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Original Research

Correlation between serum leptin and its gene expression to the anthropometric measures in overweight and obese children

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Abstract: Obesity is a multifactor disorder with evidence supporting the role of the genetic factor in its etiology. The present study evaluates the relationship between leptin G2548A (rs7799039) and leptin receptors (Gln223Arg (rs1137101) genotyping and its leptin level and the risk of childhood obesity. This case-control study was conducted on 168 overweight and obese Saudi children and 126 non obese one served as control. Fasting insulin, leptin, blood glucose, lipid profile levels were measured. HOMA- IR, and BMI were assessed. Genotyping of leptin and leptin receptor gene variants was done by SNP real-time PCR method. GG genotype and G allele of rs1137101 were significantly higher in overweight and obese children than controls. It showed significant association with risk of obesity OR 7.1 [95% CI: 3.4 – 14.8] and OR 2.8 [95% CI: 2.0 – 4.1] respectively. Leptin level was significantly greater in patients than controls ($p<0.000$*) with GG and AG genotypes having the highest level of leptin when compared with another genotype in the obese group. As regards, rs7799039 AA genotype showed significant higher leptin level than other genotypes in the same group with a non-significant difference in genotypes distribution between obese and controls. rs1137101 variant of leptin receptor and fasting leptin level are correlated with overweight and obesity in Saudi children. The GG genotype of leptin receptors rs1137101 and higher serum leptin levels can be used as risk factors for childhood obesity.

Key words: Leptin; Polymorphism; Obese; Receptors; Real time PCR.

Introduction

Obesity prevalence continues to increase in both developed and developing countries. It became a major health problem (1). Obesity that begins in childhood usually persists into adulthood and is associated with adverse outcomes (2). The World Health Organization defines overweight as > 85th centile and obesity as > 97th centile; for children (3).

The prevalence of childhood obesity has risen in recent years. The International Association for the Study of Obesity (IASO) and International Obesity Task Force (IOTF) estimate that 200 million school children are either overweight or obese (4).

In the United States, overweight prevalence among 2- to 19-year-olds was reported at 14.9% while obesity one was at 16.9% (5). Child obesity will continue to be a problem without improved understanding of multifactorial etiology responsible for it, includes genetic, behavioral and environmental factors and identification of preventive methods (6).

Leptin (LEP) is neuroendocrine hormone reduces food intake and increases energy expenditure by binding to leptin receptors (LEPR) (7). Several polymorphisms of LEP and LEPR genes were studied in different populations for its potential association with obesity and its related complications. Leptin *and LEPR genes* variants may result in leptin resistance and occurrence of a large amount of fat tissue which lead to Obesity with hyperleptinemia (8).

The aim of this study was to determine the prevalence of leptin G2548A (rs7799039) and leptin receptors Gln223Arg (rs1137101) single nucleotide polymorphism and its possible association with obesity in Saudi children.

Materials and Methods

This study was conducted on 294 children attending the GIT and endocrine clinic at the MCH in Al Madinah Almonaurah KSA in a period of 6 months (from January 2014 to June 2014). They were classified by BMI according to World Health Organization (WHO) criteria as follows: normal range (BMI 18.5–24.9 kg/m2) no = 126, overweight and obese (BMI 25.0–30 kg/m2) no = 168. Before the enrollment, the guardian of the participants gave their signature in a consent document after they were informed about all the implications of the study. Appropriate ethical and biosecurity conduct was ensured by the ethical committee of the MCH. None of

the participants presented with signs or symptoms of an acute or chronic disease, besides obesity. There is no past history of glucose intolerance, medication, and a stable weight for at least the last three weeks. Individuals with infectious diseases, hypertension, and history of cardiovascular disease, malignancy, renal and metabolic diseases such as T2DM were not included.

Physical examination and medical history

All participants completed a questionnaire to gather personal and family medical history. Blood pressure and heart rate were measured 3 times at 3-minute intervals with the subject in the sitting position, and before the first measurement, they had a relaxation period of at least 15 minutes.

Anthropometric measurements

Height was measured using a stadiometer (Seca GmbH & Co. KG., Hamburg,Germany, to the nearest 1.0 mm), while body weight (to the nearest 0.1 kg), BMI, and body composition (including total muscle and body fat mass percentage) were determined using bioelectrical impedance analysis (TANITA Corporation, TBF304, Tokyo, Japan). Waist and hip circumferences were measured to the nearest 0.1 cm with an anthropometric fiberglass tape (GULICK, accuracy 1 mm; North Coast Medical Inc., Gilroy, CA) following the procedures recommended by Durnin. Four measurements of skinfold thickness (biceps, triceps, subscapular, and suprailiac) were obtained from the right side of the body employing a caliper skinfold Harpenden (with a maximum opening of 80 mm, accuracy of 0.2 mm and constant pressure of 10 g/mm2; Holtain Ltd., Croswell,UK), according to the procedures recommended by the Anthropometric Indicators Measurement Guide. We also calculated the waist/hip ratio as an indicator of preferential accumulation of fat in the abdomen, rather than in the extremities.

