Impact of Leptin Receptor Gene GLN223ARG Polymorphism on Obesity in Jeddah City

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Abstract: Obesity is a major global epidemic problem. Obesity results from the combined effects of genes, environment, lifestyle and the interactions of these factors. The leptin receptor gene plays a critical role in the regulation of body weight. Genetic variations of leptin receptor gene may play a role in the pathophysiology of human obesity. In this study, the association between the LepR gene polymorphism and obesity in Jeddah population was evaluated by determine the distribution of alleles frequency of the leptin receptor GLN223ARG polymorphism in 180 volunteers (94 males & 86 females). Each gender was divided into 2 groups: Children and Teenager (6-17 years old), and Adults (18-27 years old). As well as each group were divided into 2 sub-groups according to BMI obese and non obese (control). When comparison the obese and non obese groups, results demonstrated that no significant difference between genotype distribution and body mass index (BMI), weight, hip, waist and waist-hip ratio (WHR). In contrast, there was a significant increase in GG genotype (OR= 19.11, 95%CI: 0.95-384.95, *P*= 0.01) compared to AA genotype, and GG genotype (OR= 25.24, 95%CI: 1.25-509.46, *P*= 0.006) compared to AG genotype in males children and teenagers, which suggests that genetic polymorphisms (GLN223ARG) of leptin receptor gene may play a role in prevalence of obesity in children and teenagers males group.
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1. Introduction

Obesity is a major global epidemic problem and it is a complex disorder resulting from a net imbalance between genetic, energy intake and expenditure. Obesity results from the combined effects of genes, environment, lifestyle and the interactions of these factors **(Bouchard, 1994 and Mantzoros, 1999**). Obesity is the most common nutritional problem in the United States (**Devlin, 2002)** and increasing in Kingdom of Saudi Arabia (KSA) (**Al-Nozha *et al.*, 2005)**. Previous studies in KSA, conducted from 1990 to1993, have shown an overall prevalence of obesity of 22.1% and approximately 53% of Saudi adults are either overweight or obese **(Al-Nozha *et al.*, 2005).** Obesity rate in Saudi Arabia was much higher, especially among females **(Al-Othaimeen *et al.*, 2007).** This indicates that health authorities need to address the problem, for example with a campaign for nutrition education and physical activity for the general population.

Childhood obesity increases the risk of obesity in adulthood. **Whitaker *et al.*, (1997)** found that obesity is an increasingly important predictor of adult obesity among older children. Moreover, overweight in adolescence predicts a broad range of adverse health effects **(Must *et al.*, 1992)** Therefore, it is important to identify the cause of obesity, and to prevent obesity in childhood.

The name leptin was derived from the Greek word λεπτοσ: root leptos, meaning thin, because it induces weight loss when injected in mice **(Mouzon *et al.*, 2006).** This name was proposed by **(Halaas *et al.*, 1995)** for the fat-regulating hormone Leptin is an adipose-derived cytokine present in the circulation in amounts proportional to fat mass **(Mergen *et al.*, 2007)** encoded by the obese (Ob) gene localized on human and mouse 7 and 6 chromosomes, respectively. The Ob gene was first discovered in adipose tissue of the obese mouse through positional cloning. The full coding sequence contains 167 amino acids and represents a 21-amino-acid signal peptide and 146-amino-acid circulating bioactive hormone. In both human and mouse, the leptin gene is composed of three exons and two introns **(Bagchi and Preuss, 2007).**

The Ob gene is highly conserved among vertebrates. Mouse leptin shares 84% sequence identity to the product of the human Ob gene. When mutated Ob gene, the Ob gene is no longer deliver its appetite-suppressing message. The mice consequently develop a syndrome that resembles extreme obesity and type 2 diabetes in humans **(Munsch and Beglinger, 2005).**

Leptin circulates in blood as a 16 kDa monomeric protein, **(Munsch and Beglinger, 2005; Castracane and Henson, 2006; Mouzon *et al.*, 2006),** which is mainly but not exclusively produced by white adipose tissue (WAT) as a negative feedback response to maintain body weight by regulating appetite and fat metabolism **(Ahima, 2006; Prieur *et al.*, 2008)**. Leptin acts as a signal to help the

body decide when it has eaten enough food to feel full. Leptin levels in WAT and plasma are related to energy stores, such that leptin increases in well fed state and decreases during fasting **(Ahima, 2006)**. Leptin levels fits the criteria for a feedback signal from body energy stores to the brain as defined by **Kennedy in 1953:** leptin levels drop during starvation, when fat depots are depleted to support the organism's basic energy needs, and leptin levels rise during refeeding where fat depots are replenished **(Kennedy 1953; Münzberg *et al*., 2005).**

