Association of leptin receptor genetic variants (LEPR) with obesity and leptin level in unexplained infertility in northern Indian population

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ARTICLE INFO

Keywords:

BMI
RFLP
Unexplained infertility
Obesity
Leptin

ABSTRACT

Background: The leptin receptor (LEPR) gene, is a member of the class I cytokine receptor superfamily, with six known isoforms. Animal as well as human modal both studies indicate that LEPR have important and powerful roles in the mechanism of obesity. This study aims to determine the correlation of the LEPR genetic variant with obesity and leptin level in the obese and control groups in the northern Indian population.

Methods: 109 fertile controls and 120 unexplained infertile cases were recruited. Serum leptin level was measured by active human ELISA kit using an ELISA reader. Leptin receptor gene polymorphism A668G, A326G and G1968C were analyzed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP).

Results: The anthropometric parameters are slightly higher in obese cases as compared to control group ($p > 0.005$), whereas waist circumference and hip circumference shows a significant p -value ($p < 0.005$) between obese cases and controls. The leptin level is found increased in obese cases than controls and this difference is significant with $p < 0.05$. The higher frequency of GG genotype of (Gln223Arg) variant in obese cases with significant difference found in between obese cases and control groups $p < 0.001$. Similarly GG and CC genotype of (Lys109Arg) and (Lys656Asn) respectively found higher frequency with significantly different $p < 0.001$ in obese group as compared to control group.

Conclusion: Genetic variants (Gln223Arg), (Lys109Arg) and (Lys656Asn) in LEPR gene are significantly associated with anthropometric parameter obesity and leptin level in northern Indian population.

1. Introduction

An abnormal increase of fat in the subcutaneous connective tissue defined as obesity. It is the outcome of an interaction between genetic and environmental factors.^{1,2} In genetic factors, one of the variations at the leptin receptors (LEPR) genes are responsible for the morbid obesity in mouse models as well as in some cases of obesity in humans.³ The adipose tissue-derived hormone leptin is produced in proportion to fat stores.⁴ Leptin levels permit energy expenditure in many processes like reproduction, growth, autonomic nervous system, endocrine and immune system.^{5,6} Animal as well as human modal both studies indicate that LEPR have important and powerful roles in the mechanism of obesity.⁷ The sequence variants for LEPR gene has been screened in many studies, but mutation that associated with obesity have not been reported.^{8,9} Gene of leptin receptor is located on the chromosome of

1p31 in human and has five isoforms.^{9–11} LEPR has two main isoform i.e., long arm and short arm isoform due to changes in length of the cytoplasmic domain, but in human four isoforms were identified; one is long isoform with 1165 amino acid responsible for leptin signaling and rest 3 are splicing short isoforms.¹² Defects in leptin production cause severe obesity.¹³ Leptin receptor is located in adipose tissue, stomach, endometrium, liver, spleen, and placenta. Leptin receptor acts via negative feedback between its concentration in blood serum and its receptors expression on the cell surface.¹⁴ Plasma leptin levels are closely correlated with the amount of adipose tissue.¹⁵ Reproductive function is also depends on the energy reserves stored as fat in adipose tissue as other physiological functions.¹⁶

This study aims to determine the correlation of the LEPR genetic variants with obesity and leptin level in the obese and control groups in the northern Indian population.

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<https://doi.org/10.1016/j.cegh.2019.09.003>

Received 28 December 2018; Received in revised form 30 July 2019; Accepted 20 September 2019

Available online 24 September 2019

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2. Subjects and methods

2.1. Subjects

This case-control study was approved by institutional ethics committee (ref no-53ECMIIB/P1) of King George's Medical University, Lucknow. The work has been done in the Department of Obs & Gynae K.G.M.U Lucknow. Subjects (Cases & Control) were recruited under following inclusion and exclusion criteria. **Inclusion criteria (Case)**- Age group between 18 and 40 years, Infertile female with BMI for A 1 group ≥ 25 and for A2 group $18 < 25$, Females having unexplained infertility of at least one year. **Controls**- Age group 18–40 years, Healthy subject with BMI for B1 group ≥ 25 and for B2 group $18 < 25$, Female having at least one conception. **Exclusion criteria (Case)**- Pregnancy, Subjects with any endocrinal disorders, diabetes mellitus, chronic illness like genital tuberculosis, Su any chronic medication, mullerian anomalies. **(Controls)**- Subjects on hormonal contraception within last one year, any illness, endocrinal disorder, autoimmune/immunocompromised condition and taking chronic medication. Informed consent was obtained from each subject. The 229 female were included in the study, of which 109 fertile controls and 120 unexplained infertile cases were recruited from Out Patient Door (OPD) of Department of Obs & Gynae K.G.M.U Lucknow. Total 5 ml venous blood were collected from enrolled subjects, 3 ml for serum vile and 2 ml in EDTA vile for DNA isolation. Serum leptin level was measured by active human ELISA kit using an ELISA reader.