Laboratory techniques and procedures

After 12 hours overnight fasting, 12 ml of venous blood were withdrawn from every subject by sterile vein-puncture and divided into three tubes; 4 ml of blood were transferred into two EDTA tubes: one of them was used for quantitative colorimetric determination of glycated hemoglobin using kits supplied by Teco diagnostics, USA and the other EDTA tube for genotyping of leptin gene, One ml of blood was transferred into sodium fluoride tube for enzymatic colorimetric determination of blood glucose using Spinreact kit, SPAIN, and 5 ml into tubes without additive, left 10 minutes for coagulation, then centrifuged at 3000 rpm for 10 minutes then sera were used for colorimetric determination of serum TC, HDL, LDLc, TG and fasting serum insulin level. 2 ml of blood were transferred into EDTA containing a tube, centrifuged for 10 minutes at 4000 r.p.m. The clear supernatant plasma was kept frozen at -80° C until determination of plasma leptin level by enzyme-linked immune-sorbent assay method using DRG® leptin ELISA kit, GERMANY with a detection range (7.36 ± 3.73) in Females(9(. Serum insulin was determined by enzyme-linked immune-sorbent assay method using DRG® Insulin ELISA kit, GERMANY (10). Assessment of insulin resistance was done by homeostatic model assessment (HOMA) according to (11). HOMA- IR = fasting glucose (mg/dl) x fasting insulin (μIU/mL) / constant (405).

Genotyping of leptin G2548A (rs7799039) and leptin receptors (Gln223Arg (rs1137101) polymorphism

DNA was extracted from blood samples using Gene JET Whole Blood Genomic DNA Purification Mini extraction Kit, Thermo Fisher Scientific, USA. DNA was eluted and stored at -20 C for further PCR procedure.

Leptin gene G2548A (rs7799039) was genotyped using allelic discrimination assay by real-time PCR technique using a TaqMan probe, Applied Biosystems, USA. The maxima probe qPCR Master Mix (40X), primers and probes were supplied from Thermo Fisher Scientific; the forward primer was; 5′-TTTCCTGTAATTTTCCCGTGAG and the reverse primer was; 5′-AAAAGCAAAGACAGGCATAAA.10 µl of master mix was added to 1.25 µl of the genotyping assay of primer/ probe mix and 3.75 µl of DNAase-free water. 5 µl of genomic DNA extract for every sample and 5 µl of DNAase-free water for the negative control reaction were applied. The following cycling conditions were adjusted: Initial denaturation was done at 95°C for 10 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, primer annealing at 50°C for 60 seconds then extension at 72°C for 2 minutes and the last extension at 72°C for 1 minute. Analysis of data was accomplished using 7500 Real-Time PCR instrument, version 2.0.1, Applied Biosystems.

Leptin Receptor gene Gln223Arg (rs1137101) polymorphism was genotyped using allelic discrimination assay by real-time PCR technique using a TaqMan probe, Applied Biosystems, USA. The maxima probe qPCR Master Mix (40X), primers and probes were supplied from Thermo Fisher Scientific; the forward primer was; 5-ACCCTTTAAGCTGGGTGTCCCAAATAG-3 and the reverse primer was; 5-AGCTAGCAAATATTTTTGTAAGCAATT-3.10 µl of master mix was added to 1.25 µl of the genotyping assay of primer/ probe mix and 3.75 µl of DNAase-free water. 5 µl of genomic DNA extract for every sample and 5 µl of DNAase-free water for the negative control reaction was applied. The following cycling conditions were adjusted: Initial denaturation was done at 95°C for 10 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, primer annealing at 50°C for 60 seconds then extension at 72°C for 2 minutes and the last extension at 72°C for 1 minute. Analysis of data was accomplished using 7500 Real-Time PCR instrument, version 2.0.1, Applied Biosystems.

Statistical analysis

Results were collected, tabulated and statistically analyzed by IBM personal computer and statistical package SPSS version 20. Hardy-Weinberg equilibrium was computed to exclude any bias of results and we concluded that the genotype frequencies in this population are not significantly different than what would be expected as it was in Hardy-Weinberg frequencies. Student t-test used for comparison between two groups having quantitative variables. Chi-square test ($\chi 2$): was used to study the association between two qualitative variables. Mann Whitney and Kruskal–Wallis tests for comparison two and three groups of not normally distri-

Table 1. Comparison between the two studied groups according to different parameters.