The leptin receptor plays a critical role in the regulation of body weight, fat storage and energy homeostasis. In several studies it could be shown that LepR variations can lead to extreme obesity. The leptin receptor is encoded by the diabetes (db) gene **(Matsuoka *et al*., 1997).** Were the first to isolate and describe the leptin receptor (LepR) in mice. The human LepR gene is found on chromosome 1p31, spans about 70 kb, and contains 20 exons **(Thompson* *et al.,* 1997).** Leptin is predominantly produced by adipocytes, but leptin receptors are located in many tissues, in organs involved in energy storage, metabolism and digestion, such as skeletal muscle, adipose tissue, pancreas, stomach, small intestine, colon and liver. There are many peripheral tissues as sites of LepR, such as brain (the choroid plexus and hypothalamus), the lungs, kidneys, adrenals, ovaries, uterus and testes. LepR can be also found in tissue related to immunity, such as spleen, thymus, lymph nodes haematopietic cells and T-cells **(Frühbeck, 2001; Margetic *et al*., 2002; Bjorbaek and Kahn, 2004; Bagchi and Preuss, 2007)**

Single Nucleotide Polymorphisms (SNPs) are the most common genetic variants. SNPs are a single base substitution of one nucleotide with another throughout the human genome, in both coding and non-coding regions. This type of polymorphisms is the most common variations among individuals **(Venter *et al.,* 2001).** Several such polymorphisms exist in the leptin receptor gene including these at codons 109, 204, 223, 343, 492, 656, 976 and 1019. Were the first to identify a single nucleotide polymorphism in the coding region of the leptin receptor gene, GLN223ARG or Q223R. The GLN223ARG polymorphism, characterized by an adenine (A) to guanine (G) transition at position 668 of codon 223 in exon 6 in the extracellular domain of LepR, results in an amino acid substitution (a glutamine with an arginine) (CAG to CGG) **(Gotoda *et al*., 1997; Thompson*et al.,* 1997; Richert *et al*., 2007).**

The GLN223ARG mutation has shown greater consistency in their association with obesity in different population studies. It has been suggested that the change of amino acid glutamine (GLN or Q) by an arginine (ARG or R) causes a change of electric charge from neutral to positive, which can affect the functional characteristics of the receptor and is thought to be associated with an impaired signaling capacity of the leptin receptor and with higher mean circulating levels of leptin. Numerous analyses of LepR SNPs have been published over the last decade. The SNP GLN223ARG (A>G) has been studied extensively in a wide range of populations, several studies have associated this variant with obesity but results were discordant **(Matsuoka *et al.,* 1997; Yiannakouris *et al.,* 2001; Duarte *et al.,* 2006; Duarte *et al.,* 2007; Constantin *et al.,* 2010)**.

The GLN223ARG leptin receptor polymorphism investigated in this study results from the substitution of amino acid from glutamine **(CAG)** to arginine **(CGG)** in the extracellular domain of the receptor. This may results in altered leptin binding and therefore, receptor dimerization and signaling capacity of the leptin receptor. To investigate the frequency of the GLN223ARG alleles in Jeddah population, DNA extracted from blood samples, digestion of the PCR product with the restriction enzyme *MspI* for detection of the alleles of GLN223ARG and Statistical analysis.

2- Materials and Methods

Human subjects:

This study was approved by the ethical Committee (unit of biomedical ethics) from King Abdulaziz University; all participants gave their written informed consent of their participation. The study included 180 participant (94 males and 86 females) randomly selected from Jeddah city population, aged 6 to 27 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 and weight (waist (WC), and hip circumferences (HC) only for females). 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 two groups: from 6-17 years old (Children and Teenager), and from 19-27 years old (Adults). As well as each group were divided into two sub-groups according to BMI (Healthy "control" and obese). Classification of BMI for adults over the age of 20 years according to the World Health Organization (WHO) (Healthy BMI < 25and Obese BMI ≥25), while the children and teens, BMI is age- and sex- specific which often referred to as BMI-for-age. We used the BMI-for-age growth charts (for girls & boys) from Centers for Disease Control and

Prevention (CD) and the American Academy of Pediatrics (AAP). After BMI is calculated, the BMI number is plotted on the BMI-for-age growth charts to obtain a percentile ranking (Healthy BMI < 85th percentile and Obese BMI≥85th percentile). The practical works of this research were conducted at the Biology Graduate Studies laboratories, sixth building at King Abdulaziz University, Jeddah.

