Impact of Leptin Receptor Gene LYS109ARG Polymorphism on Obesity in Jeddah City

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Abstract: Obesity was identified as a worldwide health care problem towards the end of the 20th century. Obesity is defined as an unhealthy excess of body fat results from genetic, metabolic, behavioral and environmental factors. Leptin plays a pivotal role in regulating the energy balance and fat storage by its receptor. Single nucleotide polymorphisms of leptin receptor gene may play a role in the pathophysiology of human obesity. In this study, the association between the OB-R gene polymorphism and obesity in Jeddah city population was evaluated by determining the distribution of allele's frequency of the leptin receptor LYS109ARG polymorphism (rs1137100) in 123 volunteers (60 males & 63 females) aged between (15-60 years). Each gender was divided into three groups according to body mass indexnormal "control", overweight and obese. Genotypes were determined for all subjects by using polymerase chain reaction , followed by a cut using restriction enzyme . When comparing between the body mass index (BMI) of AA and AG genotypes, a significant difference (P=0.001) was observed. Also, there was a significant increase in AA genotype compared to AG genotype in female obese (OR=0.14, 95%CI: 0.03-0.70, P=0.01). This result suggests that genetic polymorphism of leptin receptor gene (LYS109ARG) may play a role in female obese.

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1. Introduction

Obesity is being described as a global epidemic because of its prevalence globally in both the developed and the developing countries and constitutes a significant universal issue of public health because of its close association with numerous chronic diseases [1,2]. It is well recognized that the causes of obesity are complex and multifactorial including metabolic, hormonal, genetic, and psychosocial factors. Effective prevention and treatment modalities can be achieved by understanding the pathogenesis of obesity better. Research about the pathophysiology and treatment of obesity has been directed to this area in the recent years [3]. Obesity and overweight are increasing in Saudi Arabia with an overall obesity prevalence of 35.5% [4]. According to World Health Organization statistics, the prevalence of obesity is estimated to be 39.1% in females and 28.6% in males in Saudi Arabia [5], which confirms necessity to take measures health to confront this epidemic through health education of Saudi society and identify its methods processed with more medical and scientific researches.

Obesity is defined as an abnormal increase of fat in the subcutaneous connective tissue to the extent that health may be impaired. Obesity and overweight have many causes, including genetic, metabolic, behavioural and environmental. Obesity is the result of an interaction between genetic and environmental factors [3,6]. From the genetic factors, which have been conducted in many studies are leptin receptors polymorphism. These studies showed leptin receptors polymorphisms role in increasing the fat proportion in the body, leading to obesity, depending on its vital role in regulation of energy homeostasis and fat storage.

Single nucleotide polymorphisms (SNPs) are the DNA sequence mutations that occur when a single base pair is altered at one position in the genome. Mutations can occur in coding regions (exons) of the DNA, as well as in non-coding regions (introns) [7,8]. In leptin receptor genes, seven polymorphic sites were identified. Two of these polymorphisms, replacing lysine to arginine at codon 109 (AAG to AGG) in exon 4 (LYS109ARG) and replacing glutamine to arginine at codon 223 (CAG to CGG) in exon 6 (GLN223ARG), are amino acid substitutions in the extracellular domain of the leptin receptor, one polymorphism is a silent substitution, and four occur in non-coding regions of the leptin receptor [9,10].

Recent studies in Saudi Arabia were investigated the association between GLN223ARG & SER343SER leptin receptor polymorphisms and obesity prevalence in Jeddah population. The first study showed that there were no association between leptin receptor gene GLN223ARG polymorphisms and obesity in adults males and females, and children females and teenagers groups, while appeared a significant association in

males children and teenager group [11]. The second study showed that there was no association between leptin receptor gene SER343SER polymorphisms and obesity in both gender: while a significant association in underweight males group was observed [12].

This study aims to detect the presence of LYS109ARG leptin receptor polymorphism in obese subjects for both genders in Jeddah city. To investigate the frequency of the LYS109ARG alleles in Jeddah population, DNA extracted from blood samples, Genotypes were determined for all subjects by using polymerase chain reaction (PCR), digestion of the PCR product with the restriction enzyme *HaeIII* for detection of the alleles of LYS109ARG and statistical analysis.