2.2. Anthropometric, biochemical and leptin level

The anthropometric data of two groups is summarized in Table-1. Age matched (19–41) year healthy control and case enrolled in the study. Body weight (Kg), height (m), BMI (kg/m^2), waist (cm) and hip (cm) circumference were measure a calibrated scale.

2.3. LEPR genes genotyping

Genomic DNA was extracted by using Phenol-Chloroform method from 2 ml blood collected in EDTA vile. Approx. 50 ng of the DNA was used for each PCR reaction. Leptin receptor gene polymorphism Gln223Arg, Lys109Arg and Lys656Asn were analyzed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Genomic DNA was amplified by PCR Thermocycler (Applied Biosystems, Germany) using following PCR conditions: 94 °C for 4 min, 35 cycles at 94 °C for 30 s, 58 °C for 40 s, 72 °C for 40 s, and finally 72 °C for 8 min for Gln223Arg variant, 95 °C for 5 min, 31 cycles at 94 °C for 30 s, 58 °C for 30 s, 72 °C for 40 s, and finally 72 °C for 6 min for Lys109Arg variant and 94 °C for 4 min, 30 cycles at 94 °C for 30 s, 58 °C for 40 s, 72 °C for 40 s, and finally 72 °C for 8 min for Lys656Asn variant. The primers used for amplification of LEPR gene Gln223Arg, Lys109Arg and Lys656Asn polymorphism are as follows: (i) forward primer 5'- AGTTCAAATAGAGGTCCAAATCA-3' and reverse primer 5'- TTCTGAGGTTGTGTCAC TGGCA-3' (ii) forward primer 5'- AGTTCAAAT AGAGGTCCAAATCA-3' and reverse primer 5'- TTCTGAGGTTGTGTCAC TGGCA-3', (iii) forward primer 5'-AGTTCAAATAGAGGTCCAAATCA-3' and reverse primer 5'-TTCTGAGGTTGTGTCAC TGGCA -3' respectively. The PCR results for Gln223Arg, Lys109Arg and Lys656Asn Polymorphism were determined by the digestion of PCR product for 18hr using MspI HaeIII and AccII enzymes (NEB, UK) for Gln223Arg, Lys109Arg and Lys656Asn respectively. Gel electrophoresis with 3% agarose was performed to analyze the digestion.

2.4. Data analysis

Continuous data were summarized as Mean $\pm$ SD while discrete (categorical) in no. and percentage. Continuous groups were compared by independent Student's *t*-test while categorical by chi-square (χ^2) test.

Table 1

Anthropometric characteristics and leptin level in the obese and control group.

S. No.	Variables	Controls(n = 109)	Cases (n = 120)	p-value
1.	Age (yrs)	29.58 $\pm$ 4.01	29.53 $\pm$ 4.43	0.925
2.	Height (cm)	145.78 $\pm$ 6.58	147.39 $\pm$ 6.69	0.067
3.	Weight (kg)	51.54 $\pm$ 6.90	52.29 $\pm$ 8.92	0.480
4.	BMI (kg/m^2)	24.31 $\pm$ 3.19	24.10 $\pm$ 3.90	0.671
5.	WC (cm)	82.95 $\pm$ 9.64	95.65 $\pm$ 10.99	< 0.001
6.	HC (cm)	91.49 $\pm$ 7.36	99.89 $\pm$ 10.11	< 0.001
7.	WHR	0.91 $\pm$ 0.14	0.96 $\pm$ 0.12	0.005
8.	Leptin (pg/ml)	3.75 $\pm$ 1.84	6.89 $\pm$ 2.46	< 0.001

Pearson correlation analysis was done to assess association between the variables. Genotypic distribution was analyzed for Hardy-Weinberg equilibrium (HWE) and genetic variants for LEPR gene A668G, A326G and G1968C for obese and controls female were compared by chi-square (χ^2) analysis. All of the tests were performed using SPSS version 19.0 software. $P < 0.05$ was considered as statistically significant.

3. Results

The anthropometric data and leptin level of all the subjects are summarized in Table 1. The anthropometric parameters are slightly higher in obese cases as compared to control group but the p-value is found no significant whereas, waist circumference and hip circumference shows a significant p-value ($p < 0.005$) between cases and controls. The leptin level is found increased in obese cases than controls and this difference is significant with $p < 0.05$.