	Obese (n = 168)	Control (n = 126)	p
Sex			
Male	89(53%)	61(48.5%)	0.09
Female	79(47%)	65(51.5%)	
Age (years)	10.2±2.2	9.9±1.8	0.149
BMI (kg/m^2)	26.7±2.7	19.3±1.2	<0.001*
Leptin (ng/ml)	33.9(18.4–74.6)	14.5(7.9–19.2)	<0.001*
Serum insulin (µIU/ml)	28(1.7–40)	15(2.3–19)	<0.001*
Fasting blood Glucose (mg/dl)	104.0±20.0	92±8	<0.001*
HOMA IR (%S)	7.3(0.5–15.7)	3.5(0.5–4.5)	<0.001*
Total cholesterol (mg/dl)	219.9±38.2	160.1±11.0	0.024*
Triglycerides (mg/dl)	97.5(29–181)	105(92–128)	0.037*
HDLc (mg/dl)	48(43–55)	52(32–80)	0.001*
LDLc (mg/dl)	145.8(126.5–218)	125.5(72.5–149.6)	0.04*
Waist circumference (cm)	81.5±7.6	66.1±3.3	<0.001*
Hip circumference (cm)	88.5±5.8	78.6±6	<0.001*
Waist /hip ratio	0.9±0.1	0.8±0.1	<0.001*

Qualitative data were described using number and percent and was compared using Chi square test , while normally quantitative data was expressed in mean ± SD and was compared using student t-test, abnormally distributed data was expressed in median (Min. – Max.) And was compared using Mann Whitney test. *: Statistically significant at p ≤ 0.05.

buted variables were used. P-value < 0.05 was considered statistically significant.

Results

The study was conducted on a total number of 294 subjects divided into two groups as follows; 168 obese persons as a group I and 126 healthy persons (control) as group II. There was a statistically significant difference between the two studied groups regarding; serum leptin, insulin, FBG, lipid profile, HOMA-IR, BMI, waist circumference, hip circumference, waist/hip ratio (P= 0.037). While there was no significant difference as regards age and sex (Table 1).

As regards Leptin receptor rs1137101 genotype distribution between the two studied groups, it showed a significant difference, with increased frequency of the GG and AG genotypes and G allele in the obese group and increased AA genotype and A allele frequency in the control group (P <0.001; Table 2 and Figure 1,2). The results also showed that the GG genotype of Leptin receptor rs1137101(A/G) increases the risk of obesity by 7.1- fold and AG genotype increases the risk by 2.4-fold, combined AG + GG increases the risk of obesity by 3.3- fold, while the G allele increases the risk by 2.8-fold, as shown in Table 2 and figure 1.

As regards Leptin rs7799039 genotype distribution between the two studied groups, there was a non-significant difference between the obese group and the controls as shown in table 2 and figure 2.

When comparing the three different genotypes of Leptin receptor rs1137101 (A/G) (combined GG& AG

Table 2. Comparison between the two studied groups according to rs1137101 and rs7799039**.**

	Obese (n = 168)	Control (n = 126)	p	OR	95% C.I
rs1137101					
AA®	42(25%)	66(52.4%)	<0.001*	1.0	
AG	72(42.9%)	48(38.1%)	0.411	2.4*	1.4 – 4
GG	54(32.1%)	12(9.5%)	<0.001*	7.1*	3.4 – 14.8
AG + GG	126(75%)	60(47.6%)	<0.001*	3.3*	2 – 5.4
Allele					
A	156(46.4%)	180(71.4%)		1.0	
G	180(53.6%)	72(28.6%)	<0.001*	2.8*	2.0 – 4.1
rs7799039					
GG®	44(26.2%)	36(28.6%)	0.650	1.0	
AG	66(39.3%)	42(33.3%)	0.295	1.3	0.7 – 2.3
AA	58(34.5%)	48(38.1%)	0.528	1.0	0.6 – 1.8
AG+AA	124(73.8%)	90(71.4%)	0.650	1.1	0.7 – 1.9
Allele					
G	154(45.8%)	114(45.2%)			
A	182(54.2)	138(54.8%)	0.886	1.0	0.7 – 1.4

Qualitative data were described using number and percent and was compared using Chi square test. *: Statistically significant at p ≤ 0.05.