Methodology:

Genetic Analysis:

Genomic DNA was extracted from whole blood which stored in EDTA coated tubes by using Qiagen-QIAamp DNA Blood Mini Kit. The concentration of genomic DNA was determined by the quantitative method, which is based on the optical density measurement. Was 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 (A_{260}/A_{280}). Pure DNA should have an A_{260}/A_{280} ratio of 1.7 - 1.9.

Amplification for GLN223ARG gene:

Genotyping of the LepR polymorphism was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay with previously described primer pairs **(Sorli Guerola, 2008).** Forward primer: 5'- TCC TCT TTA AAA GCC TAT CCA GTA TTT-3', and Reverse primer: 5'- AGC TAG CAA ATA TTT TTG TAA GCA AT- 3'. PCR amplification products were obtained using (QIAGEN HotStarTaq Master Mix Kit) a final volume of 50µl and prepared as [2µl genomic DNA (2µg/µl), 25µl HotStar Taq Master Mix, 19µl RNase free water, 2 µl of each primer] in a Thermal cycler (mastercycle personal, Eppendorf, Germany). The amplification conditions were as follows: initial denaturation at 95°C for 15 min followed by 35 cycle of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. 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 (Moecular Imager® Gel Doc™ XR + Systems with Image Lab™ 2.0 Software, BioRad, USA). The ethidium bromide (fluorescent dye) intercalates between bases of DNA causing the visualization of the bands. The gel was then photographed.

Genotyping of rs1137101SNP in GLN223ARG gene:

The resulting DNA fragment was 368bp in length. The genotypes for this SNP were determined by restriction fragment length polymorphism (RFLP) procedure. They were prepared as follows: in a labeled clean and dry Eppendorf tubes 10µl of PCR product, 16.3µl of sterile, deionized water, 0.2µl of 100X BSA, 2µl of 10X RE Buffer, and mixed by pipetting. Finally, 1µl of restriction enzyme (*MspI*) were added. The tubes were incubated for 4 hours at 37□C followed by heat inactivation for 15 minutes at 65˚C. After that, the genotypes were resolved after running it on 2% (w/v) agarose gels electrophoresis.

Statistical Analysis:

Statistical analysis of the data was performed using the Statistical Package for Social Science (SPSS for windows, version 16) (SPSS Inc., Chicago, IL, U.S.A). Descriptive data were given as mean ± standard deviation (SD). Differences among groups were tested using t-test for unpaired data. The Fisher's exact test and Chi-square test were applied to test the association between genotypes and clinical groups. Contingency analysis was applied to calculate the odds ratio (OR) and 95% confidence interval (CI) 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* value less than or equal 0.05 (Two-sided). Hardy-Weinberg Equilibrium was tested by goodness-of fit $\chi 2$ test to compare the observed genotypes frequencies to the expected genotypes frequencies among the obese and non obese, chi-sequare test featuring one degree of freedom.

3- Results

Table 1 shows males' group data; there were a significant difference between the obese group and non-obese group in weight, BMI and waist ($P< 0.05$). Table 2 shows females' group data, which represents a significant difference between the obese group and non-obese group, in weight, BMI, hip and waist ($P< 0.05$).

Figure 1 shows the genotyping results of GLN223ARG leptin receptor polymorphism. A single band of 368 bp shows the presence of allele A (GLN/GLN), while the presence of 2 bands of 245 and 123 bp shows the presence of allele G (ARG/ARG). There are 2 bands for the G allele because this product contains a digestion site for the *MspI* enzyme, which is absent when A is present. Therefore, the PCR product containing the G allele is cleaved by *MspI* and produces 2 bands of low molecular weight.

Table 3 shows the distribution of genotype and allele frequencies for GLN223ARG polymorphism in males' children and teenagers group (6-17 years old). The obese group genotyping were 40.9 % (n=9) homozygous AA, 36.4 % (n= 8) heterozygous AG

and 22.7 % (n= 5) homozygous GG compared to the non-obese group (control) (45.7 % (n= 16) homozygous AA, 54.3 % (n=19) heterozygous AG, the GG homozygous genotype was undetected in this group. In obese group, the frequency of the A and G alleles were 59.1 and 40.9 respectively compared to control group (A and G alleles frequency were 72.9 and 27.1 respectively). In contrast, there were no significant differences in alleles frequencies between obese and non-obese group in this age class (*P*= 0.12).