2- Materials and Methods

Human subjects:

This study was approved by the ethical Committee (unit of biomedical ethics) (No. 377-10) from King Abdulaziz University; all participants gave their written informed consent of their participation. The study included 123 adult participant (60 males and 63 females) randomly selected from Saudis residents in Jeddah, aged 15 to 66 years. The blood samples of the subjects were collected from King Abdulaziz University Hospital in Jeddah All the participants underwent complete physical examination. At the time of blood collection, information was recorded for all subjects, including height, weight. All volunteers were asked to answer a questioner about family history of obesity and genetic diseases. Exclusion of pregnant women and people who use any treatment course in this study. Each gender was divided into three groups: normal, overweight and obese according to BMI. Classification of BMI for adults according to the World Health Organization (WHO) (Healthy BMI < 25, overweight BMI 25-30 and Obese BMI ≥30). The practical works of this research were conducted at the Biology Postgraduate Studies laboratories, sixth building at King Abdulaziz University, Jeddah.

Genetic Analysis:

Genomic DNA was extracted from whole blood which stored in EDTA coated tubes by using Qiagen-QIAamp DNA Blood Mini Kit (QIAGEN, Germany). The concentration of genomic DNA was determined by the quantitative method, which is based on the optical density measurement. The samples were quantified using spectrophotometric analysis using 6800 UV/Vis Spectrophotometer (JENWAY, UK). The purity was determined by calculation the ratio of absorbance at 260 nm to absorbance at 280 nm (A260/A280). Pure DNA should have an A260/A280 ratio of 1.7 - 1.9.

Amplification for LYS109ARG gene

Genotyping of the Ob-R polymorphism was carried out using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay with previously described primer pairs (Table 1) [13]. PCR amplification products were obtained using Maxima Hot Start Green Master Mix Kit (Fermentas Co, USA). Polymerase chain reaction was performed using the following: 3µl genomic DNA (2µg/µl), 25µl Hot Start Green Master Mix, 20µl nuclease free water, 1µl of each primer] where the final volume of 50µl. The amplification reactions were performed in a Thermal cycler (mastercycler personal, Eppendorf, Germany). The amplification conditions were as follows: initial denaturation at 95°C for 4 min followed by 35 cycle of denaturation at 94°C for 30s, annealing at 50°C for 45s and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min [13]. The PCR product was purified by using the isolate PCR kit (Bioline Inc., USA). The DNA bands were Visualized under UV light and photographed using gel documentation (G: BOX EF, Chemi, ChemiHR16, ChemiXT16 and ChemiXL Camera Hardware, Syngene, USA). The ethidium bromide (fluorescent dye) intercalates between bases of DNA causing the visualization of the bands. The gel was then photographed.

Table 1: Sequences of primer for LYS109ARG leptin receptor gene (rs1137100)

Primer name	Sequence (5' to 3')	Size (bp)	Melting temp. (□C)	Amplicon size (bp)
Lys109Arg_forward	TTTCCACTGTTGCTTTCGGA	20	56.4	101
Lys109Arg_Reverse	AAACTAAAGAATTTACTGTTGAAACAAATGGC	32	64.8	

Genotyping of rs1137100 SNP in LYS109ARG gene

The resulting DNA fragment was 101 bp in length **[13].** The genotypes for this SNP were determined by restriction fragment length polymorphism (PCR-RFLP) procedure. They were prepared as follows: in a labeled clean and dry Eppendorf tubes 20µl of PCR product, 5 µl of sterile, deionized water, 3µl of 10X NEBuffer 4, mixed by pipetting. Finally, 2µl of restriction enzyme (*HaeIII*) were added (New England Biolabs Inc., USA). The tubes were incubated for overnight at 37°C followed by heat inactivation for 20 minutes at 80˚C. After that, the genotypes were resolved after running it on 5% Polyacrylamide gel electrophoresis.

Statistical Analysis:

All statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive data were given as mean ± standard deviation (SD). The associations between BMI and other parameters with genotypes were performed using analysis of independent samples T- test. A case-control study was designed to compare patients (who had overweight or obese) with control (who had normal weight).

