141–43.

8. Chung WK, Power-Kehoe L, Chua M, Chu F, Aronne L, Huma Z, et al(1997). Exonic and intronic sequence variation in the human leptin receptor gene (LEPR). Diabetes 46:1509-11.

9. Ebenbichler CF, Kaser S, Laimer M, Wolf HJ, Patsch JR and Illsley NP (2002). Polar expression and phosphorylation of human leptin receptor isoforms in paired, syncytial, microvillous and basal membranes from human term placenta. Placenta 23: 516-21.

10. Echwald SM, Sorensen TD, Sorensen TIA, et al (1997). Amino acid variants in the human leptin receptor: lack of association to juvenile onset of obesity. Biochem Biophys Res Commun 233:248 – 252

11. Eisenberg A, Biener E, Charlier M, Krishnan RV, Djiane J, Herman B, Gertler A(2004). Transactivation of erbB2 by short and long isoforms of leptin receptors. FEBS Lett. 565:139-42.

12. Friedman JM, Halaas JL(1998). Leptin and the regulation of body weight in mammals. Nature 395:763-70.

13. Garofalo C, Koda M, Cascio S, et al (2006). Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: possible role of obesity-related stimuli. Clin Cancer Res 12: 1447-53.

14. Garofalo C, Surmacz E(2006). Leptin and cancer. J Cell Physiol 207:12-22.

15. Gotoda T, Manning BS, Goldstone AP, et al (1997). Leptin receptor gene variation and obesity: lack of association in a white British male population. Hum Mol Genet 6:869–76

16. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al(1995). Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-46.

17. Ishikawa M, Kitayama J and Nagawa H (2004). Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer. Clin Cancer Res 10: 4325-31.

18. Kitawaki J, Koshiba H, Ishihara H, Kusuki I, Tsukamoto K and Honjo H(2000). Expression of leptin receptor in human endometrium and fluctuation during the menstrual cycle. J Clin Endocrinol Metab 85: 1946-50.

19. Konopleva M, Mikhail A, Estrov Z, et al (1999). Expression and function of leptin receptor isoforms

in myeloid leukemia and myelodysplastic syndromes: proliferative and anti-apoptotic activities. Blood 93: 1668-76.

20. Lahiri DK, Nurnberger JI (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research 19(19): 5444.

21. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, et al(1996). Abnormal splicing of the leptin receptor in diabetic mice. Nature 379: 632-35.

22. Malik KF, Young WS 3rd(1996). Localisation 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.

23. Mix H, Widjaja A, Jandl O, et al (2000). Expression of leptin and leptin receptor isoforms in the human stomach. Gut 47: 481-86.

24. Park KS, Shin HD, Park BL, Cheong HS, Cho YM, Lee HK, Lee JY, Lee JK, Oh B, Kimm K (2009). Polymorphisms in the leptin receptor (LEPR)-putative association with obesity and T2DM. J Hum Genet 51:85-91.

25. Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI (2001). A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women. Hum Genet 108:233-23.

26. Sierra-Honigmann MR, Nath AK, Murakami C, García-Cardeña G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR(1998). Biological action of leptin as an angiogenic factor. Science 281:1683-86.

27. Silver K, Walston J, Chung WK, et al (1997). The Gln223Arg and Lys656Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity. Diabetes 46:1898 –900.

28. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, 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.

29. Sweeney G (2002). Leptin signalling. Cell Signal 14: 655-63.

30. Tartaglia LA (1997).The leptin receptor. J Biol Chem 272: 6093-96.

31. Tsuchiya T, Shimizu H, Horie T and Mori M(1999). Expression of leptin receptor in lung: leptin as a growth factor. Eur J Pharmacol 365: 273-79.

32. Wang XJ, Yuan SL, Lu Q, Lu YR, Zhang J, Liu Y and Wang WD (2004). Potential involvement of leptin in carcinogenesis of hepatocellular carcinoma. WorldJ Gastroenterol 10: 2478-81.

33. White DW, Kuropatwinski KK, Devos R, Baumann H, Tartaglia LA (1997). Leptin receptor (OB-R)

signaling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. J Biol Chem 272(7):4065–71.