When comparing the obese and control groups results, the frequency of the homozygote GG were highly significant (*P*= 0.006) because there was no GG genotype in control group. There was an increased frequency of the A allele in control group (72.9) compared to obese group (59.1) and an increased frequency of the G allele in obese group (40.9) compared to control group (27.1).

Table 1: Descriptive of males group data (*n* = 94).

Age class	Variables	BMI class		*P* value
		Non obese	Obese	
6-17 (y) *n*= 57	***n***	35 (61.4%)	22(38.6%)	
	Age (Years)	12.9±3.31	13.52±2.80	0.46
	Weight (Kg)	37.97±12.42	67.37±21.57	0.00*
	Height (m)	1.47±0.18	1.53±0.14	0.18
	BMI (Kg/m^2)	17.02±2.59	28.25±7.03	0.00*
	Waist (cm)	65±6.87	87.95±15.42	0.00*
18-27 (y) *n*= 37	***n***	19 (51.4%)	18 (48.6%)	
	Age (Years)	20.21±1.61	20.94±2.5	0.29
	Weight (Kg)	16±8.79	99.17±18.36	0.00*
	Height (m)	1.71±0.05	1.73±0.05	0.45
	BMI (Kg/m^2)	20.75±2.59	33.29±6.24	0.00*
	Waist (cm)	77.05±7.01	1.03±16.04	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.

When comparing AA and AG genotype the odd ratio (OR) was (0.75), this indicated there were no effect and association. When comparing between AA and GG genotype (*P*= 0.01) the odd ratio was (19.11), which indicated that the odds of obese is about 19 times higher in GG genotype compared to control (RR=12.47, 95% CI: 0.75-207.2). When comparing between AG and GG genotype (*P*= 0.006) the odd ratio was (25.24), indicated that the odds of obese are about 25-fold higher in GG genotype than control (RR=15.71, 95% CI: 0.94-261.9).

Table 4 shows the distribution of genotype and allele frequencies of the obese males were 50% (n= 9) homozygous (AA), 50% (n= 9) heterozygous (AG) and the homozygous (GG) genotype was undetected, compared to the non obese males (control), the results were 47.4% (n= 9) homozygous (AA), 42.1% (n= 8) heterozygous (AG) and 10.5 (n= 2) homozygous (GG). In obese group, the frequency of the A and G alleles were 75 and 25 respectively; compared to control group, the frequency of the A and G alleles were 68.4 and 31.6 respectively. In contrast, there were no significant differences in frequencies of alleles between obese and non-obese group in this age class (*P*= 0.53).

When comparing the obese and non-obese groups results, the frequency of the homozygous AA and heterozygous AG (*P*= 0.86), the homozygous AA and GG (*P*= 0.47), and heterozygous AG and homozygous GG (*P*= 0.47) were not significantly difference. Also, the frequency of dominant model AA and AG+GG genotype were not significantly difference (*P*= 0.86). There was an increased frequency of the A allele in the obese group (75) compared to non-obese group (68.4), and an increased frequency of the G allele in non-obese group (31.6) compared to obese group (25). When comparing AA and AG genotype the odd ratio was (1.13) and AA and GG genotype the odd ratio was (0.2), this indicates –nearly- non existence for the effect and association.

Table 2: Descriptive of females group data (n = 86).

Age class	Variables	BMI class		P value
		Non obese	Obese	
6-17 (y) n= 50	n	33 (66%)	17 (34%)	
	Age (Years)	13.05±2.61	12.24±3.23	0.34
	Weight (Kg)	38.52±10.93	54.82±17.53	0.00*
	Height (m)	1.46±0.13	1.44±0.12	0.51
	BMI (Kg/m^2)	17.68±2.89	25.10±5.55	0.00*
	Hip (cm)	79.69±10.09	93.71±11.83	0.00*
	Waist (cm)	66.20±8.94	85.12±15.25	0.00*
	WHR	0.83±0.05	0.91±0.10	0.00*
18-27 (y) n= 36	n	19 (52.8%)	17 (47.2%)	
	Age (Years)	21.37±2.73	21±1.93	0.64
	Weight (Kg)	50.42±8.33	78.41±12.45	0.00*
	Height (m)	1.58±0.04	1.59±0.05	0.63
	BMI (Kg/m^2)	20.24±3.24	31.14±4.26	0.00*
	Hip (cm)	93.24±6.86	1.16±9.62	0.00*
	Waist (cm)	75.03±6.55	93.65±9.16	0.00*
	WHR	0.81±0.05	0.81±0.05	0.85

* Significance (P<0.05). Values are expressed as mean ± standard deviation (SD), and were compared by t-test. BMI; Body mass index.WHR; waist-hip ratio.n; no. of sample.