The Fisher's exact test and Chi-square test were applied to test the association between genotypes and clinical groups. Moreover, a contingency analysis was applied to calculate the odds ratio (OR) at 95% confidence interval (CI) was used to estimate the relative risk and strength of association for their various genotypes or their combinations. Statistical significance was defined as the probability of $P \leq 0.05$ (two-sided).

3- Results

Table 2 shows comparing data (males and females). There were a significant difference between the normal group, compared to both overweight and obese groups in weight and BMI ($P< 0.05$). Table 3 shows male's group data. There were a significant difference between the normal group and overweight group in weight and BMI ($P< 0.05$). Also a significant difference in weight and BMI between the normal group and obese group was found ($P< 0.05$). Table 4 shows female's group data, which represents a significant difference between the normal group and overweight group in weight and BMI ($P< 0.05$). A significant difference in weight and BMI between the normal group and obese group was also found ($P< 0.05$).

Table 2: Descriptive of data for all volunteers (*n*=123)

Variables	BMI class			*P.* value	
	Normal	Overweight	Obese	Ov. Wt *vs.* N	N *vs.* Ob
n	51 (41.5%)	23 (18.7%)	49 (39.8%)		
Age (Years)	19±3.21	20.6±3.04	26.04±11.29	0.04*	0.00*
Weight (Kg)	50.53±9.75	74.56±12.11	95.46±16.72	0.00*	0.00*
Height (m)	1.61±0.08	1.64±0.10	1.64± 0.11	0.19	0.11
BMI (Kg/m2)	19.4±3.16	27.44±2.09	35.22±4.46	0.00*	0.00*

*Significance ($P < 0.05$). Values are expressed as mean±standard deviation (SD), and were compared by t-test. BMI; Body mass index. *n*; no. of sample. Ov .Wt *vs.* N: Overweight vs. Normal. N *vs.* Ob: Normal vs. Obese.

Table 3: Descriptive of males' group data (*n*= 60)

Variables	BMI class			*P.* value	
	Normal	Overweight	Obese	Ov. Wt *vs.* N	N *vs.* Ob
n	20 (33.3%)	13 (21.7%)	27 (45%)		
Age (Years)	18.7±2.75	20.69±3.40	21.07±5.03	0.07	0.04*
Weight (Kg)	52 ±12.13	78.30±14.49	1.02±17.39	0.00*	0.00*
Height (m)	1.66±0.10	1.68±0.11	1.68±0.13	0.59	0.73
BMI (Kg/m2)	18.51±3.17	27.12±2.59	36.43±4.91	0.00*	0.00*

*Significance ($P < 0.05$). Values are expressed as mean±standard deviation (SD), and were compared by t-test. BMI; Body mass index. *n*; no. of sample. Ov .Wt *vs.* N: Overweight *vs.* Normal. N *vs.* Ob: Normal *vs.* Obese.

Table 4: Descriptive of females' group data (*n*= 63)

Variables	BMI class			*P.* value	
	Normal	Overweight	Obese	Ov. Wt *vs.* N	N *vs.* Ob
n	31 (49.2%)	10 (15.9%)	22 (34.9%)		
Age (Years)	19.19±3.51	20.3±2.67	32.13±13.77	0.28	0.00*
Weight (Kg)	49.59±7.93	69.7±5.65	86.45±10.44	0.00*	0.00*
Height (m)	1.57±0.42	1.58±0.57	1.59±0.05	0.73	0.09
BMI (Kg/m2)	19.98±3.07	27.85±1.19	33.72±3.38	0.00*	0.00*

*Significance ($P < 0.05$). Values are expressed as mean±standard deviation (SD), and were compared by t-test. BMI; Body mass index. *n*; no. of sample. Ov .Wt *vs.* N: Overweight vs. Normal. N *vs.* Ob: Normal vs. Obese.

Figure 1 shows the genotyping results of LYS109ARG leptin receptor polymorphism. A single band of 101 bp shows the presence of allele A, while the presence of 2 bands of 70 and 31 bp shows the presence of allele G. There are 2 bands for the G allele because this product contains a digestion site for the *HaeIII* enzyme, which is absent when A is present. Therefore, the PCR product containing the G allele is cleaved by *HaeIII* enzyme and product 2 bands of low molecular weight. The homozygote GG genotyping (two bands only) were absent in this study results.