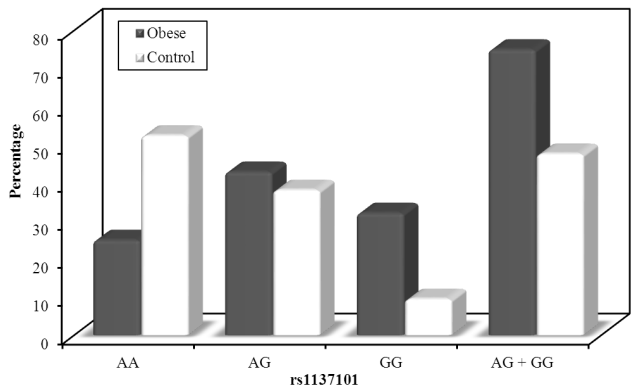

Figure 1. Comparison between the two studied groups according to rs1137101.

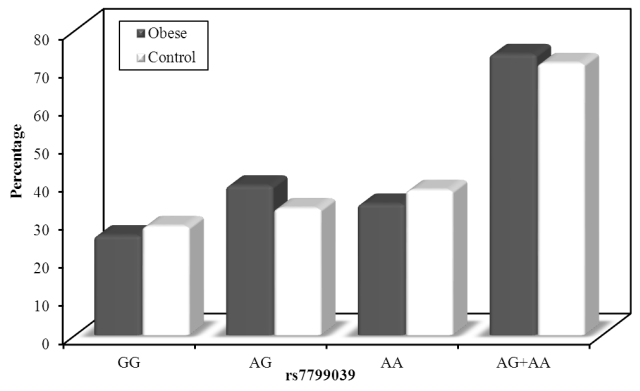

Figure 2. Comparison between the two studied groups according to rs7799039.

and AA), there was a significant difference increase in fasting blood glucose, Insulin, leptin, total cholesterol levels, BMI, HOMA-IR, Waist circumference (cm) and waist to hip ratio in combined GG and AG genotypes when compared to the wild genotype in obese group. While there was a significant difference in only fasting blood glucose level and HOMA-IR in the controls as shown in table 3.

When comparing the three different genotypes of Leptin rs7799039 (G/A) (combined AA & GA and GG), there was a significant difference increase in fasting blood glucose, Insulin, leptin, total cholesterol, LDL levels, BMI, HOMA-IR, Waist circumference (cm) and waist to hip ratio in combined GG and AG genotypes when compared to the wild genotype in obese group. While there was a significant difference in only triglyceride level in the controls as shown in table 4.

Multivariate logistic regression for risk of childhood obesity showed that the most common risk factor is fasting leptin level OR; 10.8 (8.32-28.92), followed by GG genotype of rs 1137101 OR; 2.45(1.02-5.90), HOMA-IR OR; 2.26(1.75-2.92) and HDL level OR; 1.07(1.02-1.12) as shown in table 5.

Discussion

Childhood obesity is an important health problem as its prevalence increase leads to many complications in children like, hypertension and dyslipidemia which was seen only in the adult. Genetic predispositions for weight gain with a positive energy balance are the most common causes of this obesity (12). The genotype of the individual affects the response to an environmental agent or a behavior (13).

leptin (LEP), leptin receptor (LEPR) susceptibility genes code for proteins which have a central role responsible for maintaining energy balance through food consumption and energy expenditure (14).

In the present study, there was a statistically significant difference between the two studied groups regarding; serum leptin, insulin, FBG, lipid profile, HOMA-

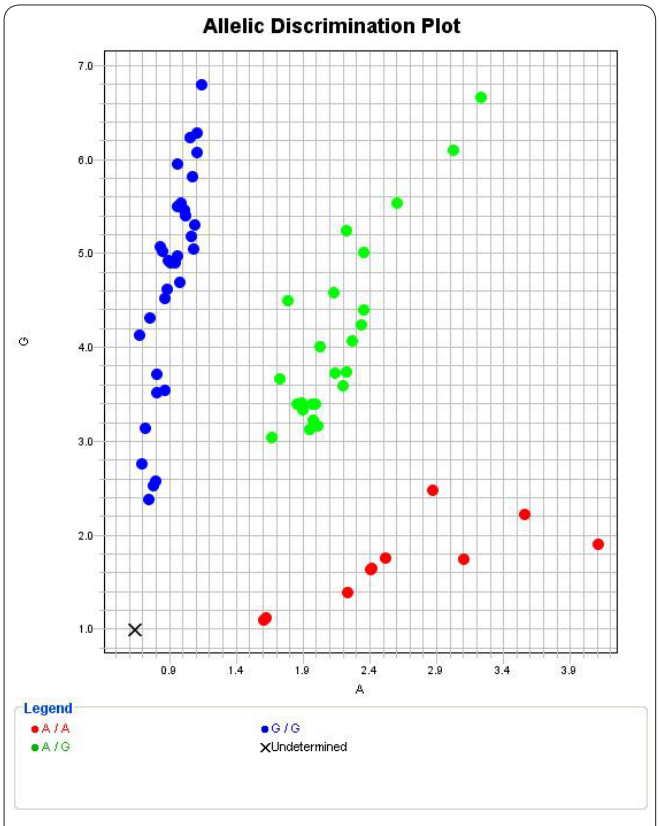

Figure 3. Allelic Discrimination plot of leptin receptors Gln223Arg (rs1137101) polymorphism.