Genotype and allelic frequency of LEPR gene (Gln223Arg), (Lys109Arg) and (Lys656Asn) in the obese cases and control groups is given in Table-2. The higher frequency of GG genotype of (Gln223Arg) variant in obese cases with significant difference found in between obese cases and control groups $p < 0.001$. Similarly GG and CC genotype of (Lys109Arg) and (Lys656Asn) respectively found higher frequency with significantly different $p < 0.001$ in obese group as compared to control group. When all these 3 LEPR genetic variants compared using Hardy-Weinberg Equation, the significant difference in the frequency of genotype was found.

Genetic variants of LEPR gene (Gln223Arg), (Lys109Arg) and (Lys656Asn) were correlated with anthropometric parameters i.e., obesity (non obese and obese) and leptin (low and high) level in the obese cases and control groups shown in Table 3. On comparing between controls and cases, the χ^2 test showed significant ($p < 0.05$,

Table 2

Distribution of leptin receptor gene variants among cases and controls.

Genetic variant	Genotype/ Allele frequency	Controls (n = 109) (%)	Cases (n = 120) (%)	χ^2 value	P value
LEPR Gln223Arg	AA	67 (61.5)	42 (35.0)	17.16	< 0.001
	AG	34 (31.2)	56 (46.7)		
	GG	8 (7.3)	22 (18.3)	18.20	< 0.001
	Minor (A)	168 (77.1)	140 (58.3)		
LEPR Lys109Arg	Major (G)	50 (22.9)	100 (41.7)	17.48	< 0.001
	AA	52 (47.7)	31 (25.8)		
	AG	45 (41.3)	53 (44.2)	19.53	< 0.001
	GG	12 (11.0)	36 (30.0)		
LEPR Lys656Asn	Minor (A)	149 (68.3)	115 (47.9)	19.47	< 0.001
	Major (G)	69 (31.7)	125 (52.1)		
	(GG)	58 (53.2)	31 (25.8)	18.60	< 0.001
	(GC)	42 (38.5)	65 (54.2)		
	(CC)	9 (8.3)	24 (20.0)	18.60	< 0.001
	Minor (G)	158 (72.5)	127 (52.9)		
	Major (C)	60 (27.5)	113 (47.1)		

Table 3
Correlation of leptin receptor gene polymorphisms with Leptin level and obesity.

SNP	Leptin	Group	Genotype			χ^2 value	P value
			Wild (%)	Heterozygous (%)	Mutant (%)		
LEPR Gln223Arg	Low	Controls (n = 71)	40 (56.3)	24 (33.8)	7 (9.9)	1.37	0.505
		Cases (n = 40)	21 (52.5)	12 (30.0)	7 (17.5)		
	High	Controls (n = 38)	27 (71.1)	10 (26.3)	1 (2.6)	22.28	< 0.001
		Cases (n = 80)	21 (26.3)	44 (55.0)	15 (18.8)		
LEPR Lys109Arg	Low	Controls (n = 71)	35 (49.3)	31 (43.7)	5 (7.0)	7.16	0.028
		Cases (n = 40)	17 (42.5)	13 (32.5)	10 (25.0)		
	High	Controls (n = 38)	17 (44.7)	14 (36.8)	7 (18.4)	10.08	0.006
		Cases (n = 80)	14 (17.5)	40 (50.0)	26 (32.5)		
LEPR Lys656Asn	Low	Controls (n = 71)	39 (54.9)	26 (36.6)	6 (8.5)	8.07	0.018
		Cases (n = 40)	11 (27.5)	25 (62.5)	4 (10.0)		
	High	Controls (n = 38)	19 (50.0)	16 (42.1)	3 (7.9)	9.08	0.011
		Cases (n = 80)	20 (25.0)	40 (50.0)	20 (25.0)		
Obesity							
LEPR Gln223Arg	Non obese	Controls (n = 70)	46 (65.7)	17 (24.3)	7 (10.0)	6.88	0.032
		Cases (n = 77)	34 (44.2)	31 (40.3)	12 (15.6)		
	Obese	Controls (n = 39)	21 (53.8)	17 (43.6)	1 (2.6)	14.56	0.001
		Cases (n = 43)	8 (18.6)	25 (58.1)	10 (23.3)		
LEPR Lys109Arg	Non obese	Controls (n = 70)	40 (57.1)	25 (35.7)	5 (7.1)	10.42	0.005
		Cases (n = 77)	27 (35.1)	32 (41.6)	18 (23.4)		
	Obese	Controls (n = 39)	12 (30.8)	20 (51.3)	7 (17.9)	8.69	0.013
		Cases (n = 43)	4 (9.3)	21 (48.8)	18 (41.9)		
LEPR Lys656Asn	Non obese	Controls (n = 70)	42 (60.0)	24 (34.3)	4 (5.7)	20.76	< 0.001
		Cases (n = 77)	18 (23.4)	47 (61.0)	12 (15.6)		
	Obese	Controls (n = 39)	16 (41.0)	18 (46.2)	5 (12.8)	3.01	0.223
		Cases (n = 43)	13 (30.2)	18 (41.9)	12 (27.9)		