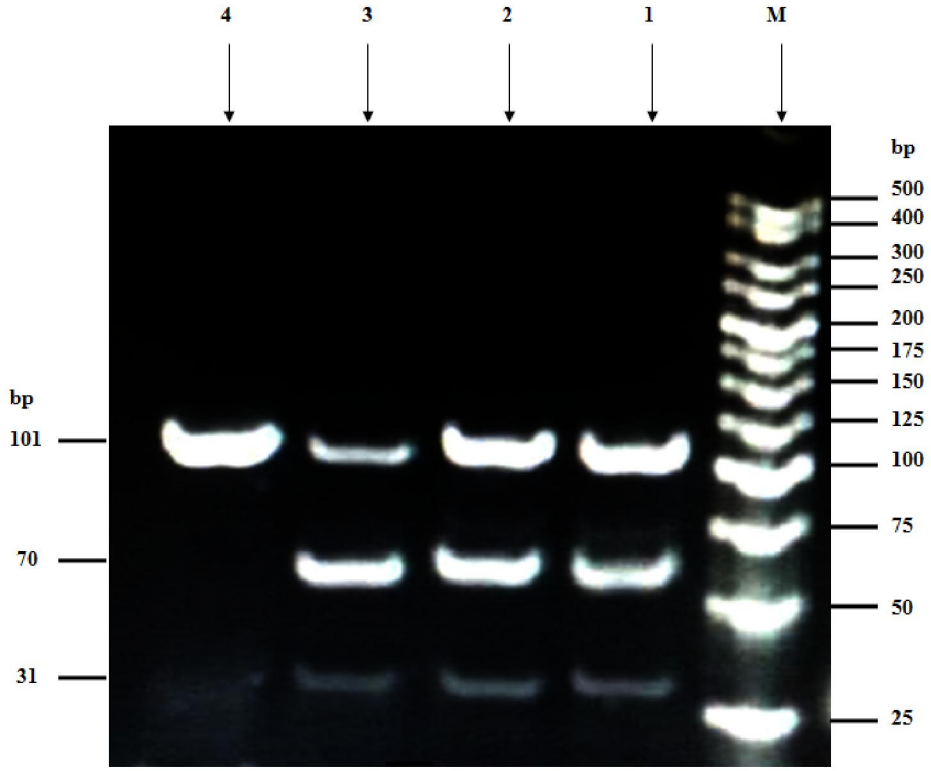

Figure 1: Photograph of 5% Polyacrylamide gel showing the digested PCR products for LYS109ARG leptin receptor polymorphism genotyping. Lane M: DNA marker. Lanes 1,2,3: Heterozygous AG three bands of size 101, 70 and 31 bp. Lane 4: Homozygous AA one band of size 101 bp.

Table 5 shows the genotypes distribution in all data for LYS109ARG polymorphism. The genotype frequencies were 77.2% AA homozygous **(*n*= 95)** and 22.8% AG heterozygous **(*n*= 28)**, with 88.6% and 11.4% A and G allele frequencies, respectively. The data revealed that, there were significant differences in body weight (*P*=0.003) and BMI (*P*=0.001) between AA (homozygous) and AG (heterozygous) genotypes. Data with AA genotype showed the higher values in weight (76.06 ± 24.79) and BMI (28.35 ± 8.15), while data with AG genotype showed the lowest values in weight (62.32 ± 19.31) and BMI (23.32 ±6.11).

Table 6 shows males' group data at the genotypes distribution for LYS109ARG polymorphism. The genotype frequencies were 85% AA homozygous **(*n*= 50)** and 15% AG heterozygous **(*n*=9)**, with 92.5% and 7.5% A and G allele frequencies, respectively. There were no significant differences in weight and BMI between different genotypes. However, males with AG genotype showed the lowest values in weight (69±26.27) and BMI (23.72±8.02).

Table 7 shows females' group data of the genotypes distribution for the LYS109ARG polymorphism. The genotype frequencies were 69.8% AA homozygous **(*n*=44)** and 30.2% AG heterozygous **(*n*=19)**, with 85% and 15% A and G allele frequencies, respectively. Females with AA and AG genotypes showed significant differences in weight (*P*=0.04) and BMI (*P*=0.02). Females with AG genotype showed the lowest values in weight (59.16 ±14.81) and BMI (23.13 ±5.22).