34. 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 86:4434-39.

35. Zabeau L, Lavens D, Peelman F, Eyckerman S, Vandekerckhove J, Tavernier J(2003). The ins and outs of leptin receptor activation. FEBS Lett 546(1):45-50.

36. Zhang YY, Gottardo L, Mlynarski W, Frazier W, Nolan D, Duffy J, Marescotti MC, Gervino EV, Johnstone MT, Mantzoros CS, Avogaro A, Doria A(2007). Genetic variability at the leptin receptor (LEPR) locus is a determinant of plasma fibrinogen and C-reactive protein levels. Atherosclerosis 191:121-27.

Table 1 : Genotype distribution of LEPR Q223R polymorphism in Breast Cancer cases and Controls

	Cases	Controls	OR(95% CI)	P-value	Z statistics	χ
P-value						
All Women	(n =194)	(n =186)				
Allele frequencies						
LEPR (Q)	262(67.52)	246(66.12)	1.0			
LEPR (R)	126(32.47)	126(33.87)	0.94(0.69-1.27)	0.68	0.41	0.110
df= 1 0.74						
Genotype frequencies						
LEPR (Q/Q)	85(43.8)	68(36.6)	1.0			
LEPR (Q/R)	92(47.4)	110(59.1)	0.7 (0.44-1.02)	0.0622	1.865	
LEPR(R/R)	17(8.8)	8(4.3)	1.70(0.69-4.18)	0.2472	1.157	6.567*
df=2 0.0375*						
LEPR (Q/Q)	85(43.8)	68(36.6)	1.0			
LEPR (Q/R) + (R/R)	109(56.2)	118(63.4)	0.74(0.49-1.12)	0.1498	1.440	1.788
df=1 0.18						

* *χ2* value significant

Table 2 : Association of LEPR Q223R polymorphism with respect to breast cancer and Menopausal status, Estrogen receptor and Progesterone receptor status at disease onset.

			OR(95% CI)	P-value	Z statistics	$\chi 2$	P-value
1. Menopausal Status	Pre	Post					
(N=194)	(n =97)	(n =97)					
Allele frequencies							
LEPR (Q)	135(69.59)	127(65.46)	1.0				
LEPR (R)	59(30.41)	67(34.54)	0.83(0.54-1.27)	0.39	0.87		0.58
df=1 0.45							
Genotype frequencies							
LEPR (Q/Q)	44(45.36)	41(42.27)	1.0				
LEPR (Q/R)	47(48.45)	45(46.39)	0.97(0.54-1.76)	0.93	0.09		
LEPR(R/R)	6 (6.19)	11(11.34)	0.51(0.17-1.50)	0.22	1.23		1.620
df=2 0.45							
LEPR (Q/Q)	44(45.36)	41(42.27)	1.0				
LEPR (Q/R) + (R/R)	53(54.63)	56(57.73)	0.88 (0.5-1.56)	0.66	0.43		0.08
df=1 0.77							
2. Estrogen status	+ve	-ve					
(N=146)	(n =73)	(n =73)					
Allele frequencies							
LEPR (Q)	100(68.49)	100(68.49)	1.0				
LEPR (R)	46(31.51)	46(31.51)	1.0(0.61-1.64)	1.00	0.00		0.02
df=1 0.89							

Genotype frequencies

LEPR (Q/Q)	32(43.84)	33(45.2)	1.0			
LEPR (Q/R)	36(49.3)	34(46.6)	1.10(0.56-2.15)	0.80	0.26	
LEPR(R/R)	5(6.85)	6(8.2)	0.86(0.24-3.10)	0.82	0.23	0.163
df=2 0.92						

LEPR (Q/Q)	32(43.84)	33(45.2)	1.0			
LEPR (Q/R) + (R/R)	41(56.16)	40(54.79)	1.06(0.55-2.03)	0.87	0.17	0.00
df=1 1.0						

3. Progesterone status

	+ve	-ve				
(N=146)	(n =68)	(n =78)				