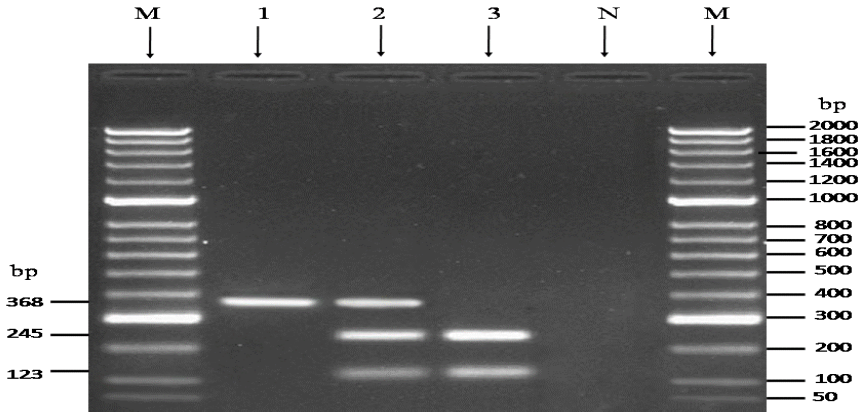

Figure 1: Photograph of a 2% (w/v) agarose gel showing the digested PCR products for GLN223ARG leptin receptor polymorphism genotyping. Lane M: DNA marker. **Lane 1:** Homozygous AA (GLN/GLN); genetype produce one band of size 368 bp. **Lane 2:** Heterozygous AG (GLN/ARG); genetype produce three bands of size 368, 245 and 123 bp**. Lane 3:** Homozygous GG (ARG/ARG); genetype produce two bands of size 245 and 123 bp. **Lane N:** A negative control (- DNA).

Table 5 shows the distribution of genotype and allele frequencies for GLN223ARG polymorphism in children and teenagers females group (6-17 years old). The obese group genotyping were 47.1% (n= 8) homozygous (AA), 35.3% (n= 6) heterozygous (AG) and 17.6% (n= 3) homozygous (GG) compared to the non obese group (control) which were 48.5% (n= 16) homozygous (AA), 36.4% (n= 12) heterozygous (AG) and 15.2% (n= 5) homozygous (GG). In obese group, the frequency of the A and G alleles were 64.7 and 35.3 respectively, compared to control group the frequency of the A and G alleles were 66.7 and 33.3 respectively. In contrast, there were no significant differences in frequencies of alleles between obese and non obese group in this age class (P= 0.84).

When comparing the obese and control groups' results, the frequency of the homozygous AA and the heterozygous AG (P= 1), or the homozygous AA and GG (P= 1), and the heterozygous AG and the homozygous GG (P= 1) were not significantly difference. Also, the frequency of dominant model AA and AG+GG genotype were not significantly

difference (*P*= 0.92). The frequency of the G allele was increased in obese group (35.3) compared to control group (33.3), whereas the frequency of the A allele was increased in control group (66.7) compared to obese group (64.7). When comparing AA and AG genotype the odd ratio was 1 (95% CI: 0.27-3.66), AA and GG genotype the odd ratio was 1.2 (95% CI: 0.22-6.34), and AA and AG+GG genotype the odd ratio was 1.06 (95% CI: 0.33-3.42), this indicated there were no effect and association.

Table 6 shows the distribution of genotype and allele frequencies for GLN223ARG polymorphism in adults females group (18-27 years old). The obese group genotyping were 47.1% (n= 8) homozygous (AA), 41.2% (n= 7) heterozygous (AG) and 11.8% (n= 2) homozygous (GG), compared to the non-obese group (control) were 42.1% (n= 8) homozygous (AA), 47.4% (n= 9) heterozygous (AG) and 10.5% (n= 2) homozygous (GG). In obese group, the frequency of the A and G alleles were 67.6 and 32.4 respectively compared to control group, the frequency of the A and G alleles were 65.8 and 34.2 respectively. In contrast, there were no significant differences in frequencies of alleles between obese and non-obese group in this age class (*P*= 0.86). When comparing the obese and non-obese groups' results, the frequency of the homozygous AA and heterozygous AG (*P*= 0.71), or the homozygous AA and GG (*P*= 1), and the homozygous GG and heterozygous AG (*P*= 1) were not significantly difference. Also, the frequency of dominant model AA and AG+GG genotype were not significantly difference (*P*= 0.76).