Table 5: The distribution of genotypes in all data for LYS109ARG polymorphism (*n*= 123)

Variables	Frequency%		*P*. value
	AA (*n*= 95) 77.2%	AG (*n*= 28) 22.8%	AA *vs*. AG
	Mean ±SD	Mean ±SD	
Age (Years)	22.68 ± 8.93	20.14 ±4.34	0.14
Weight (Kg)	76.06 ± 24.79	62.32 ± 19.31	0.003*
Height (m)	1.63 ± 0.10	1.63 ± 0.08	0.86
BMI (Kg/m^2)	28.35 ± 8.15	23.32 ±6.11	0.001*
Results are expressed as mean±standard deviation (SD), and were compared by t-test (P< 0.05). *Significance (P < 0.05). BMI; Body mass index. *n*; no. of sample.			

Table 6: Male's group data of the distribution of genotypes for the LYS109ARG polymorphisms (*n*=60)

Variables	Frequency%		*P*. value
	AA (*n*= 50) 85%	AG (*n*=9) 15%	AA *vs*. AG
	Mean ±SD	Mean ±SD	
Age (Years)	20.24±4.21	20±3.87	0.87
Weight (Kg)	82.62±26.84	69±26.27	0.16
Height (m)	1.68 ± 0.12	1.70±0.09	0.62
BMI (Kg/m^2)	29.28±8.79	23.72±8.02	0.08
Results are expressed as mean ± standard deviation (SD), and were compared by t-test (P< 0.05). BMI; Body mass index. *n*: no. of sample.			

Table 7: Female's group data of the distribution of genotypes for the LYS109ARG polymorphisms (*n*=63)

Variables	Frequency%		*P*. value
	AA (*n*=44) 69.8%	AG (*n*=19) 30.2%	AA *vs*. AG
	Mean ±SD	Mean ±SD	
Age (Years)	25.52 ±11.76	20.21 ±4.64	0.01*
Weight (Kg)	68.45 ±19.90	59.16 ±14.81	0.04*
Height (m)	1.58 ±0.054	1.60 ±0.044	0.27
BMI (Kg/m^2)	27.29 ±7.29	23.13 ±5.22	0.02*
Results are expressed as mean ± standard deviation (SD), and were compared by t-test (P< 0.05). *Significance (P < 0.05). BMI; Body mass index. *n*: no. of sample.			

Table 8 shows the distribution of genotypes and allele frequencies for LYS109ARG polymorphism in all male and female data. The genotypic frequencies in the overweight group were 73.9% homozygous AA (n=17) and 26.1% heterozygous AG (n=6), the genotypic frequencies in the obese group were 91.8% homozygous AA (n=45) and 8.2% heterozygous AG (n=4), compared to the normal group (control) which were 64.7% homozygous AA (n=33) and 35.3% heterozygous AG (n=18). In overweight group, the frequency of the A and G alleles were 87% and 13% respectively, and in the obese group, the frequency of the A and G alleles were 96% and 4% respectively, compared to control group, A and G alleles frequency were 82% and 18% respectively. In contrast, there were no significant differences in alleles frequencies between overweight and normal group (P=0.48) but there were significant differences in alleles frequencies between obese and normal group (P=0.002).

When comparing the overweight and control groups results, the frequency of the homozygous AA and heterozygous AG were not significantly difference (P=0.4). There was an increased frequency of the A allele in overweight group (87%) compared to control group (82%) and an increased frequency of the G allele in control group (18%) compared to overweight group (13%). So there was an increased frequency of the AA genotype in overweight group, and an increased frequency of the AG genotype in

control group. When comparing AA and AG genotypes the odd ratio (OR) was 0.65 (95% CI: 0.22-1.93), this value is indicating the protective effect of AG genotype.

When comparing the obese and control groups results, the frequency of the homozygous AA and heterozygous AG were significantly different (*P*=0.001). While there was an increased frequency of the A allele in obese group (96%) compared to control group (82%) and an increased frequency of the G allele in control group (18%) compared to obese group (4%). There was an increased frequency of the AA genotype in obese group, and an increased frequency of the AG genotype in control group. When comparing AA and AG genotypes the odd ratio was 0.16 (95% CI: 0.05-0.53), this value is indicating the protective effect of AG genotype.