Allele frequencies

LEPR (Q)	91(66.91)	109(69.87)	1.0			
LEPR (R)	45(33.09)	47(30.13)	1.15(0.7-1.9)	0.59	0.54	0.174
df=1 0.677						

Genotype frequencies

LEPR (Q/Q)	27(39.7)	38(48.72)	1.0			
LEPR (Q/R)	37(54.4)	33(42.31)	1.58(0.80-3.12)	0.19	1.31	
LEPR(R/R)	4(5.88)	7(8.97)	0.80(0.21-3.0)	0.75	0.32	2.234
df=2 0.327						

LEPR (Q/Q)	27(39.7)	38(48.72)	1.0			
LEPR (Q/R) + (R/R)	41(60.2)	40(51.2)	1.44(0.75-2.79)	0.28	1.09	0.858
df=1 0.354						

28

Table 3 : Genotype distribution of LEPR Q223R polymorphism in Breast Cancer cases with BMI

			OR(95% CI)	P-value	Z statistics	$\chi2$	P-value
BMI	Less weight	Obese					
(N=151)	(n = 29)	(n = 122)					
Allele frequencies							
LEPR (Q)	42(72.41)	156(63.93)	1.0				
LEPR (R)	16(27.59)	88(36.07)	0.68 (0.36-1.27)	0.224	1.22	1.140	
df=1 0.29							
Genotype frequencies							
LEPR (Q/Q)	14(48.28)	47(38.52)	1.0				
LEPR (Q/R)	14(48.28)	62(50.82)	0.76(0.33-1.74)	0.51	0.65		
LEPR(R/R)	1(3.45)	13(10.66)	0.26(0.031-2.15)	0.21	1.25	1.894	
df=2 3.88							
LEPR (Q/Q)	14(48.28)	47(38.52)	1.0				
LEPR (Q/R) + (R/R)	15(51.72)	75(61.48)	0.67 (0.3-1.52)	0.34	0.96	0.565	
df=1 0.45							

Table 4 : Genotype distribution of LEPR Q223R polymorphism in Breast Cancer cases with Stage of the disease

	I	II	III &IV	χ2	P-value
Stage of the Disease					
Allele frequencies					
LEPR (Q)	12(75)	103(64.38)	118(68.60)		
LEPR (R)	4(25)	57(35.62)	54(31.40)	1.161	
df=1 0.56					
Genotype frequencies					
LEPR (Q/Q)	5(62.5)	33(41.25)	38(44.19)		
LEPR (Q/R)	2(25.0)	37(46.25)	42(48.84)	3.016	
df=4 0.56					
LEPR(R/R)	1(12.5)	10(12.5)	6(6.98)		
LEPR (Q/Q)	5(62.5)	33(41.25)	38(44.19)		
LEPR (Q/R) + (R/R)	3(37.5)	47(58.75)	48(55.81)	1.353	
df=2 0.51					

Table 5 : Association of LEPR Q223R polymorphism and breast cancer risk by breast cancer stage

Genotype	Controls(n)	Stage I(n)	OR(95% CI)	P	Stage II(n)	OR(95% CI)	P	StageIII& IV(n)	OR(95% CI)	P
LEPR (Q/Q)	68(36.6)	5(62.5)	1.0		33(41.25)	1.0		38(44.19)	1.0	
LEPR (Q/R)	110(59.1)	2(25)	4.04(0.8-21.43)	0.10	37(46.25)	1.44 (0.83-2.52)	0.2	42(48.84)	1.46(0.92-2.49)	0.16
LEPR(R/R)	8(4.3)	1(12.5)	0.59(0.06-5.69)	0.65	10(12.5)	0.39(0.14-1.08)	0.07	6(6.98)	0.75(0.24-2.31)	0.61
LEPR (Q/Q)	68(36.6)	5(62.5)	1.0			33(41.25)	1.0 1.0	38(44.19)		
LEPR (Q/R) + (R/R)	118(63.4)	3(37.5)	2.89(0.67-12.48)	0.15	47(58.75)	1.22(0.71-2.08)		0.47	48(55.81)	1.37(0.82-2.31)	0.23

This page is intentionally left blank

30