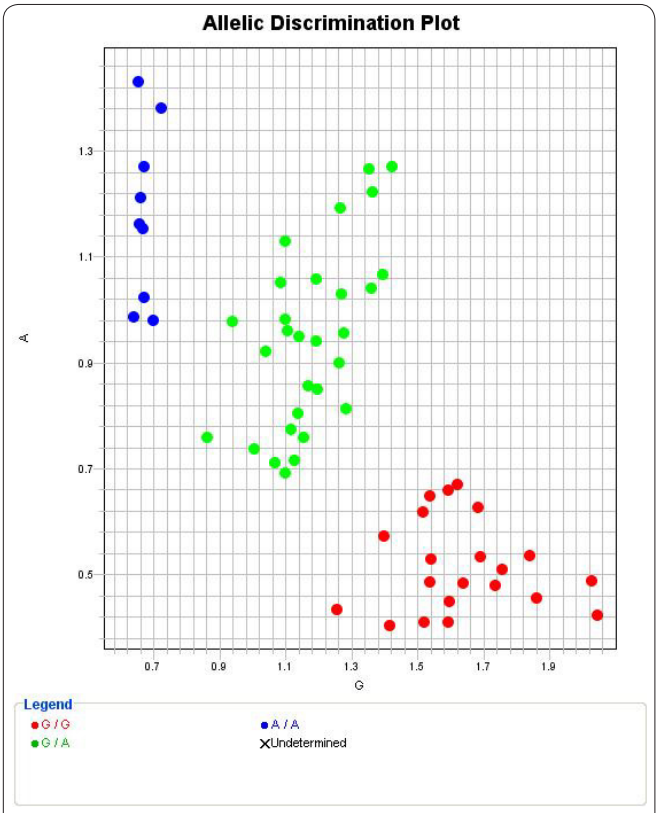

Figure 4. Allelic Discrimination plot of leptin G2548A (rs7799039) Polymorphism.

Table 3. Relation between rs1137101 and different parameters in each group.

	rs1137101					
	Obese (n = 168)		p	Control (n = 126)		p
	AG + GG (n = 126)	AA® (n = 42)		AG + GG (n = 60)	AA® (n = 66)	
Sex						
Male	67(53.2%)	22(52.4%)	0.929	42(70%)	48(72.7%)	0.735
Female	59(46.8%)	20(47.6%)		18(30%)	18(27.3%)	
Age (years)	10.4 ± 2.1	9.7 ± 2.5	0.065	9.6 ± 1.9	10.1 ± 1.8	0.074
BMI (kg/m^2)	27.1 ± 2.6	25.6 ± 2.5	0.001*	19.3 ± 1	19.3 ± 1.3	0.861
Leptin (ng/ml)	35.5(18.4–74.6)	32.5(19.6–39.2)	<0.001*	14.4(8.5 – 19.2)	14.5(7.9 – 17.5)	0.537
Serum insulin (µIU/ml)	28(1.7 – 40)	19.4(2.3 – 32.7)	<0.001*	15(12 – 19)	16(2.3 – 19)	0.422
Fasting blood glucose (mg/dl)	108.5 ± 18.9	90.7 ± 17.1	<0.001*	97.5 ± 6.2	86.9 ± 5.8	<0.001*
HOMA IR	7.3(0.5 – 15.7)	4(0.5 – 10.3)	<0.001*	3.6(3.1 – 4.5)	3.4(0.5 – 3.8)	<0.001*
Total cholesterol (mg/dl)	223.5 ± 37.6	208.8 ± 38.3	0.030*	158 ± 11.1	152.6 ± 8.8	0.243
Triglycerides "TG" (mg/dl)	101(38 – 181)	87(29 – 169)	0.078	102(92 – 120)	105(95 – 128)	0.051
HDLc (mg/dl)	52(32 – 80)	48(39 – 73)	0.507	48(43 – 55)	48(45 – 52)	0.653
LDLc (mg/dl)	147.6(80.6–217.8)	113.2(72.2–199.6)	0.090	149(132 – 169)	140.4(140.4–155)	0.06
Waist circumference (cm)	83.6 ± 7.6	75.2 ± 1.9	<0.001*	66.2 ± 3.4	66 ± 3.3	0.733
Hip circumference (cm)	89 ± 6	87.1 ± 5.1	0.048*	79.1 ± 6	78.1 ± 5.9	0.352
Waist /hip ratio	0.9 ± 0.1	0.9 ± 0	<0.001*	0.8 ± 0.1	0.9 ± 0.1	0.563

Qualitative data were described using number and percent and was compared using Chi square test or Fisher Exact test, while normally quantitative data was expressed in mean ± SD and was compared using student t-test, abnormally distributed data was expressed in median (Min. - Max.) and was compared using Mann Whitney test. *: Statistically significant at $p \leq 0.05$.