Table 8: Genotypes and Allele frequencies in all data (*n*= 123)

Genotypes	Frequencies%			*P* Value[1]	OR[1] (95%CI) RR[1] (95%CI)	*P* Value[2]	OR[2] (95%CI) RR[2] (95%CI)
	Normal (n=51)	Over weight (n=23)	Obese (n=49)				
AA	64.7 (n=33)	73.9 (n=17)	91.8 (n=45)	Reference			
AG	35.3 (n=18)	26.1 (n=6)	8.2 (n=4)	[a]0.4	0.65 (0.22-1.93) 0.74 (0.34-1.62)	[b]0.001*	0.16 (0.05-0.53) 0.23 (0.08-0.64)
Alleles							
A	82 (n=84)	87 (n=40)	96 (n=94)	Reference			
G	18 (n=18)	13 (n=6)	4 (n=4)	0.48	0.7 (0.26-1.90) 0.74 (0.31-1.74)	0.002*	0.20 (0.07-0.61) 0.23 (0.08-0.66)

Data are presented as number of cases with frequency. OR: Odds Ratio. RR: Risk Ratio. CI: Confidence Intervals. *Significance ($P < 0.05$). 1 Between overweight *vs*. control groups. 2 Between obese *vs*. control groups. [a]: AA *vs*. AG, *P*-value Person Chi-Square test. [b]: AA *vs*. AG, *P*-value Fisher Exact test.

Table 9 shows the distribution of genotype and allele frequencies for LYS109ARG polymorphism in males group. The genotypic frequencies in the overweight group were 84.6% homozygous AA (n=11) and 15.4% heterozygous AG (n=2), the genotypic frequencies in the obese group were 92.6% homozygous AA (n=25) and 7.4% heterozygous AG (n=2), compared to the normal group (control) genotyping were 75% homozygous AA (n=15) and 25% heterozygous AG (n=5). In overweight group, the frequency of the A and G alleles were 92.3% and 7.7% respectively, in obese group, the frequency of the A and G alleles were 96.3% and 3.7% respectively, compared to control group, the frequency of the A and G alleles were 87.5% and 12.5% respectively. In contrast, there were no significant differences in frequencies of alleles between overweight and normal groups (*P*=0.6), and between obese and normal groups (*P*= 0.13).

When comparing the overweight and normal groups results, the frequency of the homozygous AA and heterozygous AG (*P*=0.6) was not significantly difference. There was an increased frequency of the A allele in overweight group (92.3%) compared to normal group (87.5%), an increased frequency of the G allele in normal group (12.5%) compared to overweight group (7.7%). There was an increased frequency of the AA genotype in overweight groups and an increased frequency of AG genotype in normal group. When comparing AA and AG genotypes the odd ratio was 0.55 (95% CI: 0.09-3.35), this indicating the protective effect of AG genotype.

When comparing the obese and normal groups results, the frequency of the homozygous AA and the heterozygous AG (*P*=0.11) was not significantly different. The frequency of the A allele was increased in obese group (96.3%) compared to normal group (87.5%), whereas the frequency of the G allele was increased in normal group (12.5%) compared to obese group (3.7%). There was an increased frequency of the AA genotype in obese group, and an increased frequency of the AG genotype in normal group. When comparing AA and AG genotypes the odd ratio was 0.24 (95% CI: 0.04-1.40), this indicating the protective effect of AG genotype.

Table 9: Genotypes and Allele frequencies in males group (*n*=60)

Genotypes	Frequencies %			*P* value[1]	OR[1] (95% CI) RR[1] (95%CI)	*P* value[2]	OR[2] (95% CI) RR[2] (95% CI)
	Normal (n=20)	Over weight (n=13)	Obese (n=27)				
AA	75% (n=15)	84.6% (n=11)	92.6% (n=25)	Reference			
AG	25% (n=5)	15.4% (n=2)	7.4% (n=2)	[a]0.6	0.55 (0.09-3.35) 0.61 (0.14-2.71)	[b]0.11	0.24 (0.04-1.40) 0.30 (0.06-1.38)
Alleles							
A	87.5% (n=35)	92.3% (n=24)	96.3% (n=52)	Reference			
G	12.5% (n=5)	7.7% (n=2)	3.7% (n=2)	0.6	0.58 (0.10-3.26) 0.62 (0.13-2.94)	0.13	0.27 (0.05-1.47) 0.30 (0.06-1.45)

Data are presented as number of cases with frequency. OR: Odds Ratio. RR: Risk Ratio. CI: Confidence Intervals. 1 Between overweight vs. control groups. 2 Between obese vs. control groups. [a]: AA *vs.* AG, *P*-value Fisher Exact test. [b]: AA *vs.* AG, *P*-value Fisher Exact test.