Table 7 shows the comparison results between all obese children and teenagers (males and females) and all obese adults (males and females). There was an increased frequency of the GG genotype in obese children and teenagers (4 times) than obese adults and an increased frequency of the AA and AG genotype in obese adults.

Table 3: Genotypes and Allele frequencies in males' children and teenagers

Genotypes	Frequencies %		*P* value	OR (95% CI)	RR (95% CI)
	Non obese (n= 35)	Obese (n= 22)			
AA	45.7 (n= 16)	40.9 (n= 9)	Reference		
AG	54.3 (n= 19)	36.4 (n= 8)	[a]0.62	0.75 (0.23-2.39)	0.87 (0.48-1.56)
GG	0	22.7 (n= 5)	[b]0.01	19.11 (0.95-384.95)	12.47 (0.75-207.2)
			[c]0.006	25.24 (1.25-509.46)	15.71 (0.94-261.9)
AG + GG	54.3 (n= 19)	59.1 (n= 13)	[d]0.72	1.22 (0.41-3.58)	1.09 (0.69-1.73)
Alleles					
A	72.9 (n= 51)	59.1 (n= 26)	Reference		
G	27.1 (n= 19)	40.9 (n= 18)	0.12	1.86 (0.84-4.13)	1.51 (0.89-2.54)

Data are presented as number of cases with frequency.OR: Odds Ratio.RR: Risk Ratio. CI:Confidence Intervals.[a]: AA *vs.* AG, *P*-value Person Chi-Square test.[b]: AA *vs.* GG, *P*-value Fisher Exact test. [c]:AG *vs.* GG, *P*-value Fisher Exact test. [d]: AA *vs.* AG+GG, *P*-value Person Chi-Square test.

Table 4: Genotypes and Allele frequencies in adults males group (18-27 Years old) (*n*= 37)

Genotypes	Frequencies %		*P* value	OR (95% CI)	RR (95% CI)
	Non obese (n= 19)	Obese (n= 18)			
AA	47.4 (n= 9)	50 (n= 9)	Reference		
AG	42.1 (n= 8)	50 (n=9)	[a]0.86	1.13 (0.3-4.24)	1.06 (0.54-2.11)
GG	10.5 (n=2)	0	[b]0.47	0.2 (0.01-4.75)	0.24 (0.01-4.44)
			[c]0.47	0.18 (0.01-4.28)	0.22 (0.01-4.05)
AG + GG	52.6 (n= 10)	50 (n= 9)	[d]0.86	0.9 (0.25-3.27)	0.95 (0.51-1.78)
Alleles					
A	68.4 (n= 26)	75 (n= 27)	Reference		
G	31.6 (n= 12)	25 (n= 9)	0.53	0.72 (0.26-2)	0.79 (0.38-1.65)

OR: Odds Ratio.RR: Risk Ratio. CI: Confidence Intervals. [a] AA *vs.* AG, *P*-value Person Chi-Square test: AA *vs.* GG, *P*-value Fisher Exact test. [c]: AG *vs.* GG, *P*-value Fisher Exact test. [d]: AA *vs.* AG+GG, *P*-value Person Chi-Square test.

Table 5 : Genotypes and Allele frequencies in children and teenagers females group (6-17 Years old) (*n*= 50)

Genotypes	Non obese (n= 33)	Obese (n= 17)	*P* value	OR (95% CI)	RR (95% CI)
AA	48.5 (n= 16)	47.1 (n= 8)	Reference		
AG	36.4 (n= 12)	35.3 (n= 6)	[a]1	1 (0.27-3.66)	1 (0.48-2.1)
GG	15.2 (n= 5)	17.6 (n= 3)	[b]1	1.2 (0.22-6.34)	1.15 (0.33-3.92)
			[c]1	1.2 (0.21-6.8)	1.13 (0.35-3.69)
AG + GG	51.6 (n= 17)	52.9 (n= 9)	[d]0.92	1.06 (0.33-3.42)	1.03 (0.59-1.79)
Alleles					
A	66.7 (n= 44)	64.7 (n= 22)	Reference		
G	33.3 (n= 22)	35.3 (n= 12)	0.84	1.09 (0.46-2.6)	1.06 (0.6-1.87)

OR: Odds Ratio.RR: Risk Ratio. CI: Confidence Intervals. [a]: AA *vs.* AG, *P*-value Person Chi-Square test. [b]: AA *vs.* GG, *P*-value Fisher Exact test. [c]: AG *vs.* GG, *P*-value Fisher Exact test. [d]: AA *vs.* AG+GG,*P*-value Person Chi-Square test.