Table 4. Relation between rs7799039 and different parameters in each group.

	rs7799039					
	Obese (n = 168)		p	Control (n = 126)		p
	GA + AA (n = 124)	GG® (n = 44)		GA + AA (n = 90)	GG® (n = 36)	
Sex						
Male	73(58.9%)	24(54.5%)	0.62	66(73.3%)	24(66.7%)	0.454
Female	51(41.1%)	20(45.5%)		24(26.7%)	12(33.3%)	
Age (years)	10.1 ± 2.1	10.6 ± 2.4	0.160	9.5 ± 1.7	10.7 ± 1.9	0.065
BMI (kg/m^2)	26.3 ± 2.8	27.9 ± 1.9	<0.001*	19.2 ± 1	19.5 ± 1.5	0.269
Leptin (ng/ml)	33(18.4 – 49.6)	37.5(19.6 – 74.6)	0.004*	14.5(8.5 – 19.2)	13.8(7.9 – 18.4)	0.845
Serum insulin (µIU/ml)	28(2.8 – 38.5)	29.3(1.7 – 40)	0.012*	15(12 – 19)	15(2.3 – 19)	0.844
Fasting blood glucose (mg/dl)	100.9 ± 20.5	113 ± 15.7	0.042*	91.6 ± 8.2	92.8 ± 7.6	0.308
HOMA IR	7.3(0.6 – 12.8)	8.6(0.5 – 15.7)	0.024*	3.5(2.4 – 3.9)	3.4(0.5 – 4.5)	0.205
Total cholesterol (mg/dl)	247.2 ± 29	210.2 ± 36.4	<0.001*	156.1 ± 10.6	160.4 ± 11.6	0.085
Triglycerides (mg/dl)	94(29 – 181)	123(38 – 181)	0.013*	112.5(95 – 128)	102(92 – 120)	0.002*
HDLc (mg/dl)	52(32 – 80)	52(32 – 67)	0.236	48(43 – 55)	48(45 – 52)	0.164
LDLc (mg/dl)	144.2(72.2–217.8)	182.2(109–211.6)	<0.001*	145.5(127 – 169)	144.7(126.5–155)	0.270
Waist circumference (cm)	79.3 ± 5.7	87.7 ± 8.8	<0.001*	66.1 ± 3.4	66.2 ± 3.2	0.960
Hip circumference (cm)	88.2 ± 5.8	89.6 ± 5.6	0.175	78.8 ± 6.1	78.1 ± 5.6	0.569
Waist /hip ratio	0.9 ± 0.1	1 ± 0.1	<0.001*	0.8 ± 0.1	0.9 ± 0.1	0.715

Qualitative data were described using number and percent and was compared using Chi square test or Fisher Exact test, while normally quantitative data was expressed in mean ± SD and was compared using student t-test, abnormally distributed data was expressed in median (Min. - Max.) and was compared using Mann Whitney test. *: Statistically significant at $p \leq 0.05$.

Table 5. Multivariate analysis logistic regression for risk of obesity.

	B	SE	Sig.	OR	95% CI	
					LL	UL
Sex	0.212	0.364	0.561	0.636	0.606	2.520
HOMA.IR	0.817	0.131	<0.001*	2.263	1.751	2.925
Triglycerides (mg/dl)	0.025	0.009	0.06	0.976	0.959	0.993
HDL (mg/dl)	-0.070	0.025	0.005*	1.072	1.022	1.126
LDL(mg/dl)	0.005	0.008	0.513	1.005	0.989	1.022
RS 1137101	0.899	0.448	0.045*	2.457	1.021	5.909
RS 7799039	-0.238	0.366	0.515	0.788	0.385	1.615
Leptin (ng/ml)	9.537	2.207	<0.001*	10.8	8.32	28.92

B: Unstandardized Coefficients , OR: Odds ratio, CI: Confidence interval, LL: Lower limit , UL: Upper Limit

IR, BMI with an increase in leptin levels in obese group than controls.

Under normal condition, leptin production suppresses the need to eat by inhibiting orexigenic neuropeptides release in the hypothalamus but in an obese subject, a state called "leptin resistance may be developed due to the mutation in leptin receptors (15).