Table 10 shows the distribution of genotype and allele frequencies for LYS109ARG polymorphism in females group. The overweight group genotyping were 60% homozygous AA (n=6) and 40% heterozygous AG (n=4), the genotypic frequencies in the obese group were 90.9% homozygous AA (n=20) and 9.1% heterozygous AG (n=2), compared the normal group (control) were 58.1% homozygous AA (n=18) and 41.9% heterozygous AG (n=13). In the overweight group, the frequency of the A and G alleles were 80% and 20% respectively, in the obese group, the frequency of the A and G alleles were 95.5% and 4.5% respectively, compared to the normal group, the frequency of the A and G alleles were 79% and 21% respectively. In contrast, there were no significant differences in frequencies of alleles between overweight and normal groups (*P*=1), but there were significant differences in frequencies of alleles between obese and normal group (*P*=0.02).

Table 10: Genotypes and Allele frequencies in females group (*n*=63)

Genotype	Frequencies %			*P* value[1]	OR[1] (95%CI) RR [1] (95%CI)	*P* value[2]	OR[2] (95%CI) RR[2] (95%CI)
	Normal (n=31)	Over Weight (n=10)	Obese (n=22)				
AA	58.1% (n=18)	60% (n=6)	90.9% (n=20)	Reference			
AG	41.9% (n=13)	40% (n=4)	9.1% (n=2)	[a]1	0.92 (0.21-3.95) 0.95 (0.40-2.27)	[b]0.01*	0.14 (0.03-0.70) 0.22 (0.05-0.87)
Allele							
A	79% (n=49)	80% (n=16)	95.5% (n=42)	Reference			
G	21% (n=13)	20% (n=4)	4.5% (n=2)	1	0.94 (0.27-3.30) 0.95 (0.35-2.60)	0.02*	0.18 (0.04-0.84) 0.22 (0.05-0.91)

Data are presented as number of cases with frequency. OR: Odds Ratio. RR: Risk Ratio. CI: Confidence Intervals. *Significance ($P < 0.05$). 1 Between overweight *vs*. control groups. 2 Between obese *vs*. control groups. [a]: AA *vs.* AG, *P*-value Fisher Exact test. [b]: AA *vs.* AG, *P*-value Fisher Exact test.

When comparing the overweight and normal groups results, the frequency of the homozygous AA and heterozygous AG (*P*=1) was not significantly different. The frequency of the A allele was an increased in overweight group (80%) compared to normal group (79%), whereas the frequency of the G allele was an increased in normal group (21%) compared to the overweight group (20%). There was

an increased frequency of the AA genotype in overweight group, and an increased frequency of AG genotype in normal group. When comparing AA and AG genotypes the odd ratio was 0.92 (95% CI: 0.21-3.95), this indicated there were no effect and association.

When comparing the obese and normal groups results, the frequency of the homozygous AA and heterozygous AG (P=0.01) were significantly different. There was an increased frequency of the A allele in obese group (95.5%) compared to normal group (79%), and an increased frequency of the G allele in normal group (21%) compared to the obese group (4.5%). There was an increased frequency of the AA genotype in obese group, and an increased frequency of AG genotype in normal group. When comparing AA and AG genotypes the odd ratio was 0.14 (95% CI: 0.03-0.70), and this value is showing the protective effect of AG genotype.

4- Discussion

In this research, we study the distribution of alleles of the leptin receptor gene LYS109ARG polymorphism in obese and overweight subjects and compare this with those obtained from normal subjects. The techniques used were PCR analysis of leptin receptor polymorphisms in DNA extracted from peripheral blood samples. PCR analysis was used for genotyping. The LYS109ARG leptin receptor polymorphism investigated in this study was studied by detecting gene mutation across the replacement of Lysine to Arginine at codon 109 (A/G) (AAG to AGG). This may results in altered leptin binding and therefore, receptor dimerisation and signalling capacity of the leptin receptor.