Table 6: Genotypes and allele frequencies in adults females group (18-27 Years old) (*n*= 36)

Genotypes	Non obese (n= 19)	Obese (n= 17)	*P* value	OR (95% CI)	OR (95% CI)
AA	42.1 (n= 8)	47.1 (n= 8)	Reference		
AG	47.4 (n= 9)	41.2 (n= 7)	[a]0.71	0.78 (0.19-3.13)	0.88 (0.44-1.78)
GG	10.5 (n= 2)	11.8 (n= 2)	[b]1	1 (0.11-8.95)	1 (0.17-5.77)
			[c]1	1.29 (0.14-11.54)	1.22 (0.21-7.04)
AG + GG	57.9 (n= 11)	53 (n= 9)	[d]0.76	0.81 (0.22-3.06)	0.91 (0.51-1.65)
Alleles					
A	65.8 (n= 25)	67.6 (n= 23)	Reference		
G	34.2 (n= 13)	32.4 (n= 11)	0.86	0.92 (0.34-2.46)	0.95 (0.49-1.82)

OR: Odds Ratio. RR: Risk Ratio. CI: Confidence Intervals. [a]: AA *vs.* AG, *P*-value Person Chi-Square test. [b]: AA *vs.* GG, *P*-value Fisher Exact test. [c]: AG *vs.* GG, *P*-value Fisher Exact test. [d]: AA *vs.* AG+GG, *P*-value Person Chi-Square test.

Table 7: Comparison genotypes between all obese children and teenagers and all obese adults.

Genotypes	Children and teenager (*n*= 39)	Adults (*n*= 35)
AA	43.6% (*n*= 17)	48.6% (*n*= 17)
AG	35.9% (*n*= 14)	45.7% (*n*= 16)
GG	20.5% (*n*= 8)	5.7% (*n*= 2)

4- Discussion

In this research, the distribution of alleles of the leptin receptor gene GLN223ARG polymorphism in obese subjects and compared this with those obtained from non-obese subjects was studied. The techniques used were PCR analysis of leptin receptor polymorphisms in DNA extracted from peripheral blood samples. PCR analysis was used for genotyping. The GLN223ARG leptin receptor polymorphism investigated in this study results in the substitution of amino acid from glutamine (CAG) to arginine (CGG) in the extracellular domain of the receptor. This may results in altered leptin binding and therefore, receptor dimerization and signaling 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 **(Ben Ali, *et al.*, 2009)**. Data in the literature concerning the association between the GLN223ARG polymorphism and obesity are controversial among different ethnic population.

In the current study, the results demonstrated that no significant differences with all parameters (weight, height, BMI, hip, waist and WHR) screened across genotypes in the obese group compared to non-obese group. Also, when the subject were divided into males and females, there was no significant differences in BMI and all parameters, which agree with two studies of obese males

(Echwald, *et al.,* 1997; Gotoda *et al.,* 1997), which detected no associations between GLN223ARG and BMI and leptin levels.

In contrast, a recent study in Tunisian population found that the obese men whom carrying the ARG allele had lower BMI than those whom homozygous for GLN allele **(Ben Ali *et al.,* 2009).** Also, our results disagree with Greek population study, which reported the presence of a weak association between GLN223ARG polymorphism and BMI **(Yiannakouris *et al.,* 2001)**. In Caucasian women **(Quinton, *et al.,* 2001)** found that carriage of the A allele (AA and AG) was associated with a higher mean BMI than not carrying the A allele (*P*= 0.009). In Saudi obese women) demonstrate that, GG and AG genotype had higher BMI and leptin levels were increased significantly **(Daghestani, *et al.,* 2009)**.