In this study, there was a significantly increased frequency of the GG and AG genotypes and G allele of Leptin receptor rs1137101 in the obese group. The GG genotype of rs1137101(G/A) increases the risk of obesity by 7.1- fold.

This in accordance with the study of Sabah et al who suggests that there was an association between GLN223ARG polymorphism and obesity in male children and teenagers (16).

Also, the study of Kanjana and Kittisak demonstrated that LEPR Gln223Arg polymorphism in Thai subjects was associated with increased metabolic syndrome risk (17).

The LEPR Q223R SNP (rs1137101) converts a glutamine a neutral amino acid to an arginine a positively charged amino acid (Gln/Q to Arg/R) in codon 223 (CAG to CGG) at position 668 located within the leptin-binding region of the LEPR gene. Thus, this change may be linked to impaired signaling capacity of leptin (18,19). It may also lead to increase insulin, resistance and glucose levels (20).

In contrast to the present study, several studies on different ethnic groups includes the study of Guìzar-Mendoza on in Mexican adolescents, the study of Okada et al. on Japanese school children, and the study Komşu-Ornek et al. on Turkish children reported that no significant difference in genotypes frequencies for GLN223ARG polymorphism between obese and non obese children (21,22,23).

Regarding LEP G2548A (rs7799039) polymorphism, this study shows a non-significant difference between two group but in comparison of the three genotypes in the obese group, there was a significant increase in leptin, insulin, HOMA-IR, and BMI in AA and GA genotypes when comparing to GG genotype in the same group.

In line with this, a study in Tunisian subjects reported the association of G-2548A with higher BMI and insulin levels in subjects homozygous for AA genotype (24). Also, the study in Egyptian subjects relieved an association of AA genotype of leptin rs7799039 SNP with metabolic syndrome and higher serum leptin levels (25).

While the study of Shahid et al found an association between leptin rs7799039 and obesity in children and adolescents (≤18 years of age) especially in girls, Carriers of G allele had significantly higher BMI, fasting blood glucose and serum leptin levels compared to homozygous A allele carrier (26).

Also, the study of Hoffstedt et al stated that LEP G2548A polymorphism influenced leptin expression, so, it alters plasma leptin levels (27). This may be due to the interactions of these polymorphisms with other polymorphisms of the gene, variation in distribution of genotype and the association of LEP G-2548A variant with obesity in different ethnic groups and genders (28).

It can be concluded that genetic polymorphism in the LEPR Gln223Arg rs1137101 appeared to affect the leptin concentration and the susceptibility to obesity. an association of GG genotype with increased BMI and leptin levels in obese children. While, leptin rs7799039 SNP has no association with obesity.

Conflict of interest

There is no conflict of interest.

Author contribution

Author 1 was responsible for reviewing and publication. Author 2 was responsible for laboratory work, data analysis and writing. Author 3 writed the protocol of the research. Author 4 was responsible for collection of cases.

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References

1.Mirzazadeh A, Sadeghirad B, Haghdoost AA, Bahreini F and Rezazadeh Kermani M. The prevalence of obesity in Iran in recent decade; a systematic review and meta-analysis study. Iran J Public Health. 2009;38(3):1–11.

2.Tirosh A, Shai I, Afek A, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. N Engl J Med 2011;364:1315-25.

3.Dietitians of Canada, Canadian Paediatric Society.The College of Family Physicians of Canada, Community Health Nurses of Canada. Promoting optimal monitoring of child growth in Canada: using the new World Health Organization growth charts. 2010. Available: www.dietitians.ca/growthcharts (accessed 2014 Oct. 24).

4.International Obesity Task Force. [accessed on November 22, 2013]. Availablefrom: http://www.iaso.org/iotf/obesity/obesitytheglobalepidemic/ for Saudi, Canada, South Africa, Australia and NZ estimates.

5.Ogden CL, Carroll MD, Kit BK, et al. Prevalence of obesity and trends in body mass index among US children and adolescents. JAMA 2012;307:483-90.

6.Shabana and Hasnain S. The p. N103K mutation of Leptin (LEP) gene and severe early onset obesity in Pakistan. Biol. Res. 13;49:23.

7.Cammisotto PG, Bukowiecki LJ, Deshaies Y, Bendayan M. Leptin biosynthetic pathway in white adipocytes. Biochem Cell Biol 2006;84:207–214.

8.Hinuy HM, Hirata MH, Forti N et al. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. Arq Bras Endocrinol Metabol 2008;52:611–616.