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 [14,15]. Data in the literature concerning the association between the LYS109ARG polymorphism and obesity are controversial among different ethnic populations.

In the current study, the results demonstrated that no significant differences were observed with age and height when screened across genotypes, but significant differences were found with weight and BMI, which disagree with previous studies [16,17], reported no associations between BMI and weight with LYS109ARG polymorphism. Also, our results disagree with Coimbatore population study, which found no associations between BMI and LYS109ARG polymorphism [18]. When the subjects were divided into males and females, there was no significant associations between genotype and all parameters in males group, which agree with a study on British male population [13], which showed no associations between LYS109ARG and BMI. In addition, these results disagree with study conducted by **Rosmond *et al.*** [19], on Swedish men who reported there was a significant association between BMI and LYS109ARG polymorphism. This variation in data may be due to ethnic origin of patients.

On other hand, there was a significant association between genotype and all parameters (age, weight and BMI) in females group correlated with the finding of **Han *et al.*** [20], who reported that LYS109ARG polymorphism was strongly associated with BMI in Korean female population. However, It is noticed that the female subjects who carrying the AG genotype had lower BMI (23.13 ±5.22) than those carrying the AA genotype (27.29 ±7.29). This suggests that, the G allele was related to lower body fat content, which is disagree with Caucasian women study, which reported that higher BMI was seen in G allele carriers for postmenopausal women group [21].

The present study demonstrated that there were significant differences across genotypes in the obese group compared to normal group with AA and AG genotype (P= 0.001), with the G allele being less frequency in obese group compared to normal group, which is in agreement with Brazilian individuals study conducted by **Oliveira *et al.*** [22], who found that G allele, as well as GG genotype was less frequent in obese compared to non-obese individuals. Our findings disagree with that obtained by **Marti *et al.*** [16], who observed that there were no significant differences in the genotype frequencies or allele distribution for LYS109ARG polymorphism among obese and normal subjects. This contradiction may be due to absence of GG genotype between samples. On the other hand, no significant differences in genotype and allele frequency distribution of the LYS109ARG polymorphism between overweight and normal subjects, which agree with **Yiannakouris *et al.*** [23], who stated that there were no significant differences in the allele frequency or genotype distribution between normal and overweight-obese subjects. This may be due to the appearance of obesity linked by multiple factors including behavioral and environmental factors.

In this study, results obtained from male and female groups showed that, there was an increasing frequency of the AA genotype in overweight and obese groups compared to the normal group, but no significant differences were detected. These results agree with white British population study that showed an increased frequency in AA genotype in obese men [13]. In contrast, our female results disagree with **Wauters *et al.*** [21], who reported that no significant differences across genotypes of LYS109ARG polymorphism were observed in premenopausal women, although AA genotype

showed higher leptin levels compared to the G carriers in postmenopausal women, despite the fact that the former had a significantly lower BMI. This could be due to classifying women based on menopausal state, which did not take into consideration in this study.

Our results showed that, the G allele frequency was less in obese and overweight groups compared to normal group in males, which confirmed by the study of **Masuo *et al.*** [24]. Although, the G allele frequency was less in obese and overweight groups than females normal group, a significant differences were detected between obese females and normal group. These results disagree with the finding obtained by **Wauters *et al.*** [21], who reported that there were 10% more carriers of the Arg mutation at lys109arg in obese women with impaired glucose tolerance, allele frequencies were not significantly different between women with impaired glucose tolerance and those with normal glucose tolerance. This could be due to interference other factors such as glucose metabolism, where this study were performed in nondiabetic subjects.

Conclusion

Based on the results of this study, there was no association between leptin receptor gene LYS109ARG polymorphisms and obesity in males, but it seem to be associated with obesity in females. These results suggest a possible role for leptin receptor gene LYS109ARG polymorphisms in obesity in females and recommended to conduct further studies on males to identify the impact of this gene in Saudi society. Further studies in large samples may be helpful to investigate a more subtle effect of this gene in this serious phenotype. Such studies should also consider possible interactions of this Ob-R variant with other genetic polymorphisms.

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