Childhood obesity increases the risk of obesity in adulthood. **Whitaker *et al*., (1997**) found that obesity is an increasingly important predictor of adult obesity among older children**.** Moreover, overweight in adolescence predicts a broad range of adverse health effects **(Must *et al.,* 1992).** Therefore, it is important to identify the cause of obesity, and to prevent obesity in childhood.

In this study, the subjects were subdivided according to age class into two groups' children and teenagers group, and adults group. The males children and teenagers results showed a significant increased in GG genotype in obese, an increased in GG genotype in females children and teenagers but not significant compared to non obese groups. This suggests that there was an association between GLN223ARG polymorphism and obesity in male children and teenagers but not in females, because males children and teenagers had a higher weights from 36 kg to 115kg with mean (67.37±21.57) and high BMI with an average (28.25±7.03) compared to females whom weights from 29 kg to 85 kg with mean (54.82±17.53) and BMI with an average (26 ±5.55). Our results disagreement with previous study conducted on children females and males carried out in Mexican adolescents **Guizar-Mendoza (2005),** reported that no significant difference in genotypes frequencies for GLN223ARG polymorphism between obese and non obese children Another study in Japanese school children **Endo *et al*., (2000),** reported that the GLN223ARG polymorphism is not associated with obesity in Japanese school children because the difference between obese and non obese was not significant **()**. Also, **Komşu-Ornek *et al*., (2012**) stated that there were no differences in the genotype frequencies or allele distribution for GLN223ARG polymorphism among obese, obese with metabolic syndrome and lean Turkish children, which suggest that there was no association between GLN223ARG polymorphism and obesity in Turkish children. Same finding was reported by **Pyrzak *et al*., (2009)** confirmed no association between GLN223ARG polymorphism and obesity in children

In the Kingdom of Saudi Arabia, several studies have been carried out and the numbers of obese individuals is rising in an alarming rate. In a national study on Saudi adults, 15.12% of the males and 23.97% of the females were found to be obese, while 32.82% and 29.09% respectively were overweight **(EL-Hazmi and Warsy, 2002).**

In this study, results obtained from adult males and females showed that there was an increased frequency of the AA and GG genotype in obese females and an increased frequency of the AG and AA genotype in obese males compared to control but not significant. In contrast**, Gotoda *et al.,* (1997)** reported that there was an increased frequency in AA and GG genotype on white British male population Also, AG and AA genotype was an increased in overweight and obese Caucasian women **(Wauters *et al.,* 2001)**. In Saudi women **Daghestani *et al.,* (2009)** found that GG homozygote and AG heterozygotes genetype had higher frequencies among obese subjects **(**Recent studies **(Changnon *et al.,* 1999; Duarte *et al.,* 2006; Duarte *et al.,* 2007; Ben Ali *et al.,* 2009)** demonstrated that there was an increased frequency of the AG genotype in obese compared to non obese. In contrast, **(Yiannakouris *et al.,* 2001; Constantin *et al.,* 2010)** reported an increased in GG genotype in obese compared to non obese.

This study showed no significant differences in genotypic distribution and allele frequencies of the GLN223ARG polymorphism between all adults obese and non obese subjects, which confirmed **(Ben Ali *et al.,* 2009)** study. In the other hand, **Duarte *et al.,* (2006**) conducted a study in Brazilian multiethnic subjects and found there was a significant differences in genotypic distribution in obese compared to lean or normal individuals in the co-dominant and dominant models, but not in the recessive model. Another study of Brazilian population conducted by **Duarte *et al.,* (2007),** confirmed that the association of the GLN223ARG polymorphism with obesity was related to the co-dominant and dominant model (*P*= 0.05) and (*P*= 0.03) respectively, but not with the recessive model (*P*= 0.628) Also, **Yiannakouris *et al.,* (2001)** reported a significant differences in genotype distribution of the GLN223ARG polymorphism which related to the co-dominant models (*P*= 0.02) and recessive model (*P*= 0.01) between normal weight and overweight-obese subjects in Greek population

Conclusion

Based on the results of this study, there was no association between leptin receptor gene GLN223ARG polymorphisms and obesity in females children and teenager, and adults males and females groups; while appeared a significant association in males children and teenager groups with too high risks may be due to overweight and obesity in this groups compared to others age groups. Together these results suggest a possible role for leptin receptor gene GLN223ARG polymorphisms in obesity in males' children and teenager groups and **Recommended** to conduct further studies on females' children and teenager age class and males' adults to identify the impact of this gene in Saudi society obesity. Further studies in a 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 LepR variant with other genetic polymorphisms.

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