$p < 0.01$ and $p < 0.001$) association (correlation) of all genetic variants with high Leptin. However, both LEPR Lys109Arg and LEPR Lys656Asn SNPs also showed significant ($p < 0.05$) with low Leptin. In all significant associations, the frequency of mutant genotype was higher in cases than controls. Similarly, on comparing between controls and cases, the χ^2 test showed significant ($p < 0.05$ or $p < 0.01$ or $p < 0.001$) association (correlation) of all SNPs with obesity (obese group) except LEPR Lys656Asn. However, LEPR Gln223Arg, LEPR Lys109Arg and LEPR Lys656Asn SNPs also showed significant ($p < 0.05$ or $p < 0.01$ or $p < 0.001$) with non obesity (non obese group). In all significant associations, the frequency of mutant genotype was higher in cases than controls.

4. Discussion

In our study, we demonstrated a significant difference in the anthropometric data of obesity and an increase leptin level between obesity cases and control group. A significant rise of leptin level was found in obese group as compared to control group. This might be caused by LEPR resistant and also results in altered leptin binding and therefore, receptor dimerisation and signalling capacity of the leptin receptor. Moreover, waist hip ratio significantly different in obese cases as compared to control. These results are consistent with some studies as obesity was associated with higher leptin level.^{17,18} Whereas, some study contradicted our results and shown that obesity was not associated with higher leptin level.¹⁹ The leptin gene receptor is polymorphic, which means that in different subjects, differences in genetic variants may be found. However, such mutations are extremely rare and are not likely to be responsible for the obesity, because there are many factors that involved and contribute to the appearance of obesity.²⁰ A study was done by Han et al. Showed a significant association between genotype and all parameters (age, weight and BMI) in females group,²¹ who reported that LYS109ARG polymorphism was strongly associated with BMI in Korean female populations which disagree with our findings of study. In Indian population, all three genetic variants (Gln223Arg), (Lys109Arg) and (Lys656Asn) showed different frequency (0.18), (0.30) and (0.20), which found similar to results of one meta-analysis showed 0.25 of LEPR Lys109Arg in nine different Caucasian

populations, 0.82 frequency for Korean and Japanese subjects. Similarly our frequency for 0.18 (Gln223Arg) in Indian population was similar to Pima Indians (0.32), and (0.45) and (0.85) in Caucasian or Asian population respectively. On the other hand, research by de Oliveira¹⁸ found LEPR Lys109Arg to be correlated with reduced risk of obesity whereas in our study it shows high risk of obesity. Another research studies was done in reference to (Gln223Arg) in Tehran 0.15²¹, 0.156 in Spain²², 0.169 in Brazilian,²³ 0.18 in Kuala Lumpur,²⁴ 0.227 in Jeddah city,²⁵ 0.287, 0.261 and 0.412 respectively in Malay, Chinese and Indian ethnics of Malaysian population,²⁶ supports our study. Research done in Caucasian²⁷ and Asian^{24,25} population, contrary to our data and showed that genetic variant of Gln223Arg was not associated with obesity and leptin level. Mutations in the leptin gene and the leptin gene receptor may lead to extreme obesity, this was found to be true in mice by Chen et al.,²⁸ and in humans by Clement et al.²⁹ They found, that the presence of a homozygous mutations in the leptin gene (homozygotes ob/ob) and also homozygous mutations in the leptin gene receptor (homozygotes db/db) were associated with early onset of extreme obesity due to hyperphagia, poor energy expenditure and severe insulin-resistance.^{28,29} These studies gave rise to the idea that several genotypes of the leptin gene and its receptor may lead to obesity. In the Spanish population agreed with our results, the leptin gene receptor variants (Gln223Arg) can also be associated with obesity, similar results were obtained for Brazilian population.³⁰

These Variations were found due to different ethnic rays in different study in different country due to and population. A large no. of sample size also required to use as biomarker of LEPR genetic variants and leptin level in obesity and unexplained infertility.

5. Conclusion

Genetic variants (Gln223Arg), (Lys109Arg) and (Lys656Asn) in LEPR gene are significantly associated with anthropometric parameter obesity and leptin level in northern Indian population with unexplained infertility. So, the present study concluded that these LEPR gene variants with obesity and higher leptin level could be as risk factor for unexplained infertility in Indian population.

Declaration of competing interest

All the Authors declare that they have no conflict of interest.

Acknowledgement

The authors would like to acknowledged to Department of Science and Technology (DST) India, for providing funding support for this study. The Funding ID is DST/2012/30 dated 17August2012 . We would also like to thanks the Department of Biochemistry, K.G.M.U. Lucknow, for wet lab work of the study.

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