9.Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP et al. Serum Immunoreactive- Leptin Concentrations in Normal Weight and Obese Humans, N Engl J Med. 1996; 1;334(5):292-5

10.Judzewitch R, Pfeifer M, Best J, Halter J and Port D. Chronic chlorpropamide therapy of noninsulin-dependent diabetes augments basal and stimulated insulin secretion by increasing islet sensitivity to glucose. J Clin EndocrinolMetab. 1982;55(2):321-8.

11.Matthews D; Hosker J; Rudenski A; Naylor B; Treacher D and Turner R: Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia.1985; 28:412-419.

12.Kumar S and Kelly AS Review of Childhood Obesity: From Epidemiology, Etiology, and Comorbidities to Clinical Assessment and

Treatment. Mayo Clin Proc. 2017;92(2):251-265.
13.Bouchard C. Childhood obesity: are genetic differences involved? Am J Clin Nutr.2009; 89:1494–1501.
14.Farooqi IS and O'Rahilly S. Monogenic obesity in humans. Ann Rev Med.2005; 56:443–458.
15.Chavarria-Avila E, Vázquez-Del Mercado M, Gomez-Bañuelos E, Ruiz-Quezada SL, Castro-Albarran J, Sánchez-López L, et al.The Impact of LEP G-2548A and LEPR Gln223Arg Polymorphisms on Adiposity; Leptin, and Leptin-Receptor Serum Levels in a Mexican Mestizo Population. Biomed Res Int. 2015:539408-10 pages.
16.Sabah A. Linjawi and Noor A. Hussain. Impact of Leptin Receptor Gene GLN223ARG Polymorphism on Obesity in Jeddah City. Life Sci J 2012;9(4):818-828.
17.Kanjana Suriyaprom, Rungsunn Tungtrongchitr and Kittisak Thawnasom Measurement of the levels of leptin, BDNF associated with polymorphisms LEP G2548A, LEPR Gln223Arg and BDNF Val66Met in Thai with metabolic syndrome. Diabetology & Metabolic Syndrome. 2014; 6(1):6.
18.Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS. 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.2001;86:4434–4439.
19.Richert, L., Chevalley, T., Manen, D., Bonjour,J., Rizzoli, R. and Ferrari, S. Bone Mass in Prepubertal Boys Is Associated with a Gln223Arg Amino Acid Substitution in the Leptin Receptor, The Journal of Clinical Endocrinology & Metabolism. 2007;92(11), p 4380-4386.
20.Utsunomiya K, Shinkai T, Sakata S, Hwang R, Yamada K, Chen HI, Fukunaka Y, Ohmori O, Nakamura J: Lack of association between the leptin receptor gene (LEPR) Gln223Arg polymorphism and late-onset Alzheimer disease.Alzheimer Dis Assoc Disor 2010;24:101–103.
21.Guízar-Mendoza JM, Amador-Licona N, Flores-Martínez SE, López-Cardona MG, Ahuatzin-Trémary R, Sánchez-Corona J. Association analysis of the Gln223Arg polymorphism in the human leptin receptor gene and traits related to obesity in Mexican adolescents. Journal of Human Hypertension. 2005; 19 (5):341- 6.
22.Okada T1, Ohzeki T, Nakagawa Y, Sugihara S, Arisaka O; Study Group of Pediatric Obesity and Its related Metabolism. Impact of leptin and leptin-receptor gene polymorphisms on serum lipids in Japanese obese children. Acta Paediatr. 2010;99 (8):1213-7.
23.Komşu-Ornek Z1, Demirel F, Dursun A, Ermiş B, Pişkin E, Bideci A. Leptin receptor gene Gln223Arg polymorphism is not associated with obesity and metabolic syndrome in Turkish children. Turk J Pediatr. 2012; 54(1):20-4.
24.Boumaiza I, Omezzine A, Rejeb J, Rebhi L, Ouedrani A, Ben Rejeb N, et al. Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in Tunisian volunteers. Genet. Test Mol. Biomarkers.2012; 16: 726–733.
25.Amina Alnory, Hoda Gad,, Gehan Hegazy, Olfat Shaker The association of vaspin rs2236242 and leptin rs7799039 polymorphism with metabolic syndrome in Egyptian women. Turk J Med Sci. 2016;46: 1335-1340.
26.Shahid A, Rana S, Mahmood S and Saeed S. Role of leptin G-2548A polymorphism in age- and gender-specific development of obesity.J. Biosci.2015;40:521–530.
27.Hoffstedt J, Eriksson P, Mottagui-Tabar S, Arner P: A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin. Horm Metab Res. 2002; 34:355–359.
28.Ng ZY, Veerapen MK, HonWMand Lim RL. Association of leptin/receptor and TNF-α gene variants with adolescent obesity in Malaysia. Pediatr. Int. 2014;12336.